IAEA-TECDOC-1324

Radiation synthesis and modification of polymers for biomedical applications

Final results of a co-ordinated research project
1996–2000

INTERNATIONAL ATOMIC ENERGY AGENCY IAEA

December 2002

The originating Section of this publication in the IAEA was:

Industrial Applications and Chemistry Section
International Atomic Energy Agency
Wagramer Strasse 5
P.O. Box 100
A-1400 Vienna, Austria

RADIATION SYNTHESIS AND MODIFICATION OF
POLYMERS FOR BIOMEDICAL APPLICATIONS

IAEA, VIENNA, 2002
IAEA-TECDOC-1324
ISBN 92–0–119702–0
ISSN 1011–4289

Printed by the IAEA in Austria
December 2002

FOREWORD

Radiation processing is based on the use of high energy ionizing radiation to induce chemical and biological changes in irradiated systems. The use of similar sources like glow discharge, ultraviolet, laser and visible light, and the recently developed incoherent dielectric excimer lamps are closely related. Since high energy electromagnetic and particle radiation exhibit properties of controlled penetration and intensities, which are especially suitable for synthesis and modification of polymeric biomaterials without the need of usually toxic additives, the interest to use radiation techniques in biotechnology and biomedicine is growing rapidly. These methods are being used for synthesis of functional polymers in forms of macro- and microgels, micro- and nanospheres, functionalization of surfaces and radiation processing of naturally derived biomaterials among others. Potential biomedical and biotechnological applications of these new materials include implants, topical dressings, injectable formulations, drug delivery devices, diagnostic assays, separation and purification processes, immobilized enzyme and cell bioprocesses and cell culture surfaces.

The support of the International Atomic Energy Agency (IAEA) for various activities related to the applications of radiation chemistry in biomaterial and bioengineering fields started in 1983 with the Co-ordinated Research Project (CRP) on Radiation Technology for Immobilization of Bioactive Materials. This project was concluded in 1987 with achievements published in IAEA-TECDOC-486. In the period 1988–1994 new CRPs on Radiation Processing Technology Applications in Bioengineering, and on Development of Diagnostic Reagents for Communicable Diseases using Radiation Processing Technique were implemented. These projects were followed, in 1996, by a CRP on The Use of Radiation Processing to prepare Biomaterials for Applications in Medicine with the final research co-ordination meeting held in Ankara, Turkey, on 15–19 November 2000. In parallel with these CRPs, the IAEA also organized small scientific meetings, advisory group meetings and consultants meetings in the course of the same period. Contributions of the participants of these meetings were published in IAEA reports.

This publication includes reports from the participants of the CRP on The Use of Radiation Processing to prepare Biomaterials for Applications in Medicine. Some papers selected here review the research carried out in respective centres while others reflect activities and trends in biomaterials research. The IAEA officer responsible for this publication was O. Güven of the Division of Physical and Chemical Sciences.

EDITORIAL NOTE

This publication has been prepared from the original material as submitted by the authors. The views expressed do not necessarily reflect those of the IAEA, the governments of the nominating Member States or the nominating organizations.

The use of particular designations of countries or territories does not imply any judgement by the publisher, the IAEA, as to the legal status of such countries or territories, of their authorities and institutions or of the delimitation of their boundaries.

The mention of names of specific companies or products (whether or not indicated as registered) does not imply any intention to infringe proprietary rights, nor should it be construed as an endorsement or recommendation on the part of the IAEA.

The authors are responsible for having obtained the necessary permission for the IAEA to reproduce, translate or use material from sources already protected by copyrights.

CONTENTS

Summary 1

Radiation formation of hydrogels for biomedical application 5
J.M. Rosiak, I. Janik, S. Kadlubowski, M. Kozicki, P. Kujawa, P. Stasica, P. Ulanski

The use of radiation processing to prepare biomaterials for applications in medicine 48
I. Kaetsu

Radiation synthesis of hydrogels with diprotic acid moieties and their use in the adsorption of biomolecules 62
M. Sen

Radiation synthesis of supported hydrogels for biomedical and biotechnological purposes 88
E.A. Hegazy

Radiation co-polymerization and its application in biotechnology 118
Ha Hongfei, Zhai Maolin, Li Jun, Yi Min

Gamma radiation technology of producing crosslinked polymers (hydrogels) for specific application in medicine and biotechnology 136
Z.S. Nurkeeva

Irradiation of poly (vinyl alcohol) and poly (vinyl pyrrolidone) blended hydrogel for wound dressing 151
M.T. Razzak, D. Darwis, Zainuddin, Sukirno

Nuclear methods for surface modifications of polymers by ion induced cografting 162
R.O. Mazzei, E. Smolko, D. Tadey, L. Gizzi

Preparation of patterned surfaces and microspheres using radiation processing techniques 179
A. Safrany

Affinity patterning of biomaterials using plasma gas discharge 192
A. Goessl, L. Jung, D. Bowen-Pope, A.S. Hoffman

Bibliography 197

List of Participants 199

SUMMARY

Introduction

A biomaterial is defined as any substance or combination of substances of synthetic or natural origin that can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ or function of the body without causing local adverse reaction or systemic toxicity. Biomaterials thus could either be implanted for long or short term applications, or used externally. The desired final application usually determines the design of the particular biomaterial.

There are a number of ways to synthesize new functional biomaterials or to modify surface properties of existing materials, but radiation initiation of the suitable chemical reactions has a number of advantages over classical initiation methods:

— the absence of additives (initiators, catalysts…), usually toxic materials that could contaminate the product, thus a higher possibility to produce clean materials
— the possibility of initiating the reaction at any temperature
— a wide variety of monomers and polymers to choose from including those that cannot be polymerized by classical chemical initiation
— the possibility to control the degree of crosslinking and grafting
— the possibility to control the depth of surface modification, thus surfaces of finished products can also be modified without affecting the bulk properties
— the possibility of simultaneous synthesis and sterilization
— the possibility of simultaneous immobilization of bioactive materials without any loss in their activity.

Radiation techniques are being used for synthesis of hydrogels, functional polymers, interpenetrating systems, chemical modification of surfaces, immobilization of bioactive materials, synthesis of functional micro- and nanospheres and processing of naturally derived biomaterials. Potential medical applications of these biomaterials include implants, topical dressings, treatment devices and drug delivery systems. Biotechnological applications include diagnostic assays, separation and purification systems, immobilized enzyme and cell bioprocesses and cell culture surfaces.

The main objective of the CRP on The use of Radiation Processing to Prepare Biomaterials for Application in Medicine was to co-ordinate the research carried out in the participating countries, to ensure that different research programmes complement each other and the information exchange is available to all. Furthermore, the objective was to expand the use of ionizing radiation in two major areas: synthesis of polymers and gels for medical and biotechnological applications, and modification of surfaces to achieve a specific functionality and/or to immobilize bioactive materials. The reports of participants in this document are arranged, whenever possible, according to these topics.

Synthesis of hydrogels

Hydrogels are crosslinked macromolecular networks that swell in water and biological fluids. The crosslinks are usually formed by covalent or ionic bonds, although physical crosslinks, such as entanglements, hydrogen bonds, hydrophobic or van-der-Waals interactions can also provide three-dimensional networks. Hydrogels can be synthesized either

by radiation polymerization and crosslinking of a monomer or monomer mixtures (in bulk or in solutions), or by crosslinking of polymers. The properties of the hydrogels (pore size, swelling-shrinking kinetics, water content, etc.) can be controlled by the synthesis conditions. Absorbed dose, dose rate, type of monomer(s) and its concentration (their ratio) and the irradiation temperature, all affect the properties of the gel, thus their appropriate selection permits a tailor-made preparation of a product for a specific application.

In recent years, there is an increasing interest in the synthesis of stimuli-responsive hydrogels, gels that respond to the changes in temperature, pH or solute concentration, electrical or magnetic field, by changes in shape and dimensions. These gels have numerous applications in medicine and industry from drug and hormone delivery devices, biosensors, actuators and artificial muscles, to on-off switches.

Hydrogels, whether "classical" or "stimuli-responsive", represent a major research interest to the participants of this CRP. Hydrogels were synthesized by gamma and electron beam irradiation, in a wide range of different shapes and sizes from nanogels and microgels to macrogels.

The water content of hydrogels is usually very high, and this is one of the reasons for they good biocompatibility and possibility of application in direct contact with living tissues. One of the most common such application is for wound dressing. Radiation synthesized hydrogels that were developed by a participant of this CRP are commercially produced in Poland and patented in several other countries. Collaborative research to develop new co-polymer hydrogels with improved properties for wound dressing is still continuing.

Various synthetic polymers and natural materials were used for preparation of one-component and co-polymer macrogels. Homogeneous hydrogels with acid monomers were prepared for topical delivery of antifungal drugs. Synthetic polymers and polysaccharides were combined as physical blends and as interpenetrating networks. Biodegradable and affinity-based hydrogels were also studied. The potential application for these systems is in biosensors as affinity separation support, enzyme immobilization, cell encapsulation and drug delivery devices.

Besides their use as "selfstanding" systems, hydrogels were also grafted by gamma and electron beam irradiation onto various substrates and used as blood compatible surfaces, supports for enzyme immobilization and dialysis membranes.

There is a growing interest to develop injectable micro- and nanogel drug delivery systems for various ophtalmological and dental applications. The use of such injectable hydrogels as intervertebral disk was also explored. Systems, where thermo-responsive soluble polymers were injected that solidify (sol-gel process) in the body were also developed, and their use in sustained drug release investigated.

Monodisperse functional polymer micro- and nanospheres were prepared by gamma and electron beam initiated precipitation polymerization for use in separation, protein purification and diagnostics.

Since the majority of the participants of this CRP work in the field of hydrogel synthesis, this fact is reflected in the document as well: the first seven papers describe various aspects of hydrogel synthesis. They describe the mechanism of polymerization and cross-

linking and ways to evaluate the properties of the gels. The relationship between the synthesis parameters and gel properties is also given, as well as the results of various successful medical and biotechnological applications.

Surface modification

Since the acceptance and failure of a biomaterial depends mostly on its surface properties, it is important to control and tailor these parameters. These modifications range from a simple treatment for cleaning, to specific treatments to improve biocompatibility, or to enhance or prevent protein and cell interactions. Functional groups can be introduced to surfaces for subsequent conjugation of biomolecules and preparation of cell membrane-like surfaces. Since radiation-induced processes can easily be limited to the surface area only, they are especially suited for these modifications.

In the course of this CRP, gamma and electron beam irradiation, as well as radiofrequency glow discharge (RFGD) was used to modify polymer surfaces for preparation of non-fouling, protein-repellent surfaces for potential use as implants and sensors on one hand, and to increase biomaterial bonding on selected surfaces for improved diagnostics on the other hand.

Grafting of suitable monomers by gamma and electron-beam initiated polymerization was used for biocompatible surface preparation, for hemodialysis membranes and implant applications.

Patterned surfaces were prepared by electron beam initiated grafting of stimuli-responsive hydrogels on polymers for use as potential cell-culture surfaces from where the cells can be harvested without the use of chemicals. Surfaces with cell-adhesive and cell-repellent domains for guided cell-growth were synthesized by using RFGD, heavy ion implantation and photolithography. Hybrid surfaces were created by depositing cell membrane ligands in patterns by glow discharge for cell culture and implant healing applications.

Porous membranes were prepared by irradiation of base polymers with heavy ions, alpha particles and fission fragments. Such membranes could be used as cell culture support, or could be grafted with suitable monomers (where grafting was initiated by gamma, electron beam or glow discharge) for potential use as cell guides and for separation and purification.

The last three papers included in this compilation describe various aspects of surface modification.

Conclusion

In conclusion it can be said, that the objectives of the CRP have been fully met, the research work was carried out in participating institutions as planned, and a number of interactions and joint research projects between the participants started.

The most import contributions of the participants of the CRP to the development of biomaterials through radiation processing can be summarized, as follows:

— Mechanisms of radiation-induced crosslinking of some selected hydrophilic polymers [poly (acrylic acid), poly (ethylene oxide), some polysaccharides] in aqueous solutions

were described. Results on the promising biomedical applications such as wound dressings, controlled drug delivery systems and implants were presented.

— New injectable drug delivery systems were developed using poly (ethylene glycol). Stimuli-responsiveness of poly electrolyte hydrogels were established and intelligent release and permeation devices were constructed using radiation prepared micro-porous films.
— Co-polymeric hydrogels containing diprotic acids were synthesized to be used as speciality adsorbents for biomolecules such as bovine serum albumin, amylase, invertase and some model drugs. Their release behaviours were investigated under different environmental conditions.
— Radiation grafting conditions for various monomers onto polypropylene were optimized to enhance adsorption capacity of the substrate for some enzymes and bovine serum albumin.
— Hydrogels prepared from blends of natural polymers (Kappa-carageenam) and synthetic polymers were characterized for their suitability in biomedical applications.
— Hydrogels based on vinyl ethers were prepared and their use as drain materials in opthalmology surgery tested clinically.
— Functional microspheres with uniform size and narrow distribution were prepared, immobilization and binding capacities towards bioactive molecules such as hystidine and lysosyme were determined. Surface of ELISA plates were radiation modified to improve their sensitivity for early detection of tropical diseases (e.g. schitosoma).
— Patterned surfaces were prepared by combination of gas discharge and lithographic processes to control cell attachment and growth.

The results of the studies carried out in participating centres helped for a better understanding and control of radiation effects on synthetic and natural polymers in the preparation of biomaterials. Some of the results which found industrial or pilot scale applications have been transferred to interested end users in some Member States through technical co-operation projects. Regional and country projects have been initiated for the dissemination of information and implementation of radiation synthesis of hydrogels.

The results and achievements obtained under this project were reported at international scientific conferences and published in highly acclaimed international scientific journals. It is also believed that the presentations included in this document will provide useful and updated information on the present status of biomedical application of radiation techniques and will stimulate researchers, engineers and health-care personnel to look for further solutions of the number of problems still unsolved related to biomedical materials.

RADIATION FORMATION OF HYDROGELS FOR BIOMEDICAL APPLICATION

J.M. Rosiak, I. Janik, S. Kadlubowski, M. Kozicki, P. Kujawa, P. Stasica, P. Ulanski

Institute of Applied Radiation Chemistry, Technical University of Lodz,
Lodz, Poland

Abstract. Hydrogels, i.e. materials consisting of a permanent, three-dimensional network of hydrophilic polymers and water filling the space between the polymer chains, have a number of biomedical applications, such as wound care products, dental and ophthalmic materials, drug delivery systems, elements of implants, constituents of hybrid-type organs, as well as stimuli-sensitive systems. Among various methods applied for the production of hydrogels, the radiation technique has many advantages, as a simple, efficient, clean and environment-friendly process. It usually allows to combine the synthesis and sterilization in a single technological step, thus reducing costs and production time. Efficient application and further development of this method requires broadening of the basic knowledge on the underlying radiation chemistry of polymer systems. Some selected aspects of radiation chemistry of polymers in aqueous solution are presented in this work. The experimental techniques used for studying the radiation-induced processes in polymer solutions are described with special emphasizing of determination of radiation yield of crosslinking by various methods. Also, pulse radiolysis method with different detection methods is briefly described. Selected results of our studies concerning the early stages of polymerization of water-soluble monomers are described together with the studies of mechanisms of radiation-induced crosslinking of polymers in aqueous solution. Separate section of the presentation is devoted to the radiation-induced crosslinking and degradation of polyelectrolytes (i.e. poly (poly (acrylic acid), poly (poly (methacrylic acid)) and biologically important polysaccharide, chitosan. Additionally, special attention is paid to the differences between intra- and intermolecular crosslinking. The irradiation method of changing the proportion between these two processes at the expense on intramolecular crosslinking is described. This leads to the synthesis of internally crosslinked polymeric single coils, i.e. nanogels. The selected properties of such materials are described. Some expectations as to the further research directions in the field of radiation-synthesized hydrogels and examples of their biomedical applications also presented. They include e.g. wound dressings, drug delivery systems, stimuli-responsive systems, implants and hydrogel-based hybrid-type organs.

1. INTRODUCTION

In the materials science as well as in chemistry a very important and rapidly expanding field is the design, formation and testing of materials for medical applications, i.e. biomaterials.

Biomaterial is defined as any substance (other than a drug) or combination of substances synthetic or of natural origin, which can be used for any period of time, as a whole or as a part of a system which treats, augments, or replaces any tissue, organ, or function of the body [1, 2].

Virtually every individual will have contact with biomaterials at some time during his or her life. This contact may occur in several ways:

— permanent implantation, e.g. heart valves, total joint replacement, dental restoration, intraocular lenses;
— long-term application, e.g. fracture fixation devices, contact lenses, removable dental prostheses, hemodialysis systems;
— transient application, e.g. needles for vaccination or phlebotomy, wound healing dressings, cardiopulmonary bypass and cardiac assist systems.

There are three general situations in which biomaterials are used: to sustain life or limb viability; to restore or improve function; to restore or improve contour.

Most cardiovascular and neurosurgical implants are in the first category, e.g. cardiac valves, vascular grafts, pacemakers, and hydrocephalus shunts. These implants have allowed major advances in treatment, and, although significant improvements still can be made, they are generally effective.

The second category includes biomaterials intended to restore function, such as joint replacement, fracture fixation devices, and dental implants. The success rates vary significantly in this category, ranging from excellent results in total hip replacement to lesser success rates in other joints. The biomaterials used in these devices have been improved through an increased understanding of the relevant properties, and are a key to further progress.

Facial reconstruction and breast augmentation and reconstruction are procedures representative of the third category (restore or improve contour). Even though these types of devices are not employed in life-threatening situations, they play an important role in restoring and preserving psychological and social well-being. If used properly, they have a high degree of effectiveness.

In order to consider the safety of biomaterials, a balance of risk to benefit must be recognized. Biomaterials in devices used to maintain life can carry some degree of risk in terms of time to failure and still be considered relatively safe. On the other hand, devices used to restore function or contour must have a higher degree of safety to justify their used. Overall, currently used biomaterials have been found to be safe, causing little difficulty with local tissue reaction or systemic toxicity.

Designing of biomaterial must include: the analysis of desired functions and its localization into an organism; physiology of tissue and body fluids which will be in direct contact with man-made species; choice of constituent materials and technology to achieve the product; fabrication of prototype and examination of its chemical, physical and mechanical properties; analysis of biocompatibility, end-use functioning of prototype in cell culture and in animal models; regulatory review and clinical testing. Such sequence of events forces very closely collaboration between specialists from various domains of science. Any materials to be intended for contact with living organisms have to fulfill some specific requirements, which can be divided into four groups: non-toxicity, functionality, sterilizability and biocompatibility

The main classes of materials for biomedical use are biological tissues, metals, ceramics and polymers.

Historical mentions of gold and ivory replacements of cranial defects go back to the ancient Egyptians and Romans. The use of biological tissues, amnion and placenta for wound dressings dates back to the early 1900s. The first man-made plastic used as surgical implant was celluloid, applied for cranial defect repair. Later, just before the Second World War, bakelite was used for arthoplasty of the hip. The first users applied commercial materials with no regard for their purity, biostability and post-operative interaction with the organism. Thus, these materials evoked a strong tissue reaction and were unacceptable [3].

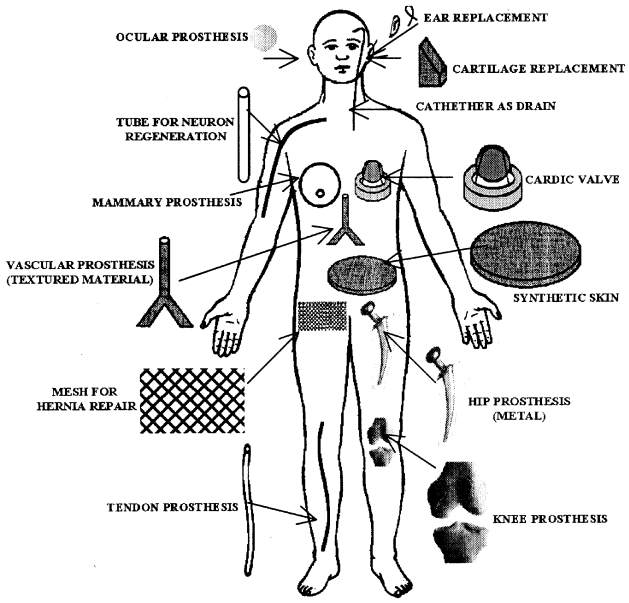


FIG. 1. Exemplary applications of biomaterials [3].

The first polymers which gained acceptance for man-made plastic was poly (poly (methyl methacrylate) — PMMA. It was observed that many pilots ended the war with PMMA splinters from their aircraft canopies embedded in their eyes, which was surprisingly very well tolerated. But the first polymer of choice, precursor of the broad class of materials known today as hydrogels, was poly (poly (vinyl alcohol), PVAL, applied in surgery under the trade name Ivalon. It was crosslinked with formaldehyde, and did withstand autoclaving temperatures. Tissue reaction to the implanted Ivalon is very mild, but after prolonged periods shrinkage and calcification has been observed. However, its applications in plastics surgery or as bone and postenucleation implants have been widely reported as successful. In the fifties Wichterle and Lim synthesized polymer, based on hydroxyethyl methacrylate, and crosslinked with diesters of methacrylic acid and mono, di and triethylene glycols [4]. Despite some technological problems in the beginning, poly (poly (hydroxyethyl methacrylate), poly-HEMA, made a tremendous career as a biotolerable material, primarily as a main component of contact lenses, but also in many other medical fields. Poly-HEMA and its various combinations with other, both hydrophilic and hydrophobic, polymers are till now the most often used hydrogel materials (see below) for medical purposes.

2. HYDROGELS — DEFINITION AND PROPERTIES

Hydrogels form a specific class of polymeric biomaterials. Precise definition of this term is not obvious. Many years ago Dorothy Jordan Lloyd stated that “the colloidal condition, the gel, is one which is easier to recognize than to define” [5]. Since this time more accurate definitions have appeared. Nowadays, hydrogels are defined as two- or multicomponent systems consisting of a three-dimensional network of polymer chains and water that fills the space between macromolecules. Depending on the properties of the polymer (polymers) used, as well as on the nature and density of the network joints, such structures in an equilibrium can contain various amounts of water; typically in the swollen state the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. Two general classes of hydrogels can be defined — physical gels (pseudogels), where the chains are connected by electrostatic forces, hydrogen bonds, hydrophobic interactions or chain entanglements (such

gels are non-permanent and usually they can be converted to polymer solutions by heating) and chemical (true, permanent) hydrogels with covalent bonds linking the chains. In this paper we deal with the formation and applications of permanent polymer networks.

According to the definition formulated above, hydrogels must be able to hold, in equilibrium, certain amounts of water. This implies that the polymers used in these materials must have at least moderate hydrophilic character. In practice, to achieve high degrees of swelling, it is common to use synthetic polymers that are water-soluble when in non-crosslinked form. Typical simple materials applied for general-purpose hydrogels are poly (poly (ethylene oxide), poly (poly (vinyl alcohol), poly (poly (vinyl pyrrolidone) and poly (poly (hydroxyethyl methacrylate). Some other polymers used for gels of special properties are mentioned in the chapter on stimuli-sensitive hydrogels. One should mention that although majority of hydrogels for biomedical purposes are made of synthetic polymers, there are also numbers of examples where crosslinked natural polymers, mainly polysaccharides, are applied.

Although hydrogels have a number of non-biomedical applications (e.g. in agriculture), it seems that their use in the field of medicine and pharmacy is the most successful and promising [6-13]. Over 30 years of research in this field resulted in the common use of hydrogels as soft contact lenses, wound dressings, drug-delivery systems, superabsorbents *etc.* with a number of products being commercially available.

A part of this success can be related to the fact that some important properties of hydrogels, e.g. the ability to absorb aqueous solutions without loosing shape and mechanical strength, are commonly met in many natural constituents of a human body, like muscles, tendons, cartilage *etc.* Besides that, hydrogels usually exhibit good biocompatibility in the contact with blood, body fluids and tissues.

Nowadays a new class of hydrogels, capable of reacting to various environmental stimuli as temperature, pH, ionic strength, solute concentration, electric field, light, sound etc., is tested for use in the so-called ”intelligent biomaterials”. For details the reader is directed to the already vast number of papers on this topic [14-17]. Certainly there is still a big gap between the artificial hydrogel fish that moves by swinging its tail in a laboratory bath [18] and an implementation of artificial muscles. However, rapid progress in this field, correlated with increasing demands for more effective medical treatment indicates that there is no exaggeration in including these systems in the list of the ”materials of XXI century”.

3. THE USE OF RADIATION TECHNIQUE IN THE FORMATION OF HYDROGELS

3.1. Introduction

Hydrogels may be synthesized in a number of ”classical” chemical ways. These include one-step procedures like polymerization and parallel crosslinking of multifunctional monomers, as well as multiple step procedures involving synthesis of polymer molecules having reactive groups and their subsequent crosslinking, possibly also by reacting the polymers with suitable crosslinking agents (e.g. [19]).

Although these methods allow to obtain products of desired properties, they have a significant drawback as far as synthesis of hydrogels for medical purposes is considered. Such

products should not contain any toxic substances (monomers, initiators, crosslinking agents, and additives) normally used in the chemical procedures. These unwanted substances have usually to be washed out from the product in a separate step. This complicates the technology and may lead to a significant increase in production costs.

Ionizing radiation has been since long recognized as a very suitable tool for the formation of hydrogels (e.g. [20, 21]). Easy process control, possibility of joining hydrogel formation and sterilization in one technological step, no necessity to add any initiators, crosslinkers, etc., possibly harmful and difficult to remove, no waste, relatively low running costs — this makes irradiation the method of choice in the synthesis of hydrogels, especially for biomedical use (although, certainly, this technique has also some limitations and not every kind of hydrogel we may think of can be synthesized in this way). Also from the point of view of radiation chemistry, crosslinking of polymers, including hydrogel formation, belongs to the most successful applications of this branch of science.

Already in the early fifties, the pioneers of the radiation chemistry of polymers began some experiments with radiation crosslinking, also with hydrophilic polymers. However, hydrogels were analysed mainly from the point of view of phenomena associated with mechanism of reactions, topology of network, and relations between radiation parameters of the processes. Fundamental monographs on radiation polymer physics and chemistry written by Charlesby [22] and Chapiro [23] proceed from this time. The noticeable interest in application of radiation to obtain hydrogels for biomedical purposes began in the late sixties as a result of the papers and patents published by Japanese and American scientists. Among others, the team of the Takasaki Radiation Chemistry Research Establishment headed by Kaetsu as well as Hoffman and his colleagues from the Center of Bioengineering, University of Washington have created the base for spreading interest in the field of biomaterials formed by means of radiation technique. Immobilization of biologically active species in hydrogel matrices, their use as drug delivery systems and enzyme traps as well as modification of material surfaces to improve their biocompatibility and ability to bond antigens and antibodies have been the main subject of their investigations [7, 24].

Design and optimization of efficient, safe and economically sound radiation-based technologies of hydrogel formation requires the knowledge of the underlying radiation chemistry. This need has been since long one of the main factors stimulating the investigations on radiolysis of polymers in aqueous solutions. Some basic findings of these studies are briefly discussed below.

3.2. Choice of methods

Hydrogels can be obtained by radiation technique in a few ways, including irradiation of solid polymer, monomer (in bulk or in solution) or aqueous solution of polymer. The first method, i.e. irradiation of hydrophilic polymer in a dry form (e.g. [25]), has some drawbacks. It may require special sample preparation (like pressing or melting), and some difficulties may be encountered in obtaining homogeneous macroscopic hydrogels. Moreover, it requires usually much higher doses of ionizing radiation to obtain a gel compared to irradiation in solution, and, furthermore, it may be difficult to remove fully the oxygen, that can promote unwanted side reactions [11]. One of the reasons for the high gelation doses in dry state is that radiation-chemical yield of radicals, that are the precursors of crosslinks, is usually lower than in aqueous solution. Also the restricted motion of the radical-bearing chains limits the

effectiveness of crosslinking. One has to note, however, that the from the necessity to use higher doses for gelation in the solid state it does not follow that more energy is needed for the formation of an average link between the chains. Radiation doses are calculated as energy per mass unit of the system. If we assume that gelation occurs when, on average, one crosslink is formed per each polymer chain [22], it is obvious that more crosslinks are needed for the gelation of 1 kg of a solid polymer sample, than for gelation of 1 kg of its dilute solution.

More frequently the method of monomer irradiation is applied (e.g. [20, 21]). In this technique polymerization takes place in the first stage, followed by crosslinking of the formed chains. This way is possibly most convenient when the chosen monomer is easily available but its polymer is not. Since many of the monomers used are harmful or even toxic (usually contrary to the corresponding polymers), particular care has to be taken when using this method for the formation of hydrogels for biomedical use to ensure that either all the monomer has reacted or its unreacted residues have been fully extracted afterwards, in a separate operation. Since during irradiation of monomer many consecutive and parallel reactions occur, the system is rather complicated and difficult for qualitative description, although some attempts were [26–30]. It is a frequent practice to add some bifunctional monomer to the mixture to increase the efficiency of crosslinking. It has to be stressed that in many cases this synthesis method works very well. There are rare cases, like with poly (poly (vinyl alcohol) gels, where it cannot be used because of the unstability of the monomer.

Especially convenient method of radiation-based synthesis of hydrogels is the irradiation of polymers in aqueous solution, since such systems, containing neither monomers nor crosslinking agents (otherwise frequently used to enhance gel formation), are easier to control and study. Also, with the application of this method, lower number of usually unwanted processes occurs, as e.g. homografting of monomer on a polymer chain that may lead to branched structures, and, last but not least, hydrogels formed in this way are suitable for biomedical use (certainly, provided that a proper polymer is chosen) with no need of further purification [31].

Typical examples of simple, synthetic polymers used for hydrogel formation by this method are poly (poly (vinyl alcohol) — PVAL, poly (poly (vinyl pyrrolidone) — PVP, poly (poly (ethylene oxide) — PEO, polyacrylamide — PAAm, poly (poly (acrylic acid) — PAA and poly (poly (vinyl methyl ether) — PVME. Gels obtained from two latter substrates belong to the group being of particular interest as the components of ”intelligent biomaterials”, since their properties are sensitive to environmental stimuli — pH, ionic strength (PAA) and temperature (PVME).

3.3. Experimental techniques for studying the radiation-induced processes in polymer solutions

Before we proceed to the brief description of radiation-induced reactions leading to the formation of hydrogels, a few words has to be said on the experimental techniques applied in studying such processes in polymeric systems. One of the basic tools is the sol-gel analysis, based on gravimetric determination of sol and gel fractions after irradiation. Simple to perform, it allows to determine the radiation-chemical yields of crosslinking and chain scission (degradation). Calculations are usually based on the Charlesby-Pinner equation [22], or on its later developed, more general version [32, 33] (for other analytical approaches see e.g. [34, 35]).

Polymer network or gel structure is completely different in behaviour from the original or partly crosslinked molecules; if flexible it can have rubberlike properties, and it may swell. The dose required to reach the gel point is D_g and the density of crosslinking due to any higher dose D is often expressed in terms of a crosslinking coefficient δ related to D and D_g; $\delta = D/D_g$. Further irradiation ($\delta > 1$) increases the fraction of the initial polymer linked together into a network or gel (gel fraction – g), while the remaining soluble fraction s decreases; $s + g = 1$. The relation between the soluble fraction s and dose D depends on the initial average molecular weight and molecular weight distribution. Theory of interaction of ionising radiation with matter assumes that these crosslinks occur at random and in proportion to dose.

When polymers are subjected to ionising radiation, crosslinking and main chain scission are usually observed. The processes ultimately cause formation of an insoluble gel, if crosslinking predominates over scission. Charlesby and Pinner [22] first obtained a simple expression relating sol fraction, s to absorbed dose D:

$$s + \sqrt{s} = \frac{p_0}{q_0} + \frac{2}{q_0 u_{2,0} D} \quad (1)$$

where p_0 is degradation density, average number of main chain scissions per monomer unit and per unit dose, q_0 is crosslinking density, proportion of monomer units crosslinked per unit dose, $u_{2,0}$ is initial weight average degree of polymerisation.

As long as the initial molecular weight distribution is of the most probable type, i.e. $M_{w,o} = 2M_{n,o}$, there is linear relation between $s + \sqrt{s}$ and the reciprocal of the dose D. Equation (1) may also be written in other forms:

$$s + \sqrt{s} = \frac{G_s}{2G_c} + \frac{9.6 \times 10^6}{M_{w,0} G_c D} \quad (2)$$

$$s + \sqrt{s} = 0.5\lambda + 0.5(4 - \lambda)\frac{D_G}{D} \quad (3)$$

where D and D_G are the absorbed and the gelation doses in kGy, respectively ; G_s and G_c are radiation yields of scission and crosslinking ($\lambda = G_s/G_c = 2p_o/q_o$).

From the plot of the dependence of $s + \sqrt{s}$ on 1/D or D_g/D it is possible to obtain the ratio $G_s/2G_c$ by extrapolating to the ordinate and to establish whether only crosslinking takes place in the polymer. Many authors find deviations in their sol/dose curves from the straight line predicted by Charlesby formula, and they ascribe it not to the structural effects of the polymer, but rather to the departure of the distribution of molecular weight from the random MWD.

Inokuti [34] extended the theory to the case of polymers whose MWD is the Schulz-Zimm type, further work of Saito, et al. [35] extended it also for polymers of the Wesslau empirical distribution. Inokuti and Saito equations may be applied only for analysing of specimens for known MWD, and described with the most probable, Schulz-Zimm or Wesslau distribution. This is a seldom case in ordinary laboratory investigations.

We proposed [33] the new general formula, which always allows to plot the relation between sol and dose in form of the straight line:

$$s + \sqrt{s} = \frac{p_0}{q_0} + \left(2 - \frac{p_0}{q_0}\right)\left(\frac{D_V + D_G}{D_V + D}\right) \quad (4)$$

or alternatively:

$$s + \sqrt{s} = 0.5\lambda + 0.5(4 - \lambda)\left(\frac{D_V + D_G}{D_V + D}\right) \quad (5)$$

where D_v is the virtual dose.

The virtual dose is a dose required for changing the distribution of molecular weight of polymer under study for such a way that the relation between weight number and average number of molecular weight would be equal 2. If the real polymer under study has initially the narrow MWD, then the virtual dose has a meaning of a dose, which should be delivered to this specimen for changing its MWD to random (i.e. with $M_w/M_n = 2$). In terms of equation (4), the value of such dose will be negative. Alternatively, if the real polymer under study has the wide MWD, the virtual dose has a positive value. It means that D_v would be a dose needed to change MWD from random to that, which is characteristic for the real specimen under study. Representative plot of the sol-gel according to the modified Charlesby-Pinner equation analysis is shown in Fig. 2.

Determination of D_v for the real polymer under study may be done as follows:

1. If there is possibility to measure of weight and number average molecular weights of the polymer under study D_v may be calculated from relation:

$$D_V = \frac{4}{3q_0}\left(\frac{1}{2u_1} - \frac{1}{u_2}\right) \quad (6)$$

where u_1 and u_2 are, respectively, number and weight average polymerization degrees of real polymer undergoing irradiation. If $u_1 = 0.5u_2$ thus $D_v = 0$, and the modified Charlesby equation (5) reduces to classical form (1).

2. If the data from gel-sol analysis are sufficiently precise, p_o/q_o can be obtained from the intercept at $D \rightarrow \infty$, and D_v may be calculated as a slope of the straight line in the co-ordinates $1/(s + \sqrt{s} - p_o/q_o)$ versus absorbed dose.

3. When the data are not sufficiently accurate, it may be preferable to calculate a value of D_v using an appropriate computer programme (which can be received from author's laboratory), which gives the closest approximation of the data points to a straight line in modified coordinates of the equation (4). It gives a remarkably accurate estimation

of D_v, D_g and p_o/q_o on the basis of variables (s and D) measured during routine gel-sol analysis.

It is worth to underline that the classical Charlesby-Pinner equation is still valid as a special case of the modified relation, when the initial MWD irradiated polymer is random.

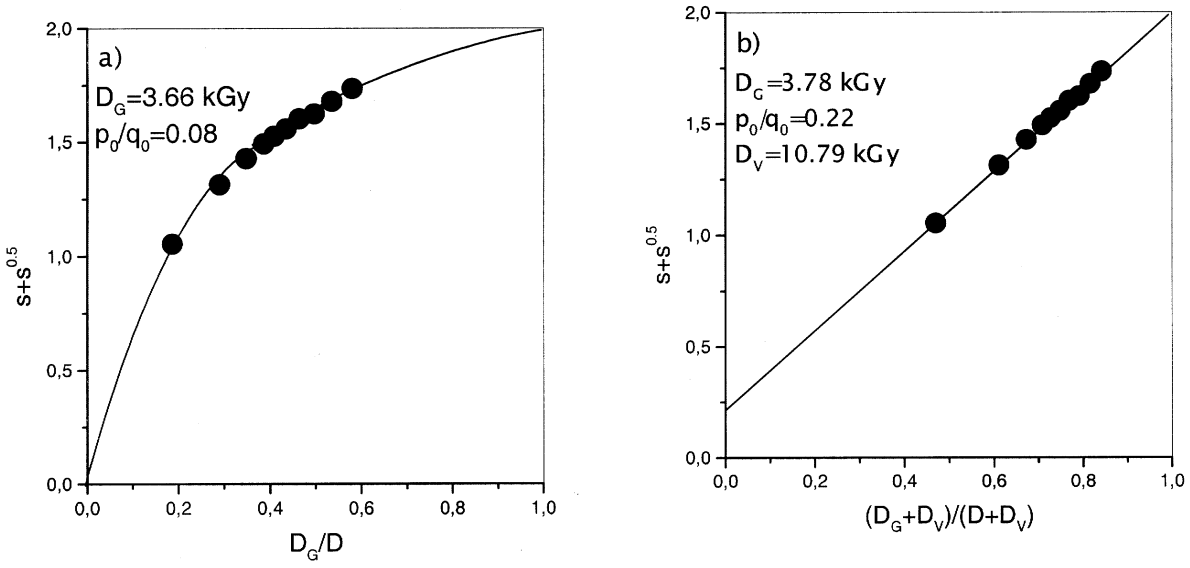


FIG. 2. Typical results of a sol-gel analysis, plotted in the co-ordinates resulting from the classical Charlesby-Pinner equation (a) and its modified version (b). Data refer to the radiation crosslinking of vinylpyrrolidone with 1% of crosslinker ethylene glycol dimethacrylate (DMGE). Dose rate: 3.3 kGy h^{-1}. Samples were irradiated in air.

The value of the radiation yield of crosslinking G_x (expressed as number of moles of crosslinking bonds per J) can be expressed as:

$$G_x = \frac{4.9 \times 10^2}{M_{w,0} D_g} \quad (7)$$

where D_g is gelation dose expressed in Gy.

Equation (7) is derived for the case when dry polymer is irradiated and only crosslinking reaction occurs in the system. If we additionally take into consideration the crosslinking in the polymer solution at the concentration c of the solution (expressed in g per dm^3) and the possibility of simultaneous degradation, equation (7) becomes:

$$G_x = \frac{4.9 \times 10^2 c}{M_{w,o} D_g \rho \left(2 - \frac{\lambda}{2}\right)} \quad (8)$$

where ρ is the solution density (in kg dm^{-3}). Thus, knowing the initial weight average molecular weight of irradiated polymer, D_g and λ from modified Charlesby-Pinner equation, the absolute values of G_x (and also G_s) can be calculated.

Also the measurements of equilibrium swelling of the hydrogels provide a way to calculate the mesh size of the gel network and crosslinking yield (Rosiak, et al. 1988a). This method allows to obtain the G_x value without any information on the polymer molecular weight. It is also applicable to the more complicated systems, where subsequently polymerization and crosslinking occur (i.e. when monomer solution is irradiated).

A crosslinked gel swells to an extent depending on the concentration of effective chains V_e, which is related to the average molecular weight between successive crosslinks:

$$V_e = \frac{\frac{\upsilon}{V_1}\left[\ln\left(1 - V_{2,s}\right) + V_{2,s} + \mu V_{2,s}^2\right]}{V_{2,r}\left[\left(\frac{V_{2,s}}{V_{2,r}}\right)^{1/3} - 0.5\left(\frac{V_{2,s}}{V_{2,r}}\right)\right]} \quad (9)$$

where υ is the specific volume of the polymer, V_1 is the molar volume of the solvent, $V_{2,r}$ and $V_{2,s}$ are polymer volume fractions of the gel samples in both relaxed and swollen state. In this context relaxed means the arrangement of the macromolecules during crosslinking, and swollen means in final solvent equilibrium (after swelling to the equilibrium value). Parameter μ is the Flory-Huggins parameter, which is a measure of the polymer-solvent interactions. Equation (9) is an extension of classical swelling theory of Flory, modified for the case of networks, where the crosslinks are introduced in solution (in relaxed state) [36, 37]. It must be emphasized here that it applies only to loosely crosslinked networks. Polymer volume fractions $V_{2,r}$ and $V_{2,s}$ of the gel samples can be calculated from the volume of the polymer in solution $V_p=W_p/d_p$, where W_p is the weight of the polymer and d_p — its bulk density (equations 10 and 11):

$$V_{2,r} = \frac{V_p}{V_r} \quad (10)$$

$$V_{2,s} = \frac{V_p}{V_s} \quad (11)$$

where V_s and V_r are volume of swollen and relaxed state, respectively.

If chain ends are ignored V_e is equal $1/M_c$ (mol/g), where M_c is number average molecular weight between two successive crosslinks. If the effect of chain ends is included, the concentration of effective chains becomes equal:

$$V_e = \frac{1}{M_c} - \frac{2}{M_{n,0}} \quad (12)$$

where $M_{n,0}$ is the initial number average molecular weight of the polymer. In fact, equation (12) is only valid, when the sol fraction is small or negligible (at late stages of crosslinking). In this case, the G_x is related to the M_c, polymer concentration in irradiated solution c (in g per dm^3) and absorbed dose D (in Gy):

$$G_x = \frac{4.9\times10^2 c}{M_c D\rho} \quad (13)$$

when no end-effects are considered. When it must be included equation (12) applies:

$$\mathrm{V_e} = \frac{\mathrm{G_x D}\rho}{4.9\times10^2 \mathrm{c}} - \frac{2}{\mathrm{M_{n,0}}} \quad (14)$$

Thus, plotting V_e against dose D, a straight line should be obtained. The slope gives G_x/c, and the intercept $2/M_{n,0}$. However, care must be taken for such extrapolation in the dose range near the gelation dose. In that case the sol fraction cannot be ignored.

Equation (14) may be also applied to the gels obtained by monomer irradiation, since in calculating G_x value, only the slope of the straight line is involved. It is, however, necessary to ensure that a complete transformation of monomer to polymer occurs before crosslinking starts, and that the sol fraction is small or negligible. If these conditions are fulfilled equation (14) becomes:

$$V_e = \frac{G_x D\rho}{4.9\times10^2 c} + A \quad (15)$$

where A is a constant. It must be emphasized here that equation (15) is only valid for doses much higher that the dose needed for complete polymerization of the monomer in the system.

The gelation process can be also followed by NMR technique based on the measurements of the spin-spin relaxation times [28, 38, 39].

More detailed information as to the reactions and processes underlying these macroscopic changes can be obtained by the methods typical for radiation chemistry — qualitative and quantitative product analysis, kinetic measurements and studies on the short-lived reactive intermediates. In contrast to the sol-gel analysis and equilibrium swelling experiments, which are performed when the gel is already present in the system, these studies are often done at the early stages of irradiation (low doses), before the macroscopic gel is formed. The most important aspect of the product study from the point of view of crosslinking and competing reactions are the changes in molecular weight. Knowing the initial value of the number-average molecular weight $M_{n,0}$ and weight-average molecular weight $M_{w,0}$ as well as the respective average molecular weights M_n and M_w after irradiation of a polymer solution with a dose D, one can calculate the radiation-chemical yields of intermolecular crosslinking G_x and chain scission G_s (in mol J^{-1})on the basis of equations (16) and (17) [22, 40, 41].

$$\mathrm{G_S} - \mathrm{G_X} = \frac{\mathrm{c}}{\mathrm{D}\rho}\left(\frac{1}{\mathrm{M_n}} - \frac{1}{\mathrm{M_{n,0}}}\right) \quad (16)$$

$$G_S - 4G_X = \frac{c}{D\rho}\left(\frac{1}{M_W} - \frac{1}{M_{W,0}}\right) \quad (17)$$

where ρ is the solution density (in kg dm^{-3}) and c is the polymer concentration expressed in g dm^3. Precise knowledge of the changes of M_n and M_w in the course of irradiation requires either their separate determination by different techniques (for example osmometry and light-scattering, see below), or measurements of the changes in molecular weight distribution by gel permeation chromatography (GPC) (*cf.* [42]). Since this is a complicated task and the quality of results obtained by the latter technique depends on the accuracy of calibration (not always easy, especially for polyelectrolytes), frequently one measures the changes of only one average molecular weight and either one confines himself to the difference of G_x and G_s, or, when justified by the reaction mechanism, one assumes that one of these values is close to zero. Equation (17) is fully applicable for samples of random initial molecular weight distribution of polydispersity ratio $M_{w,0}/M_{n,0} = 2$, but since many commercially available samples have distributions that do not greatly differ form the desired one, the equation is commonly used. The rationale behind measuring rather M_w than M_n is that the former value can be determined over a broad range, including very high molecular weights, with an absolute and precise method of light scattering, while equally good measurements of high values of M_n are more difficult to perform.

It must be emphasized here that different methods of determination of the radiation yield of crosslinking presented above are applied at various stages of the network formation. While the method based on changes in molecular weight can be applied before the gelation dose is reached and there is no gel fraction in the system, the sol-gel analysis is established for the system located well above the gelation dose (i.e. when high amount of the gel is present). Determination of the G_x based on the gelation dose value (equation (8)) is located in the middle between these two limiting approaches. Also it is important to note that the values of the radiation yield of crosslinking can vary depending on the polymer concentration, so that it is highly recommended that authors should always well characterize the investigated system, give the information about polymer concentration and about the method used in calculating yield of crosslinking.

Crosslinking and scission can be also followed by viscometric measurements. An advantage of this method is its simplicity, but recalculation of the obtained ”viscosity”-average molecular weight into M_n or M_w requires assumptions that may lead to significant errors. Furthermore, changes in viscosity as well as retention times in GPC can reflect not only true increase or decrease in molecular weight but also changes in the conformation and hydrodynamic size of macromolecules resulting from e.g. intramolecular crosslinking.

Other changes in the chemical structure of an irradiated polymer, i.e. decrease or increase in the number of specific groups, are usually more difficult to follow. Most of the products formed by irradiation are still a part of the polymer chain, so they cannot be separated. Also the concentration of such altered structures within the polymer is usually low. A more convenient way of studying these effects is to choose a low-molecular-weight model compounds, being short fragments of the polymer chain, and subject them to irradiation under otherwise similar conditions (a common practice in the studies on biopolymers [43], successfully applied as well for synthetic polymers as PVAL and PAA — see [44–46]). For these small model molecules the choice of precise and informative analytical methods (various modes of chromatography, mass spectrometry, etc.) is much broader than in the case of polymer itself.

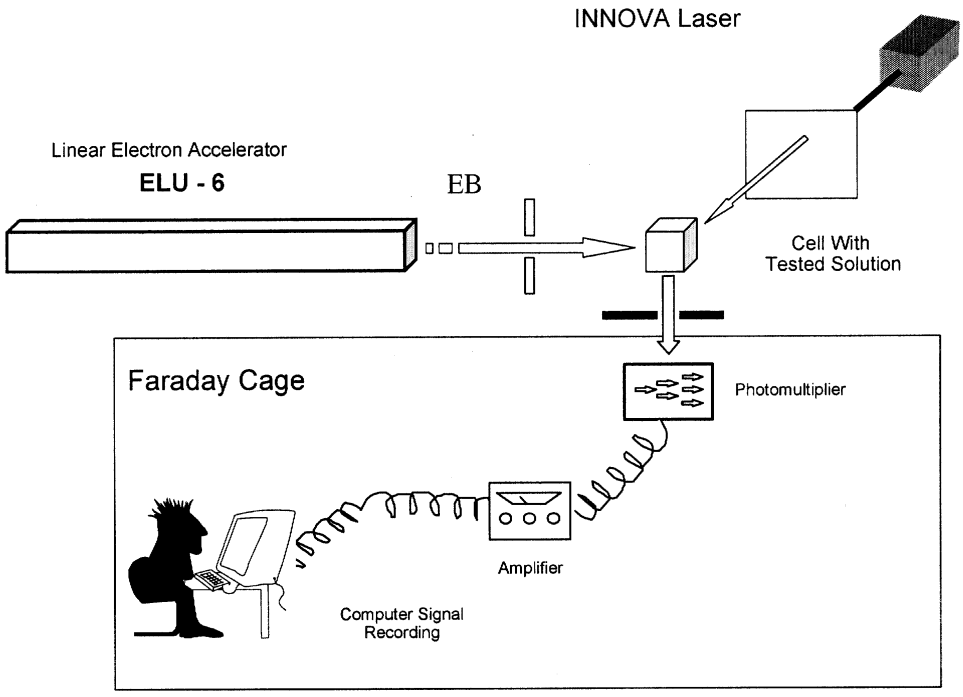


FIG. 3. Schematic representation of a pulse radiolysis system combined with right angle light-scattering detection.

Kinetic studies on the fast reactions initiated by irradiation of monomer and polymer solution, as well as identification of the reactive intermediates, can be performed by pulse radiolysis, a well-established tool in the radiation chemistry of "small" molecules. There are no significant limitations for the use of this technique for polymer solutions — in fact first papers in this field were published already in the sixties [47, 48]. Most data concerning polymers are gathered with spectrophotometry as the classical detection technique for pulse radiolysis experiments. This method is, however, not always satisfactory. Most transient species generated on the chains of simple, synthetic water-soluble polymers like PEO, PVAL, PAA, PVP or PVME, i.e. usually alkyl- and peroxyl-type macroradicals, have rather broad, featureless spectra with significant absorption only close to the low-wavelength detection limit. Moreover, the shape and time evolution of these spectra do not give direct information on such important processes as crosslinking and chain scission. For these reasons two complementary detection techniques were applied in the pulse-radiolytic studies on polymer solutions. One of them is the detection of light scattered by the polymer sample. It is a version of the above mentioned light-scattering technique, but here the laser light is scattered from a sample that is pulse-irradiated and the time-resolved changes in its intensity are recorded, providing information on the kinetics of crosslinking and/or chain scission (e.g. [49–51]). Recently, some attempts to adopt the light-scattering method to study the early stages of the radiation-induced polymerization have also been made [52]. The schematic representation of the experimental setup used for this kind of studies is shown in Fig. 3. For studies on polyelectrolytes, especially concerning the radiation-induced chain scission in such systems, a conductometric detection setup can be applied [53]. Its use is based on the fact, that when a polyion undergoes chain scission, the electrostatic potential at the newly formed ends is lowered and a part of counterions surrounding the charged macromolecule is liberated, a process that leads to the increase in the solution conductivity [54]. Light-scattering and conductivity detection methods have proven to work well both for biopolymers and their models as well as for synthetic polymers (e.g. [44, 45,55–59]).

3.4. Early stages of the radiation-induced polymerization

Kinetic parameters of the polymerization process are important parameters form both, practical and theoretical point of view. The knowledge of the reaction mechanisms let us improve the conditions of a technical process in order to obtain the final products with the best possible properties. Also, the studied of the basic steps involved in the polymerization are important in recent investigations concerning the kinetic of diffusion-controlled reactions. Pulse radiolysis with optical and light scattering detection can be used as a convenient tool in this kind of research.

The water soluble monomers studied in our laboratory were vinylpyrrolidone [20, 21, 52], cationic 2-[(methacryloyloxy)ethyl]trimethylammonium chloride (MADQUAT) [60], and anionic sodium 2-(acrylamido)-2-methylpropanesulfonate (AMPSNa) (Kujawa and Rosiak, unpublished results). These monomers are widely used in preparation of ionic hydrogels., which may be useful especially as the media with various adhesion efficiency for cell growth and proliferation. Some experiments have also been done with butyl acrylate, hydrophobic monomer used as a sensitizer for the vulcanisation of natural rubber latex with γ-irradiation [61]. Similar studies concerning polymerization of the monomers used in the production of biomaterials were also carried on by Takacs, et al. [62, 63].

The mechanism of the radiation-induced changes was studied by optical detection method and was similar in all cases. It consists of several stages:

(1) Addition of hydroxyl radicals (and hydrogen atoms) to carbon-carbon double bond of monomer with subsequent formation of monomeric radicals. The rate constant of this process is diffusion controlled.
(2) Addition of hydrated electrons to carbonyl groups and formation of radical anion. The rate constant of this reaction is also very high (~ 10^{10} $dm^3mol^{-1}s^{-1}$).
(3) The decay of radicals with parallel addition of monomer molecules to the growing chain. In case of hydrated electron adducts the decay is complex and depends on the acidity of reaction medium. At low pH radical anion undergoes fast, reversible protonation at the carbonyl oxygen. In basic solution, the reaction takes place at the β-carbon atom with subsequent formation of α centred carbon radical. The α centred carbon radicals of similar structure are formed upon the addition of hydroxyl radicals. Their decay depends on monomer concentration and dose per pulse value (Fig. 4).

The kinetics of the radical decay reflects the termination process, taking place in the reaction medium. It cannot be described by single value of the second-order rate constant. In the course of the reaction radicals grow, as the effect of propagation reaction. These growing macroradicals decay with lower rate constant, because the termination is diffusion controlled. Thus, the kinetic constant of the decay decreases with the reaction progress. The slowing down of the rate constant is more apparent when the concentrated solutions are pulsed and/or the dose per pulse is low. This effect can be also easily explained if we assume that the radical decay is diffusion controlled. Thus, at low dose/pulse values and high monomer concentration, chain growth events are favored, which leads to the formation of bigger macroradicals, which decay slower.

The light scattering (LSI) detection should give the direct information on the macroradicals growing in the solution. This process can proceeds in two ways: propagation of

the chain (by the addition of monomer molecules) or termination by recombination (biradical reaction). These two processes occur simultaneously in the solution, participating in the observed LSI signal increase. The other possibilities of LSI signal changes are diffusion of two approaching radicals and conformation rearrangement of newly formed macromolecules. While the termination reaction may be described by second-order kinetics, both propagation and diffusion processes should be the first-order. Although kinetic treatment of the recorded curves needs further refinement, a few general trends can be observed. With the increasing dose per pulse, the decrease in the limiting value of LSI signal was observed (Fig. 5). Also, the amplitude of LSI changes decreases, when the monomer concentration increases. This can be attributed to the smaller size of the formed oligomer or polymer molecules, complementary to spectroscopic detection. Further experiments connected with this topic are carried out in our laboratory. It is worth mentioning that, to the best of our knowledge, this is the first application of LSI detection to the direct observation of the macromolecular growth in the real time.

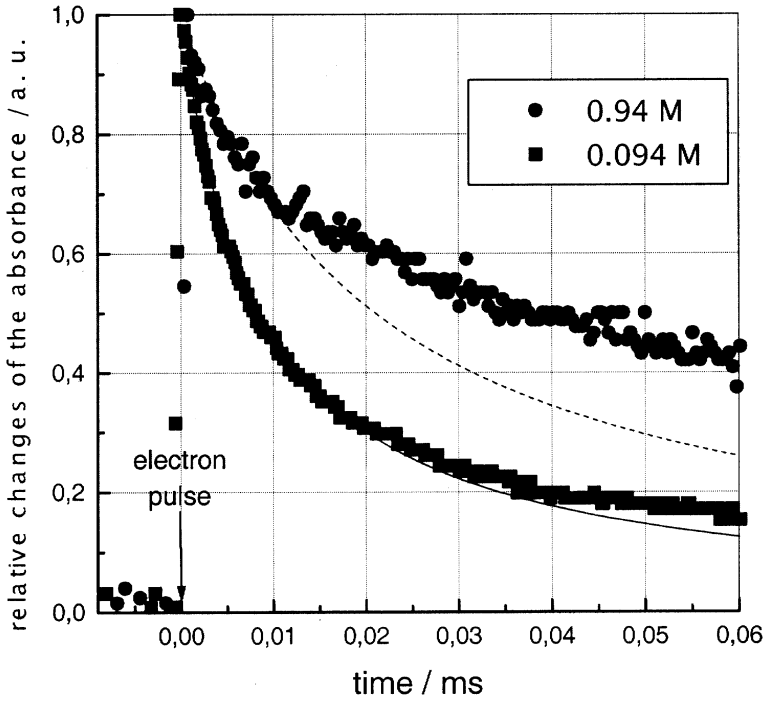


FIG. 4. Decay profiles of the radical absorption in pulsed vinylpyrrolidone solution at two different concentrations of monomer (0.94 and 0.094 M). Dose per pulse ca. 500 Gy, pulse length — 1 μs, solution saturated with nitrous oxide. Lines are calculated based on the second-order decay equation with single value of the rate constant, calculated during the initial stage of the decay. Deviation from classical behaviour is easily observed, especially at high monomer concentration.

3.5. Mechanism of the radiation induced crosslinking of polymers in aqueous solution

When polymer solution is subjected to ionizing radiation, reactive intermediates are formed. This can result from direct action of radiation on the polymer chains and from indirect effect, i.e. reaction of the intermediates generated in water with polymer molecules (for a general description of radiation-induced processes in aqueous solution, see e.g. [43]). Since the fraction of energy absorbed by each component of the polymer-water system is proportional to its electron fraction, which can be well approximated by the weight fraction, in

dilute and moderately concentrated polymer solutions the indirect effect dominates. The input of the two effects to the yield of polymer radicals is usually even more shifted to the indirect effect than it results from the weight fraction, since the yield of radicals in water is, in general, higher than in pure polymer itself. Therefore the description below will refer to the indirect mechanism only.

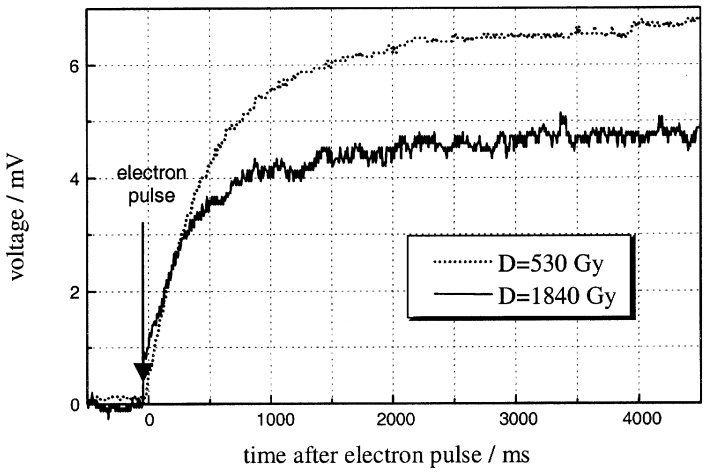


FIG. 5. Pulse radiolysis of vinylpyrrolidone solution, monomer concentration — 0.94 M. Oscilloscope traces recorded during light scattering detection at two different doses, pulse length — 1 μs, solution saturated with N_2O.

Out of the three main reactive species formed in water upon irradiation — hydrated electrons, hydroxyl radicals and hydrogen atoms — electrons exhibit low reactivity towards simple, hydrophilic hydrogel-forming polymers. This is an expected behaviour (found also for low-molecular-weight models of these polymers), since they usually do not contain functional groups being efficient scavengers of hydrated electrons. Rate constants of these reactions can be estimated by pulse radiolysis technique, by following the changes in the lifetime of hydrated electron with increasing polymer concentration. The values of the rate constant for these reactions are usually lower than 1×10^7 dm^3 mol^{-1} s^{-1} [12].

Hydroxyl radicals have been shown to be the main species responsible for reactivity transfer from water to the polymer chains. They abstract hydrogen atoms from macromolecules, thus polymer radicals are formed. Pulse radiolysis allows to determine the rate constants of these reactions, either by following the increase in the absorbance of macroradicals being formed, or, what is usually more convenient, by the competition method. Detailed studies have shown that for polymers the reaction with OH cannot be described with a single value of rate constant since such a rate depends on polymer concentration and molecular weight [64–6]. In general, the rate constants, calculated based on the molar concentration of the monomer units (and not the chains), decrease with the increase of the chain length and they are close to the diffusion-controlled limit (for discussion see e.g. [12, 66]).

From the data available for small organic molecules similar to our polymers and also from the results obtained e.g. for PAA at low pH (where e^-_{aq} are converted to H) one can conclude that hydrogen atoms react with these molecules in a similar way as OH radicals, i.e. by abstracting hydrogen atoms, however the corresponding rate constants are expected to be somehow lower.

Only in rare cases, like poly (ethylene oxide), all the hydrogen atoms in the polymer molecule are equivalent, so that only one type of macroradicals can be formed upon hydrogen abstraction by OH radicals. Otherwise hydroxyl radicals abstract hydrogen atoms from various, non-equivalent positions, so that two or more kinds of radicals of different structure are formed (Fig. 6, *cf.* [45, 57, 58, 67–69]). For the polymers listed above, these macroradicals are localized on carbon atoms. Structure of these carbon-centred polymer radicals may be of some importance for the process of crosslinking and hydrogel formation. Some macroradicals may be more prone to undergo side reactions, and even if the same type of reaction is considered — for example chain scission, it may either lead to the breakage of the main polymer chain, when the radical was localized there, or to some minor chemical changes in the side groups only, when the radical was localized on a side [70]. Since the structure of crosslinks and the energy of interchain bonds depend on the structure of the recombining radicals, one could anticipate that the localization of radicals before the crosslinks are formed may influence the structure and properties of the final product — hydrogel.

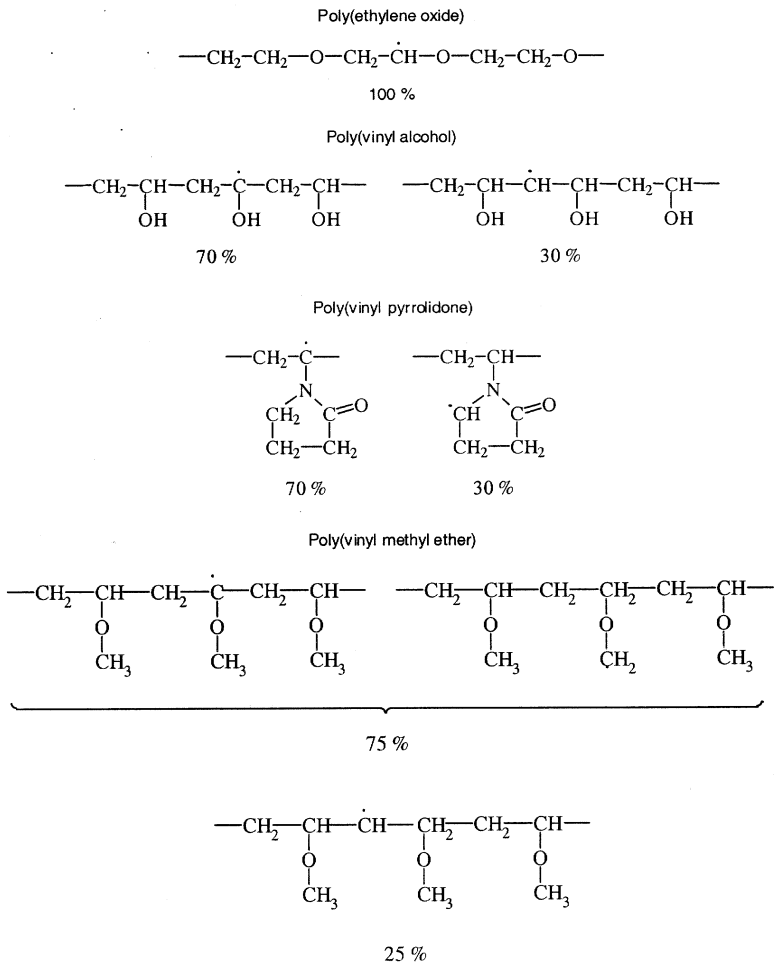


FIG. 6. Structures of polymer radicals formed upon irradiation of simple water-soluble polymers in aqueous solution [45, 57].

The fractions of radicals formed at various positions can be estimated in a number of ways — analysis of absorption spectra of transient products after an electron pulse, selective oxidation of the radicals and EPR. Straightforward EPR measurements of the radical spectra are rarely used because of the rapid decay of these transients. Only in exceptional cases, like ionized PAA, radical lifetimes are long enough to enable recording of the EPR spectra [58,

59]. An alternative method is the spin trapping, allowing transfer of the initial macroradicals into the radicals of high stability (*cf.* [58]). One can also irradiate a low-molecular-weight model, identify and quantify the products, and, by analysing the possible reaction ways, estimate the initial fractions of radicals at various positions.

On the basis of data available for PVAL, PVP and PAA one can conclude that the selectivity of OH attack on these macromolecules is not very high, i.e. there are no positions that would be the sole target of an OH attack (*cf.* Fig. 2 in [11]). In the case of PAA the fractions of radicals in the α- and β-positions to the carboxy group (*ca.* 30% and 70%, respectively) are similar to the fractions of H-atoms available for abstraction from these positions [58]. Some selectivity was observed for PVAL, where, as for simple alcohols [71], attack at the α-carbons to the hydroxy groups is preferred [45]. Also in PVP two of the possible five positions are the main attack sites [57].

From the practical point of view — formation of hydrogels — the most important reaction of macroradicals is intermolecular crosslinking, i.e. recombination of radicals localized on two different macromolecules (Fig. 7a). Although other various reactions in discussed systems are known since long [72], their importance and role in the competition with intermolecular crosslinking is not always fully recognized. If only the latter reaction would occur in irradiated aqueous polymer solution, one could expect the yield of intermolecular crosslinks, G_x, defined as number of crosslinks formed in the system upon absorption of 1 Joule of ionizing radiation energy, to be equal to the half of the initial OH and H yield. This value would then be $G_x = 1.6 \times 10^{-7}$ mol J^{-1} for deoxygenated (i.e. saturated with Ar or N_2) solutions and *ca.* 3.0×10^{-7} mol J^{-1} for solutions saturated with nitrous oxide (where the OH yield is doubled, see e.g. [43]). In fact the values of G_x are often much lower (for examples see Table I in [11]), what indicates that many of the initially formed macroradicals undergo other reactions. These other reactions include reactions between two radicals, as intramolecular crosslinking as well as inter- and intramolecular disproportionation, and also processes involving one radical, as hydrogen transfer or chain scission. These processes do not result in joining the polymer chains, thus they do not lead to the formation of macroscopic gels.

The proportion between recombination and disproportionation reactions is set by the radical structure and the possibility to control this parameter are usually very limited. What is important, model studies for PVAL and PAA show that in these systems disproportionation is the main reaction involving two radicals, while the fraction of recombination is *ca.* 20–35% for PAA model and only *ca.* 10% for the PVAL model [45, 58]. Thus the overall yields of crosslinking (both inter- and intramolecular) in the corresponding polymers are limited to these values.

We do have, however, an influence on the competition between inter- and intramolecular crosslinking. At high polymer concentration (above the critical hydrodynamic concentration, which depends on the molecular weight), when polymer chain interpenetrate, the probability that two recombining radicals are localized on different chains is relatively high. What's more, in such systems some physical entanglements may become "fixed" when the entangled chains become joined to the network in at least two points encompassing the entanglement site. If we lower the polymer concentration to a range where the macromolecules (usually having a conformation of a coil) are separate, then the probability of intermolecular recombination decreases. If the irradiation conditions allow for the simultaneous existence of

more than one radical on a single chain, then, provided the chain is flexible, the encounters of two radicals on the same chain may become faster than intermolecular recombination requiring two big chemical entities as polymer coils to diffuse to each other (Fig. 7b). Besides concentration, another parameter of equal importance for this competition is the way of irradiation, or, more precisely, the dose rate. High dose rates, as obtained by pulse-irradiation of the system with electron beam, when combined with low polymer concentration, may lead to the situation when several tens of even more than a hundred radicals are generated simultaneously on each chain. In these conditions the probability and yield of intermolecular recombination is greatly reduced. These effects have been studied in many polymeric systems (*cf.* in aqueous solution of star-shaped poly (ethylene oxide, [73]).

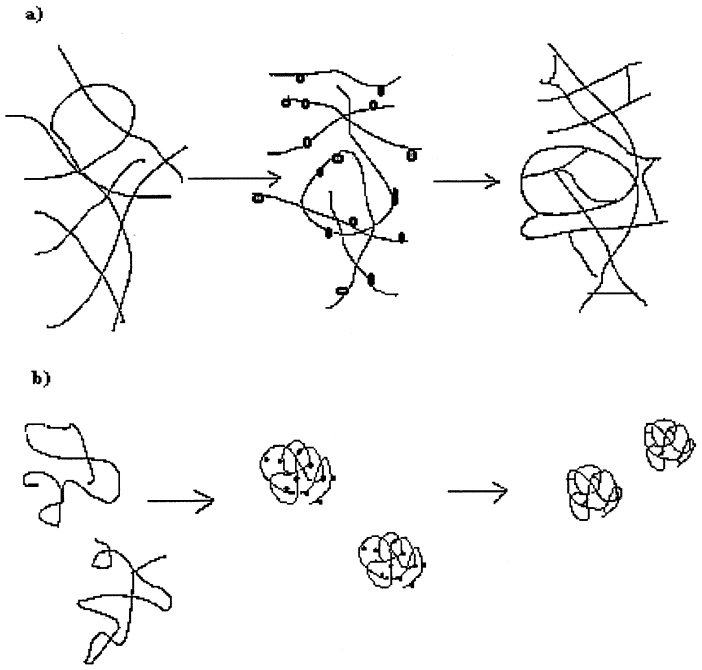


FIG. 7. Schematic representation of (a) intermolecular crosslinking due to the fixation of entanglements in concentrated polymer solution and (b) intramolecular crosslinking in diluted solution of isolated chains. Dots on macromolecule before reaction denote radical sites.

The influence of polymer concentration on the competition between inter- and intramolecular crosslinking emerges form the plots of the gelation dose *vs.* concentration. A typical curve illustrating such relationship (Fig. 8, first examples were shown by Alexander and Charlesby [72]) consists of two parts. On the high-concentration side the gelation dose is almost proportional to the polymer concentration. This is in line with the general theory of crosslinking that assumes that a gel is formed when, on average, one crosslink is formed for one macromolecule present in the system. However, when the concentration is lowered beyond some limiting value, instead of linear decrease there is a pronounced increase in the gelation dose. This is equivalent to a strong decrease in the yield of intermolecular crosslinking. This is the concentration range where intramolecular recombination prevails. The dose rate effect has been demonstrated e.g. by Ulanski, et al. [45] by pulse radiolysis with low-angle light scattering detection. When a sample of N_2O-saturated PVAL solution has been subjected to a single electron pulse of 150 Gy, almost no increase in the scattered light was detected. However, when the same dose was administered by a series of low-dose pulses, a significant increase could be observed, indicating the increase in molecular weight as a result of intermolecular crosslinking.

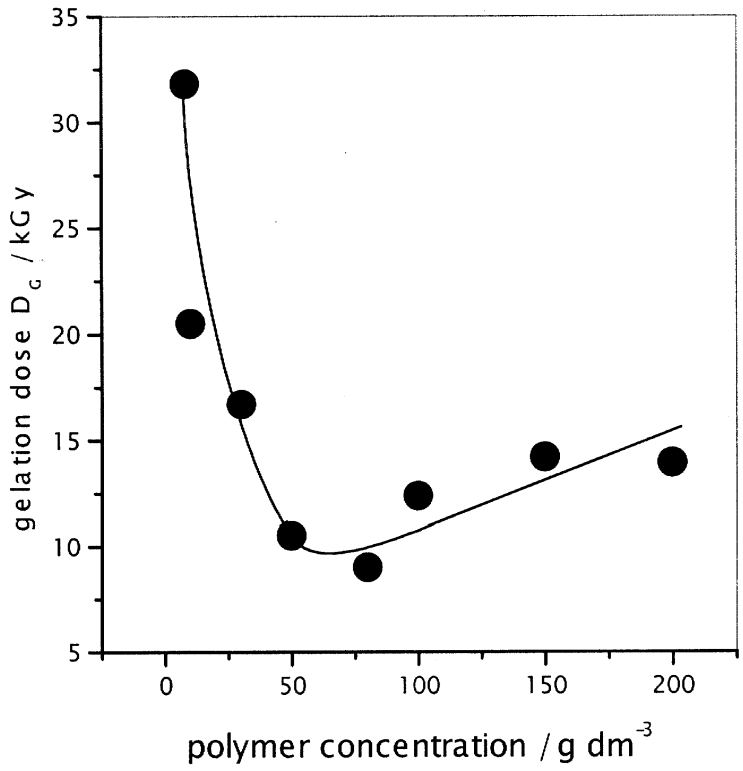


FIG. 8. Exemplary dependence of the gelation dose on the polymer concentration. Data for poly (poly (vinyl pyrrolidone) K-30, γ-irradiated in an Ar-saturated aqueous solution. Dose rate 3.0 kGy h^{-1} [79].

The competition of two recombination modes can be followed by kinetic studies as well. While intermolecular reactions follow the classical second-order kinetics, intramolecular recombination usually shows significant deviations from this simple kinetic pattern [45, 58, 75, 76]. It can be shown that in the latter case the reaction rate depends on the average number of radicals per chain, rather than on the overall radical concentration. For quantitative description of the reaction rates, a model of non-homogeneous kinetics has been successfully applied [77, 78]. For further details as well as an attempt to explain the reasons for the non-classical kinetic behaviour — see [66]. To summarize, high polymer concentration and low dose rate promotes gel formation, while for dilute solutions irradiated with high dose rates one can expect the dominance of intramolecular recombination.

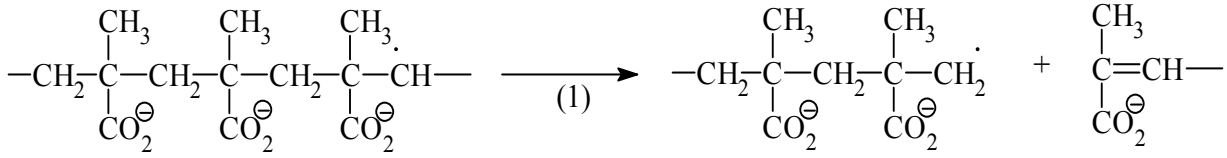
Hydrogen transfer reactions change the location of radical sites, but do not change the overall number of radicals on polymer chains, so that in general their occurrence does not decrease the number of radicals available for crosslinking. Nevertheless, some influence on the crosslinking yield and network structure can be expected, by changing the initial proportions between various radical structures that may be more or less prone to recombination and, as already mentioned above, may lead to various gel microstructures.

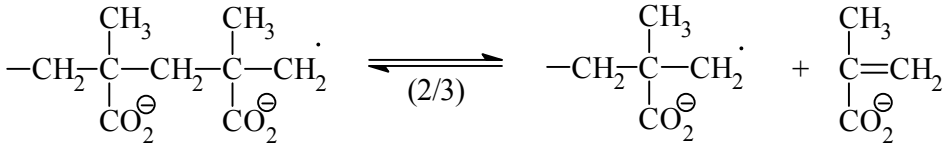
Chain scission, being in a sense a reverse process to intermolecular crosslinking, is an important reaction for our discussion. In cases when it proceeds with high yield, exceeding G_x, no gel formation occurs in the system. In deoxygenated solutions, when the chain break precursors are carbon centred radicals localized at the main chain (or its immediate vicinity), chain scission reactions are, fortunately, very slow. Thus, in most cases of the polymer considered here (polyelectrolytes being an exception), radicals recombine before chain

scission proceeds to a measurable extent. Certainly, increase in the molecular weight of the polymer, or even gel formation, do not prove the absence of degradation. However, sol-gel studies as well as analysis of the irradiation products of the model compounds allow estimation of the scission yield. For most non-ionic polymers, like polyacrylamide, poly (vinyl pyrrolidone) and poly (vinyl alcohol), these yields, under standard irradiation conditions, were found to be close to zero. However, this is no longer true for ionic polymers and in the case of the presence of oxygen in the system.

Poly (acrylic acid) requires a separate paragraph, as an exemplary polyelectrolyte. Its radiation-induced transformations in solution have been recently studied in some detail [58, 59, 74, 75, 80]. PAA can be effectively crosslinked by irradiation with no need for any additives [81], provided the irradiation is performed in acidic solution (e.g. pH2) so that most of the carboxylate groups are protonated. Under these conditions PAA resembles a non-ionic polymer and its behaviour under irradiation can be described (although still not fully) by the general rules applicable for uncharged macromolecules. Increasing pH towards neutral and alkaline reveals the specific properties of PAA as a polyelectrolyte. High density of negative charge on the chain, only in part screened by the condensed counterion atmosphere, induces coulombic repulsive forces between the chain segments and the macromolecule assumes a rod-like, relatively stiff conformation. When radicals are generated on the macromolecules, these forces prevent the radical-bearing chain segments and also the neighboring chains from an approach into the reaction distance, thus slowing down recombination and disproportionation by seven orders of magnitude in comparison with uncharged chains. Since the radical-terminating reactions are that slow (in this system macroradicals can live for hours at room temperature), competing reactions as hydrogen shift and, what's more important here, chain scission proceed with high yield and wins the competition against crosslinking. As a result of that, the molecular weight of the polymer decreases (the net yield of chain breaks exceeds 5×10^{-7} mol J^{-1} for 1×10^{-2} mol dm^{-3} PAA at pH > 9) and no gel is formed.

The chain scission processes are even more important for poly (methacrylic acid) (PMAA) [70, 82, 83]. The macroradicals of PMAA, which are very long-lived especially at high pH, undergo β-scission reaction (1), giving end-chain radical and unsaturated terminal structural element.





The rate constant of this reaction depends significantly on the acidity of the medium and it is the highest in the range of pH 7–9. Additional process taking place in the irradiated PMAA solution is very effective chain unzipping (2/3) i.e. depolymerization.

As a result of this process, methacrylic acid is formed with high yield (at pH9 a dose rate of 0.09 Gy s^{-1} and a dose of 20 Gy G(methacrylic acid)=500×10^{-7} mol J^{-1} was measured) [82, 83]. The rate of unzipping reaction depends strongly on the pH, and it is only very prominent at high pH. It is worth noting that the rate of scission for the dissociated PMAA radicals is *ca.* 70 times faster than that of PAA radicals under similar conditions [58]. This much slower rate of fragmentation is paralleled by the fact that an unzipping process (reaction (2/3)) was not observed with the PAA-derived radicals. The differences in the chemical behaviour between these two polyelectrolytes can be caused by various radical stabilization and chemical structure. In PMAA the most stable radicals in the α-position to the carboxylic group cannot be formed due to the polymer chemical structure. Formed β radicals are less stable and easily undergo the transformation via scission processes. Moreover, possible radical recombination for PMAA is hindered from sterical reasons. Additionally, some influence of the hydrophobic effects on the chain conformation, operative for poly (poly (methacrylic acid), cannot be ruled out.

PAA hydrogels formed by irradiation of its acidic solutions or by radiation co-polymerization with crosslinking agents belong to the class of stimuli-sensitive materials and respond by swelling or shrinking to the changes in pH, ionic strength and electric field [15, 84].

Since PMAA undergoes chain scission with a considerable rate constant and even depolymerize rapidly at high pH, it is impossible to crosslink poly (poly (methacrylic acid) under these conditions by ionizing radiation. In principle, this could be done at low pH and at high dose rate delivered by electron beam, when the radical lifetime is shorter and the rate of scission slower. However, at such very high dose rates intramolecular crosslinking are promoted rather than macroscopic gel formation (see above). In order to favor the intermolecular process high concentration of PMAA is required. However, at a 10% solution, some small particles are formed, but a wall-to-wall gel was not yet observed [83].

3.6. Role of radical scavengers and oxygen

Additives can alter the radiation-induced processes in aqueous solutions of polymers. Selective scavengers of transient products of water radiolysis (like *tert*-butanol for OH or nitrous oxide for e^-_{aq}) allow to study separately their reactions with polymers. Selective oxidants (e.g. tetranitromethane) can be a potent tool for identification of polymer radicals of various redox properties. Spin-traps (for example 2-methyl-2-nitrosopropane) provide the possibility of EPR studies on the otherwise short-lived species. Although many efficient radical scavengers are known and commonly applied in the laboratory (e.g. thiols or Vitamin C), there is always some interest in finding some new substances with potential scavenging properties. If they are used in biomaterials and personal care products, special care must be taken of their non-toxicity. The best solution is to find any radical scavenger, which is at the same time efficient, non-toxic and even may act as a drug in the organism. Recently, in our laboratory we have begun the studies on such a miraculous substance, melatonin [85–87].

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland of vertebrates. It is now well established that it plays an important role in regulating the circadian rhythms, and that it can be efficiently used in the treatment of some daily-rhythm disturbances, including jet lag. Systematic studies have provided convincing evidence that melatonin is a very potent antioxidant and it may be among the natural agents protecting organisms from oxygen radical damage. It has been also suggested that the role of melatonin

in the protection against radical-induced damage is mainly based on its ability to scavenge hydroxyl radicals, considered to be the most damaging of free radicals generated in living organisms (*cf.* [88–90]).

The rate constant of melatonin with hydroxyl radicals was found to be equal 1.2×10^{10} dm^3 mol^{-1} s^{-1} [85, 86]. This high value indicates that the reaction is diffusion-controlled and in fact, at least *in vitro*, melatonin is a very potent OH-scavenger. This behaviour is consisted with predictions from the computational studies [87]. The rate constant of the reaction with hydrated electron has been also measured and found 4.3×10^8 dm^3 mol^{-1} s^{-1}. Similar values of the rate constant were reported previously for some other indole derivatives. Melatonin is also very effective scavenger of tert-butanol radicals. However, it was shown that its reaction with carbon centred polymeric radicals of PAA and PVP is very slow, if it occurs at all. The main conclusion from this work is that melatonin is not universal scavenger of alkyl-type radicals, and further studies are needed to clarify this point.

Oxygen is certainly a very special additive. All the data presented above refer to deoxygenated systems. In oxygen-containing polymer solution the initially generated carbon-centred macroradicals react with oxygen to form corresponding peroxyl radicals. This is a fast, practically diffusion-controlled reaction with a rate constant in the order of 10^9 dm^3 mol^{-1} s^{-1} (values for particular polymers are listed in [59]). Even in the case of ionized PAA, when the oxygen diffusion towards the chain is slower than for neutral polymers, the rate constant is still as high as 1×10^8 dm^3 mol^{-1} s^{-1}. Chemistry of peroxyl radicals is often complex (for a review see [91], specific discussions for PVAL — [45], PVP — [57], PAA — [46, 59]). Three facts seem to be most important in the current context. Neither peroxyl nor oxyl radicals form **stable** bonds (crosslinks) upon recombination (recombination of oxyl radicals is expected to proceed with low yields only, and the formed peroxide bond cannot be considered as a stable crosslink). One of the main reaction pathways leads to the chain scission. When the radical-terminating reactions are slow, peroxyl radicals can undergo a chain reaction of hydrogen abstraction followed by a reaction of the formed alkyl radical with oxygen. In this way the degradation products — terminal peroxyl radicals — can rearrange into the mid-chain peroxyl radicals, that can initiate further chain scissions. Such chain processes have been observed for example for PAA [59].

However, it does not follow from the above presented facts that hydrogels cannot be obtained by irradiation of oxygen- or air-saturated polymer solutions. First of all, when irradiation is performed in airtight vessels, after some induction period where possibly degradation prevails, the oxygen would be used up and further irradiation would lead to crosslinking and gel formation. This method simplifies the technology and is used on a commercial scale, e.g. for the radiation formation of hydrogel dressings [92]. Structure of the polymer chains in the product would be to some extent different from the starting material due to the reactions with oxygen in the first irradiation phase. Similar situation would occur when the solution is irradiated in an open vessel, but the oxygen transport from the surface into the bulk of solution is slower than the potential oxygen consumption rate (high dose rate would be an advantage here). In such a case one can expect a non-homogeneous product due to the uneven exposure of the material to oxygen. Even when oxygen is present in the sample during irradiation, gel formation is still possible. It has been shown by Rosiak, et al. [57], that when oxygen-saturated PVP solutions are subjected to pulsed EB-irradiation at high dose per pulse (400 Gy and more), recombination of the carbon centred radicals competes quite efficiently with their reaction with oxygen. By increasing polymer concentration in order to promote intermolecular crosslinking (see discussion above) it was possible to reach a situation where

the latter process dominated over scission. These observations certainly do not impair the general rule, that gel formation is more efficient in oxygen-free systems.

3.7. Micro- and nanogels

So far in our discussion we were focusing on the formation of macroscopic hydrogels, thus intermolecular crosslinking was considered as the most desired reaction. The literature on biomaterials indicates that in some fields of medicine and pharmacy polymeric micro- and nanoparticles, including gel-type products, are applied for example as drug-delivery systems and cell markers [93–97].

Most of these materials are produced by polymerization [98–101]. Recently, nanogels of poly (poly (vinyl alcohol) have been obtained by intramolecular, chemical crosslinking of single macromolecules with glutaraldehyde [19]. It seems that similar structures, i.e. internally crosslinked macromolecules, can be obtained by radiation-induced crosslinking as well, when low polymer concentrations and high dose rates are applied (see discussion above on inter- and intramolecular crosslinking).

In preliminary tests performed in our laboratory, dilute, oxygen-free PVAL solutions, circulating in a closed loop, were repeatedly irradiated with intense (1.1 kGy) pulses of 6 MeV electrons [67]. A slight increase in molecular weight (due to some small contribution of intermolecular crosslinking) was accompanied by a decrease in the dimensions of polymer coils (i.e. gyration radius) and solution viscosity. These coil shrinkage effects, analogous to those reported by Brasch and Burchard [19], we interpret as a result of intramolecular crosslinking. Recently the same method has been applied to obtain nanogels of poly (poly (vinyl pyrrolidone) [67] and poly (poly (acrylic acid) [69, 102, 103]. The latter system is of special interest because such intramolecularly crosslinked structures of PAA have been recently proposed for stimuli-sensitive drug delivery systems [104].

Because in basic solution PAA macroradicals are long-lived and may undergo chain scission and other side reactions, the possibility of nanogels synthesis is limited to the acidic solution. However, also in this case, scission cannot be totally eliminated, and the net effect observed is the sum of three reactions: intra- and intermolecular crosslinking and chain scission. Proportion between these processes depends on the polymer concentration and the applied dose, i.e. in the initial stage of irradiation (at low dose) scission plays an important role. When combined with mostly intramolecular crosslinking, i.e. at low polymer concentration, it causes a decrease in average molecular weight. For higher PAA concentration more crosslinks are formed intermolecularly and the net effect is an increase in M_w.

An interesting effect occurs for higher doses, when some number of intramolecular crosslinks has been formed. For all concentrations tested, the molecular weight does not drop any longer, but rather shows a pronounced increase (Fig. 9). Our interpretation is that the absolute yield of chain scission does not decrease, but, for internally crosslinked structures, scission does not lead any longer to a significant decrease in molecular weight, because the formed chain fragments are still linked to the parent macromolecules. The fact that internally crosslinked macromolecules are more resistant to the degradation has already found important biomedical applications — e.g. crosslinked hyaluronic acid in synovial fluid is much more resistant to radical-induced degradation accelerated by some inflammatory diseases than the same polymer in standard, linear form [105]. As shown in Fig. 9, the dimensions of PAA coils are greatly reduced during irradiation, also when the average molecular weight increases.

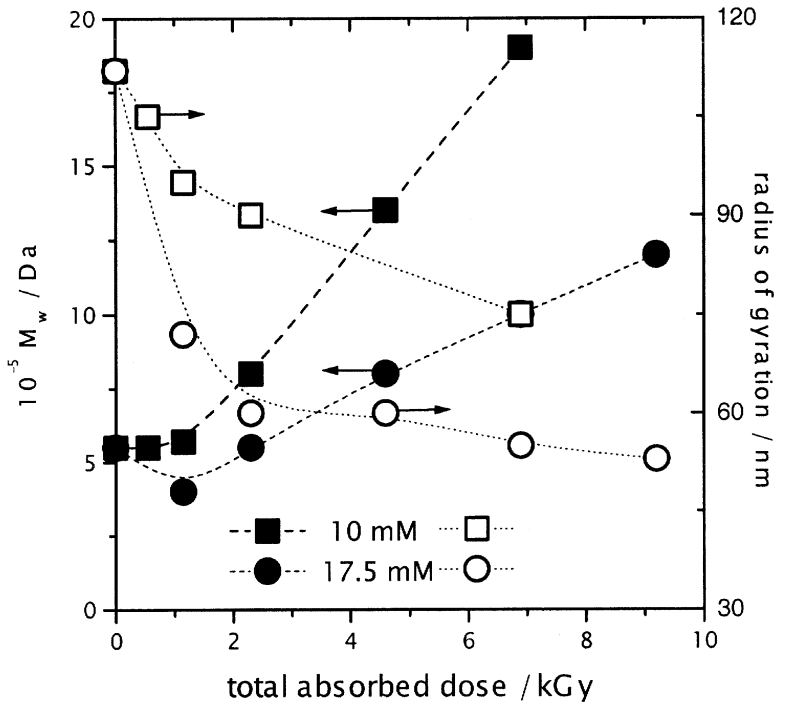


FIG. 9. Changes in the weight average molecular weight (M_w, solid symbols) and mean radius of gyration (open symbols) of poly (poly (acrylic acid) upon pulse irradiation in Ar-saturated aqueous solution of various concentrations at pH2.0. Values of R_g determined by laser light scattering at pH10.0 in the presence of 0.5 mol dm^{-3} $NaClO_4$. Dose per pulse: 1.1 kGy, pulse frequency 0.5 Hz, flow rate: 1 cm^3 s^{-1} [69, 102], for detailed description of the system see [68]).

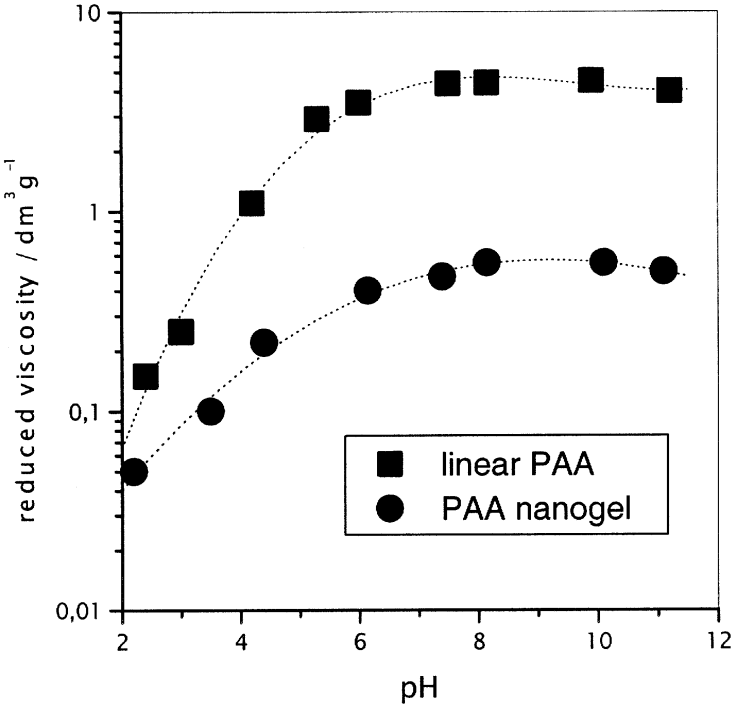


FIG. 10. Reduced viscosity of the solutions of linear (squares) and intramolecularly crosslinked (circles) PAA as a function of pH. Concentrations and average molecular weights of nanogel and linear macromolecules are the same and equal 5 mM and 550 kDa [102].

Nanogels demonstrate a number of properties which differ them from linear "parent" macromolecules. Linear macromolecules of poly (poly (acrylic acid) change their conformation together with changes in pH. In acid solutions, where carboxylic groups are protonated, chains are coiled. When pH increases degree of dissociation and linear density of charge on chain increase too. Under culombic forces between the chain segments, macromolecules assume linear conformation. It is accompanied by an increase in hydrodynamic radius, which manifests itself as strong increase in solution viscosity. The uncoiling of internally crosslinked PAA molecule under basic conditions is rather limited because of the tie points that exist inside the coil, forbidding to expand it to the same extent as for linear PAA chains. As a result, the observed viscosity changes are rather limited (Fig. 10).

3.8. Regulation of polymer molecular weight by means of ionizing radiation

So far we were concentrated on the situation, when the crosslinking reaction should dominate the kinetic pattern of the radiation-induced processes in polymer solution in order to obtain hydrogel materials. We also showed that sometimes, the interference from other side reactions is inevitable. This is especially true for polyelectrolytes and/or in the presence of oxygen in the sample. In some exceptional cases, degradation process, normally undesirable, can be profitable. This is the case of biologically important polyelectrolyte, chitosan, an ionic polysacharide derived from chitin. Due to its non-toxicity, biodegrability and many unique biomedical properties, it is of particular interest for medical and pharmaceutical purposes. In many cases the suitability of chitosan for a particular purpose and its effectiveness in exerting a specific action depends on its molecular weight [42, 106]. The molecular weight of raw chitosan depends on the starting material and the conditions of treatment, but usually it is very high (several hundreds to over one million Da).

Recently we proposed an efficient, simple and fast method of adjusting the molecular weight of chitosan to a suitable level for a particular application by subjecting the polymer in aqueous solution to ionizing radiation or to ultrasound [107, 108]. Only stoechiometric amounts of acid are required for dissolving the polymer. Both proposed procedures are simple, they run at room temperature, the degradation takes typically a few minutes only, and the final average molecular weight can be reasonably well controlled. It is worth mentioning that the scission yield in the presence of oxygen is lower than in the deoxygenated solution. Such a protective effect of oxygen is typical for the breakage of the glycosidic linkage [43], but it is in contrast to most synthetic polymer system discussed here.

3.9. Sterilization

Ionizing radiation is an efficient tool for sterilization. In fact, a great part of single-use medical products is sterilized by this technique [109]. Application of radiation for the formation of hydrogels for biomedical use offers a unique possibility to combine the formation and sterilization of the product in a single technological step. This allows to simplify the technology and reducing production costs. Since ionizing radiation in the usually used forms of gamma rays or electron beams can penetrate the packaging materials, it is usually possible to irradiate the substrates in a closed vessel or mould that serves as well as the final packaging of the product. For a description of an exemplary technological process utilizing these advantages, see the chapter on hydrogel wound dressings below.

3.10. Advantages

Use of radiation for the formation and modification of hydrogels for biomedical purposes has some general advantages. First of all it solves the problem of sterilization of products and in a few cases allows establishing a more simple and compact technology than a "conventional" one. Secondly, it allows fabricating a pure product non-contaminated with ballast materials or the residuals of toxic initiators, crosslinking agents or other additives. Last, but not least, the application of ionizing radiation originated from electron accelerators or gamma facilities is a clean technology, safe for human beings and the environment, and can lead to formation of human-friendly products.

4. SELECTED APPLICATION- AND RESEARCH FIELDS

The main areas of today's application of hydrogels as biomaterials include:

— synthetic wound care coverings,
— drug delivery systems as well as transdermal systems,
— dental materials,
— implants,
— injectable polymeric systems,
— ophthalmic applications,
— hybrid-type organs (encapsulated living cells).

These classes of products are briefly described below.

4.1. Topical applications as wound dressings

Despite of a great number of papers and patents devoted to radiation synthesis of hydrogels and propositions of their authors to apply established products to biomedical purposes, there is only limited number of successfully commercialized technologies of production of hydrogel biomaterials by means of radiation technique. Most of these products belong to the one category — synthetic wound dressings.

The hydrogel materials for wound healing are used in direct contact with living tissues. They prevent contamination of a wound by microorganisms from outside, inhibit the loss of body fluids, deliver oxygen to the wound, and generally accelerate healing processes.

A commercially successful example of such a dressing is the hydrogel dressing known under the trade name AQUA-GEL and marketed mainly in the Central Europe. It is manufactured by means of radiation technology in the form of thin swollen slides of hydrogel [20, 92]. The basic steps of this technology are shown in Fig. 11.

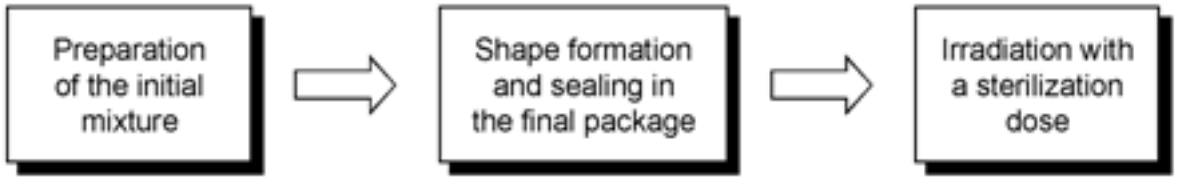


FIG. 11. Production scheme of hydrogel wound dressings [20].

The first step is the preparation of aqueous solution of the components, the main ones being poly (poly (vinyl pyrrolidone), poly (poly (ethylene glycol) and agar. After mixing at elevated temperature a homogeneous solution is formed. In the second step the moulds, which also can serve as the final packages for the dressings, are filled with the solution. After solidification of the solution upon cooling, the moulds are tightly sealed in a foil that is non-permeable for air and microorganisms. In the final step this semi-product, i.e. thermoreversible pseudo-gel, assembled in commercial boxes, is treated with ionizing radiation. Two processes — sterilization and formation of a permanent three-dimensional polymer network takes place. The product is a fully sterile permanent hydrogel in a form of a transparent sheet, 3–4 mm thick, containing over 90% of water.

The dressing produced by this technology has the following properties:

— it forms an efficient barrier for bacteria, and also for excessive loss of body fluids,
— it allows the diffusion of oxygen towards the wound,
— it is soft and elastic, but mechanically strong enough,
— it has a good adhesion to the wound and to the healthy skin, but without a tendency for excessive sticking, therefore it enables a painless removal or exchange of the dressing without disturbing the healing process,
— it is transparent, so that the healing process can be monitored without the necessity of removing the dressing,
— it enables an easy treatment of the wound with drugs (by diffusion of the drug solution from the outer surface, by injecting the drug between the dressing and the wound or by soaking the dressing in the drug solution before placing it at the wound),
— it absorbs exudes and bacterial toxins,
— it is non-antigenic and does not provoke allergic reactions,
— it soothes pain and provides optimal wound healing environment of constant humidity,
— it is sterile, easy to use and not expensive.

The radiation technology used for the production of the dressings has many advantages when compared to conventional methods:

— it is simple, easy and clean (no side products or waste),
— all the components are safe for human and environment,
— it does not require special sterile production rooms etc., but still enables to obtain a fully sterile product,
— it is flexible, i.e. can be easily tailored for various scale and method (continuous, batch).

Due to the advantages of this technology and the excellent properties of hydrogel dressings, investigations on similar hydrogel products are currently being undertaken in other countries, e.g. Indonesia, China, Italy, Japan, Brazil, Malaysia, and Iran.

It was announced that Nichban Co. Ltd of Japan begins commercial production of hydrogel dressings on the base of poly (poly (vinyl alcohol) due to similar radiation-chemical technology developed at the Takasaki Radiation Chemistry Research Establishment [110, 111].

Hydrogels can also be utilized as sprays, emulsions, ointments and creams, with or without the addition of active compounds. All the above types of wound dressings and covers can act also as slow-release drug delivery systems, or drugs can be administrated through the hydrogels *in situ*.

4.2. Drug delivery systems

Such materials in the form of hydrogel matrices enable sustained and or controlled release of embedded medicines to body fluids after their implantation, injection or other introduction into the organism. Generally speaking there are two different concepts of such systems. The first one consists of releasing of small drug molecules as a result of hydrogel swelling. The second one consists of gradual erosion of polymer matrix containing drug. In this case the diffusion of medicine into the surroundings is controlled by the rate of biodegradation. Sometimes, hydrogels are used as membrane encapsulating drugs, which allow the control of the rate of release of medicine by changing the degree of crosslinking and chemical composition of the hydrogel. Special cases of drug deposits are called "intelligent" or signal-responsive hydrogel. Small changes of the environment, e.g. pH, temperature, ion concentration, osmotic pressure, electric or magnetic field can cause huge changes of hydrogel properties and in this same way influence the rate of drug release. An example of the application of hydrogel technology is prostaglandin delivery system for the ripening of the cervix in women at full term in labour. The product constructed with poly (poly (ethylene oxide) and prostaglandin E2 has been granted a product license in the U.K. and Ireland and is marked under the trade name Propess by Roussell [112]. Another hydrogel system, with similar functions was developed in Poland using radiation technology and successfully passed clinical tests [113].

In the case of serious prolongation of pregnancy over natural time limit, or in the case when life of women and infant is in emergency, it is necessary to accelerate the beginning of delivery. In such cases therapeutic induction of childbirth must be undertaken. The essential problem may be unripened uterine cervix. Local induction of prostaglandins (E_2,$F_{2\alpha}$) is usually applied for acceleration of ripening. However, the known methods of introducing prostaglandin often cause negative side effects.

The therapeutic system for local release of prostaglandins, elaborated by author's group [113], is based on hydrogel devices obtained by irradiation of N-vinylpyrrolidone (VP). This device has the shape of thin rod of 8 mm diameter and 35 mm long, equipped with round head on the one side and surgical thread on the other side. The method of obtaining the therapeutical system is a three-stage procedure (Fig. 12). It consists of radiation polymerization and crosslinking of VP, incorporation of prostaglandin into hydrogel matrix and subsequent radiation sterilization of product. The polymerization is carried out in a special form, which enables to obtain the desired shape of devices. Placing the surgical silk thread in the monomer prior to irradiation makes it possible to obtain the rod with strongly fixed thread. Incorporation of prostaglandin into the devices is carried out by placing this rod in appropriate solution of hormone. The swelled rod is dried and then packed into foil bags. The sterilization is carried out in cobalt source with the dose of 25 kGy.

After placing by physician the rod into vicinity of the uterine cervix, hydrogel absorbs the body fluids and begins to swell. The hormone gradually diffuses into surrounding environment. The local action of this device, besides prostaglandin release, is based also on the mechanical (expanding) action on the uterine cervix, because in course of swelling the

dimensions of insert increases. In several hundred cases of childbirth being induced by means of these devices it has been found that these therapeutic systems are highly useful and safe for women in childbirth. The system may also be applied for abortion of dead fetuses.

Similar drug delivery system for the treatment of endometrial carcinoma has also been elaborated in our laboratory [114, 115]. The system contains polymeric rod with diameter of 4.5 mm and length of 30–35 mm. The rod is a mixture of poly (poly (ethylene oxide), poly (poly (ethylene glycol) and the drug, medroxyprogesterone, covered with the thin layer of latex. The rods are subsequently irradiated with a dose of 25 kGy. Depending on the latex layer thickness, the release of the active agent can be regulated. The clinical application of these devices was initiated in 1996, and so far such inserts have been used in over 70 cases. All these patients are continuously monitoring and in good health.

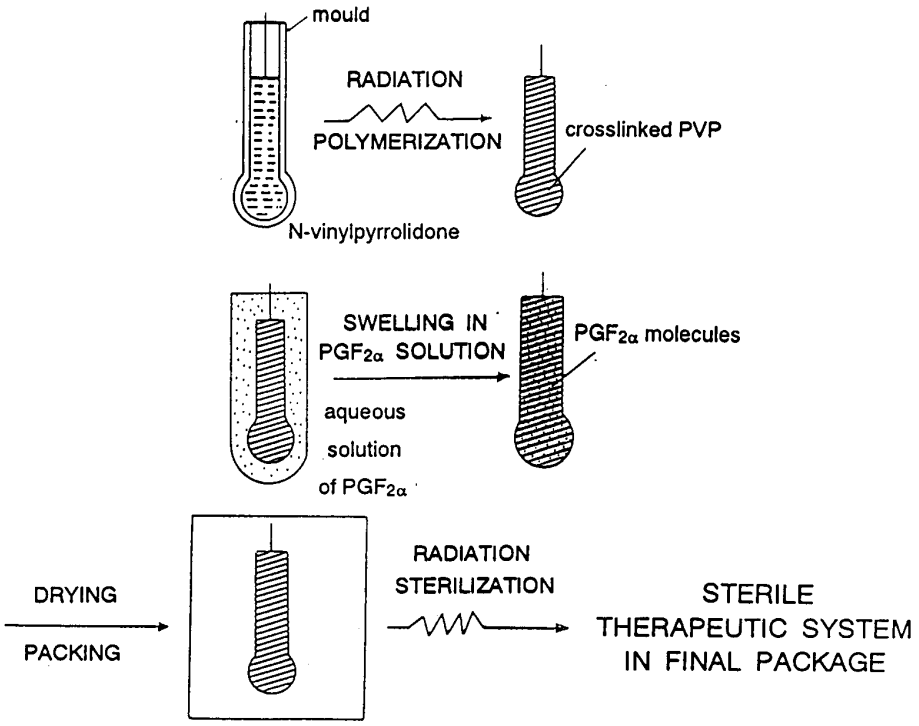


FIG. 12. Production scheme of hydrogel drug-delivery devices for the local release of prostaglandins [113].

4.3. Transdermal systems

Hydrogels are applied as the reservoir for biologically active species. The properties of skin and its interaction with the solutants as well as properties of the membrane usually placed between skin and drug container are the main factors controlling the delivery of a medicine. The first devices were developed for astronauts who become sick in space. The little patch containing scopolamine and designed to be placed behind the ear was commercialized under trade name Transderm-Scop. A number of other transdermal systems containing various therapeutic agents are now available on the market, including glycerol trinitrate, clondine, estradiol/progesterone etc. Examples of such systems are the devices for glaucoma patients, in which hydrogel strips or sacs containing pilocarpine are placed in the fornix of eye-lid. Such

systems have been introduced in the market under the trade name: Ocusert, by Alza Corporation, USA. Similar devices in the form of thin foil fabricated using radiation technology have also been clinically tested [116].

4.4. Dental applications

There are some two- and multicomponent denture base materials, containing HEMA or other hydrophilic polymers in form of a monomer or prepolymer of the jaw they are polymerized and/or crosslinked. In many cases these reactions are initiated by UV light delivered through a light guide. Some trials were undertaken to fabricate by radiation crosslinking the hydrogel dental material composed of poly (poly (vinyl alcohol) and gelatin [117].

Relatively new and extremely interesting is the application of ionic hydrogels, especially in the form of nanogels, in dental care [118]. Human teeth are penetrating by microchannels filled with a biohydrogel consisting of a fibrous protein, and a fluid phase. The gel network is bound to the organic collagen matrix and to the hydroxiapatite crystals in the walls of the dentine tubules. Fluid movements within these microchannels due to hydrostatic pressure induce sharp and/or shooting dentinal pain. Microorganisms present in the oral cavity ferment carbohydrates to form lactic acid, which causes pH decrease. Acidic environment subsequently causes dissolution of the hydroxiapatite in the hard walls of microchannels. This increases the permeability of the channels and facilitates the penetration of microorganisms. Diffusion of bacterial toxins causes inflammatory reaction in the pulp, which give symptoms such as hypersensitivity and pain. Therefore, it is of great importance to develop new prophylactic agents and methods to reduce dental decay and hypersensitive tooth necks. The intramolecularly crosslinked PAA nanogels seems to be good candidates for this purposes, as they can penetrate deeply into the microchannels due to the small dimension even in neutral pH. They can be also intermolecularly crosslinked by the multivalent metal cations, giving stable and fast-developing gel filling the channels.

4.5. Injectable polymers

Injections of collagen have been used to fill and repair cosmetic defects by plastic surgeons. The drawback of this treatment consists in relatively fast collagen resorption. Some hybrid types of materials were proposed, e.g. collagen and poly (poly (vinyl alcohol) or specially crosslinked collagen [119]. The latter belongs to the group of polymers, which undergo crosslinking under action of ionizing radiation. Other promising application includes the fabrication of microgels encapsulating the therapeutic species or being attached to them. After injection they can be sieved by part of the circulatory system. In addition, if such microparticles were equipped with antibodies, they might act as "magic bullets" targeting a receptor on the particular cell to be treated. The radiation technique allows to obtain microparticles of different shape and sizing [99].

4.6. Implants

Poly (Poly (vinyl alcohol), the first hydrogel widely used for implantation, is still a subject of intensive investigations, especially by radiation chemists. For example, a new PVAL material withstanding the autoclaving temperature for a few hours has been developed lately [120]. The hydrogels have been used as postenucleation balls, breast implants, for repair of cranial defects, noses and chins, cleft palates, as films for ear drum replacement, columels

for tympanoplasty and for other special purposes. It seems that the use of hydrogels as cartilage replacement, tendon sheaths and aortic grafts will be soon commercialized.

Radiation grafting and/or similar methods, e.g. plasma treatments, give the opportunity to modify the surface properties of implants made from various materials toward better and permanent biocompatibility [121].

One of the major problems in this area of the research concerns construction of the intervertebral disc implants. The need of such a system is obvious. The spinal disc may be displaced or damaged due to trauma or a disease process (Fig. 13). Moreover, the discs degenerate with age, the nucleus loses its water binding ability and deflates. That can potentially result in persistent and disabling back pain.

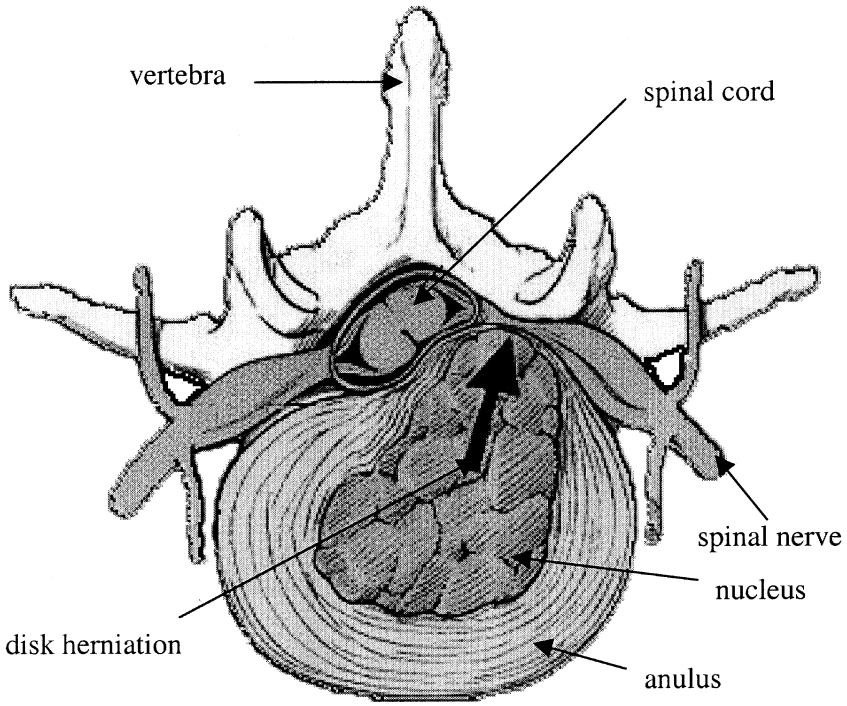


FIG. 13. Intervertebral discs herniation [123].

Whenever the nuclear tissue is herniated or removed by surgery, the disc space will narrow and may lose much of its normal stability. In many cases, to alleviate pain from degenerated or herniated discs, the nucleus or the disc as a whole is removed and the two adjacent vertebrae surgically fused together. While this treatment alleviates the pain, all discal motion is lost in the fused segment. Ultimately, this procedure places greater stresses on the discs adjacent to fused segment as they compensate for lack of motion, perhaps leading to premature degeneration of those adjacent discs.

A more desirable solution would involve replacing in part or as a hole the damaged disc with a suitable prosthesis having the ability to complement the height and motion of a disc. Therefore a substantial need exists for an easily implantable, prosthetic spinal disc of loading bearing ability and pumping action simulating the natural disc physiology.

Hydrogels seem to satisfy the major demands made on such an implant and thus the possibility of use of hydrogels in the spinal disc prosthesis construction has been investigated

for a couple of years (*cf.* US Patent No. 5824093). However, no invention has been reported to prove its functionality and become widely applied commercially available product.

In our laboratory several attempts to construct the hydrogel-based intervertebral disc implants has been made recently [123]. Our approach is based on the radiation-synthesized hydrogels of vinylpyrrolidone, HEMA and methyl methacrylate. Obtained hydrogels in the form of a rod are crushed into grains and subsequently closed into polyester fabric bag. The devices are left in water to let the hydrogel core swell to equilibrium and tested mechanically. Taking into account the present results, new hydrogels compositions of enhanced tensile parameters have to be made. This subject is currently under extensive investgation.

4.7. Ophthalmic applications

The market of contact lenses is completely dominated by products on the base of poly-HEMA. There is also a great number of various hydrogel compositions, which contain as co-polymers such compounds as poly (poly (vinyl pyrrolidone), poly (poly (vinyl alcohol), poly (poly (methacrylic acid), chitosan, etc. Co-polymerization is mainly used to improve mechanical properties of poly-HEMA and increase its oxygen permeability. Some contact lenses produced by radiation technology have been marketed in China [124].

An intraocular lens (JOL) currently used in the treatment of cataracts is generally made of poly (poly (methyl methacrylate). Despite the great number of implantations performed, in about 10% of cases the implanted JOL have had to be removed. Hydrogel intraocular lenses made of poly (poly (vinyl alcohol) have been successfully clinically tested and seem to be the next generation of such implants; contrary to the former, they can be sterilized by ionizing radiation [122]. Also the use of hydrogels as corneal implants, artificial vitreous humor, postenucleation implants and rods for retinal detachment surgery have been clinically tested with very good results.

4.8. Stimuli-responsive systems

Various polymer gels have been found to undergo reversible swelling changes in response to small changes in solvent composition, pH, temperature, intensity of light as well as magnetic and electric fields. The applications of such hydrogels in devices as actuators, artificial muscles, controlled molecular separators have been suggested [9]. There are many papers devoted to radiation and conventional synthesis of such systems, although their practical commercialization has not yet been achieved. The polymers used in these investigations include N-substitute polyacrylamide derivatives, poly (poly (acrylic acid) and poly (poly (methacrylic acid) derivatives, poly (poly (vinyl alcohol) and their combinations. Especially often poly (poly (N-isopropylacrylamide) and poly (vinyl methyl ether) are used. Their aqueous solutions show thermoresponsible characteristics. Both polymers exhibit phase separation at lower critical solution temperature (LCST), which is equal to 32 and 38°C, respectively. The hydrogels swell below and shrink above those temperatures. Recently, there is significant interest in the synthesis of the hydrogels of poly (N-isopropylacrylamide) and pH-sensitive polymers. In this case it is possible to obtain the materials, which are able to response to changes of temperature and acidity in the system [125, 126]. The temperature-sensitive radiation-synthesized PVME gels are currently under investigation in our group. Also, kinetics and mechanisms of radiation-induced crosslinking of PVME has been investigated by Janik, et al. [127].

The other class of stimuli-responsive systems includes interpolymer complexes. The stability of these complexes is controlled by various factors as the nature of the swelling medium, temperature, pH, ionic strength, and composition and nature of interacting chains. As the examples of this type materials the complex of poly (ethylene glycol) and poly (methacrylic acid) [128, 129] or poly (vinyl alcohol) and poly (acrylic acid) [130, 131] may be cited. Both these interpenetrating networks are sensitive to pH changes and may be used as matrices or membranes in drug delivery systems.

4.9. Hydrogel hybrid-type organs

Such devices designed for implantation consist of living cells surrounded by suitable membranes. The living metabolic cells, e.g. Langerhans islets, hypatocytes, hepatoma (Hep G2) etc. placed in appropriate capsules secrete specific compounds in response to the changes in body fluids. The system works as a self-controlling bioreactor. From an engineering point of view, the point of the matter is the choice of suitable materials and preparation procedures to fabricate the membrane. It should satisfy the following requirements:

— it must be permeable to water, oxygen, nutrients as well as specific secretions of living cells;
— it must be impermeable to components of the immune system;
— it should be completely "invisible" for its environment to avoid deposition of proteins and biodegradation.

There are two methods used for obtaining such systems: microencapsulation and preparation of some special larger containers. The first consisted of the entrapment of a few cells inside of macrocapsules, which can be injected into the organism. The second consisted of construction of a massive container, whose walls are semi-permeable membranes. Such devices containing a great number of cells able to substitute damaged organs can be implanted into the peritoneal cavity of a recipient. There is some information about a successful clinical application of devices prepared from poly (vinyl alcohol) as implanted artificial pancreas ([132], *cf.* Fig. 11). The appropriate connection of some functions of living cells and the properties of man-made plastics will result, in the near future, in fabrication of hydrogel, hybrid-type artificial organs.

In our paper two methods of living cells encapsulation were developed [133, 134]. These methods are based on sodium alginate crosslinking induced by calcium cations. First attempt is based on an additional covering of alginate matrix by poly (vinyl alcohol) layer crosslinked with glutaraldehyde. However, this procedure caused denaturation of cellular proteins.

The second approach is based on the additional covering of the alginate matrix containing cells by poly (methyl methacrylate) membrane. This cover was produced using interfacial precipitation method. By selecting the appropriate conditions of the encapsulation, the membranes permeable for low-molecular weight compounds (nutrients) and low-molecular weight proteins were developed. However, the high-molecular weigh immune system proteins (γ-globulin) could not diffuse though the formed cover. Described method could be a convenient tool for the encapsulation of living cells, which are not damaged and are characterised by long-term survival.

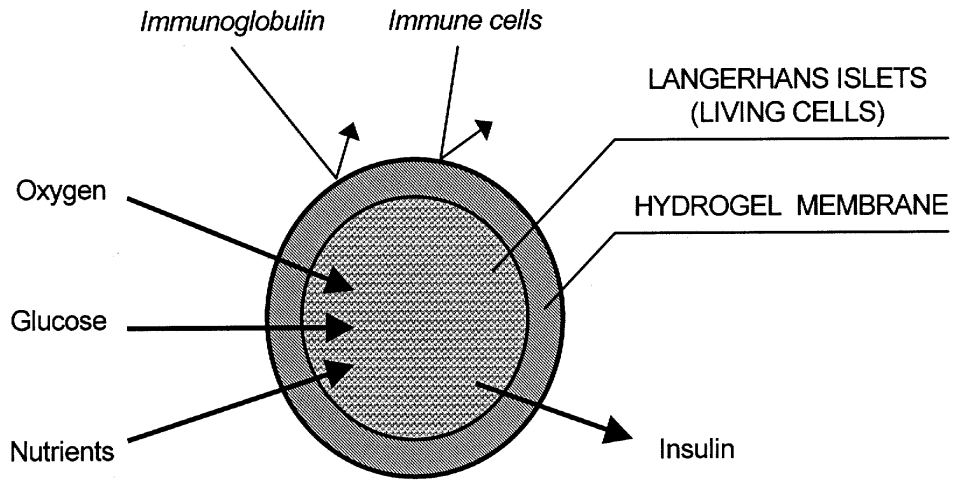


FIG. 14. Scheme of a bioartificial pancreas surrounded by a hydrogel membrane.

The following problem, which appeared in the next part of our research, is how to provide the most suitable living conditions to the cells and how to avoid their damage caused by the products of their metabolism. Studies connected with these questions are currently performed by our group. They are connected with a development of a new system of alginate gel with special, spherical, chamber-like structure [135].

5. FINAL REMARKS

Despite of many known advantages of using ionizing radiation for formation and modification of hydrogels and other biomedical products there are still a long way for radiation chemists to transfer their academic experiences to production rooms. However, the healthcare is at the top of the political, social and economic agenda in today's world and will remain a top priority into the 21st century. Therefore, this area of research and technological investigations seems to be very important and promising for those using the radiation processing technologies.

Human-friendly hydrogel systems, due to the rising trend to prolong life span and to improve the results of medical care, seem to be one of the most expected and required products. The unique advantages of radiation technology can be successfully utilized for the preparation of new commercial products, with designed functions that satisfy expectations of patients and physicians. Implants, drug delivery systems, artificial organs, and bioengineering generally are the domains in which radiation formed polymer materials begin to play an increasingly significant role. Despite a great number of investigations on radiation processes which allow for clarification of some mechanisms of reactions and elaboration of some general rules governing those phenomena, there are still some doubts and needs of further studies, both fundamental and applied. Despite many patents devoted to radiation bioengineering there are continuing needs for new products and more sophisticated biomaterials. The use of ionizing radiation in the production of human-friendly products seems to be the most promising way to broaden the range of commercial applications of radiation technology.

ACKNOWLEDGEMENTS

The authors would like to thank the International Atomic Energy Agency for its continuous support of these investigations in the framework of the Co-ordinated Research Programme F2.20.24 as well as the Technical Co-operation Projects POL/8/017 and POL/8/018. Also, a lot of thanks for the collaboration of Professor C. von Sonntag from Max-Planck-Institute, Mülheim a. d. Ruhr (Germany), and Doctors K. Makuuchi and F. Yoshii from Takasaki Radiation Chemistry Research Establishment (JAERI, Japan).

REFERENCES

[1] *High Performance Biomaterials*; ed. M. SZYCHER, TECHNOMIC, LANCASTER (1991).
[2] *Biomaterials Science*; eds. B.R. RATNER, A.S. HOFFMAN, F.J. SCHOEN, J. E. LEMONS, ACADEMIC PRESS, SAN DIEGO (1996).
[3] SILVER F. H. AND CH. DOILLON; *Biocompatibility. Interaction of Biological and Implantable Materials;* VCH Publisher Inc., New York (1989).
[4] WICHTERLE O. AND D. LIM; Hydrophilic gels for biological use; *Nature*, **185**, 117 (1960).
[5] JORDAN-LLOYD D.; in: *Colloid Chemistry*, vol. 1., J. ALEXANDER (ed.), Chemical Catalogue Company, New York, 1926, p. 767 (1926).
[6] SINGH H., P. VASUDEVAN AND A. R. RAY; Polymeric hydrogels: preparation and biomedical applications; *J. Scient. Ind. Res.*, **39**, 162 (1980).
[7] KAETSU I.; Immobilization of biofunctional substances; *Radiat. Phys. Chem.*, **18**, 343 (1981).
[8] PEPPAS N. A.; *Hydrogels in Medicine and Pharmacy*; CRC Press, Boca Raton (1986).
[9] DE ROSSI D., K. KAJIWARE, Y. OSADA AND A. YAMAUCHI; *Polymer Gels. Fundamentals and Biomedical Applications*. Plenum Press, New York (1991).
[10] ROSIAK J. M., P. ULANSKI AND A. RZEZNICKI; Hydrogels for biomedical purposes; *Nucl. Instr. and Meth. in Phys. Res. B,* **105**, 335 (1995).
[11] ROSIAK J. M., P. ULANSKI, L. A. PAJEWSKI, F. YOSHII AND K. MAKUUCHI; Radiation formation of hydrogels for biomedical purposes. Some remarks and comments; *Radiat. Phys. Chem.*, **46**, 161 (1995).11]
[12] ROSIAK J. M. AND P. ULANSKI; Synthesis of hydrogels by irradiation of polymers in aqueous solution; *Radiat. Phys. Chem.*, **55**, 139 (1999).
[13] ROSIAK J. M. AND F. YOSHII; Hydrogels and their medical applications; *Nucl. Instr. Meth. B*., **151**, 56 (1999).
[14] HOFFMAN A. S.; Environmentally sensitive polymers and hydrogels — „smart" biomaterials; *MRS Bulletin*, Sept. 1991, 42 (1991).
[15] KAETSU I., K. UCHIDA, Y. MORITA AND M. OKUBO; Synthesis of electro-responsive hydrogels by radiation polymerization of sodium acrylate; *Radiat. Phys. Chem.*, **40**, 157 (1992).
[16] HIRASA O.; Research trends of stimuli-responsive polymer hydrogels in Japan; *Journal of Intelligent Material Systems and Structures*, **4**, 538 (1993).
[17] YOSHIDA R., H. ICHIJO, T. HAKUTA AND T. YAMAGUCHI; Self-oscillating swelling and deswelling of polymer gels; *Macromol. Rapid Commun.*, **16**, 305 (1995).
[18] SHIGA T., Y. HIROSE, A. OKADA, T. KURAUCHI AND O. KAMIGAITO; Design of biomimetic machinery systems with polymer gel; In: Proc. 1st Japan International SAMPE Symposium, 28 Nov.–1 Dec. 1989, p. 659 (1989).

[19] BRASCH U. AND W. BURCHARD; Preparation and solution properties of microhydrogels from poly (vinyl alcohol); *Macromol. Chem. Phys.*, **197**, 223 (1996).
[20] ROSIAK J. M.; Hydrogel dressings. In: *Radiation Efects on Polymers*, ACS Symposium Series 475, eds: R.L. CLOUGH AND S.W. SHALABY, ACS, Washington D.C., p. 271 (1991).
[21] ROSIAK J. M.; Radiation formation of hydrogels; *J. Control. Release*, **31**, 9 (1994).
[22] CHARLESBY A.; *Atomic Radiation and Polymers*; Pergamon Press, Oxford (1960).
[23] CHAPIRO A.; *Radiation Chemistry of Polymeric Systems;* Interscience, New York (1962).
[24] HOFFMAN A. S.; A review of the use of radiation plus chemical and biochemical processing treatments to prepare novel biomaterials; *Radiat. Phys. Chem.*, **18**, 323 (1981).
[25] NEDKOV E. AND S. TSVETKOVA; Effect of γ-irradiation on the crystalline structure of ultra high molecular weight poly (ethylene oxide); *Radiat. Phys. Chem.*, **43**, 397 (1994).
[26] ROSIAK J. M., K. BURCZAK, T. CZOLCZYNSKA AND W. PEKALA; Radiation crosslinked hydrogels from acrylamide water solutions. *Radiat. Phys. Chem.*, **22**, 917 (1983).
[27] ROSIAK J. M., J. OLEJNICZAK AND A. CHARLESBY; Determination of the radiation yield of hydrogels crosslinking; *Radiat. Phys. Chem.*, **32**, 691 (1988).
[28] ROSIAK J. M., K. BURCZAK, W. PEKALA, N. PISLEWSKI, S. IDZIAK AND A. CHARLESBY; Studies on polymerization and crosslinking of aqueous acrylamide; *Radiat. Phys. Chem.*, **32**, 793 (1988).
[29] TOBITA H.; Molecular weight distribution in free-radical cross-linking co-polymerization; *Macromolecules*, **26**, 836 (1993).
[30] TOBITA H.; Kinetics of network formation in free-radical cross-linking co-polymerization; *Macromolecules*, **26**, 5427 (1993).
[31] KABANOV V. YA.; Preparation of polymeric biomaterials with the aid of radiation-chemical methods; *Russ. Chem. Rev.*, **67**, 783 (1998).
[32] ROSIAK J. M.; Gel/sol analysis of irradiated polymers; *Radiat. Phys. Chem.*, **51**, 13 (1998).
[33] OLEJNICZAK J., J. M. ROSIAK AND A. CHARLESBY; Gel/dose curves for polymers undergoing simultaneous crosslinking and scission; *Radiat. Phys. Chem.*, **37**, 499 (1991).
[34] INOKUTI M.; Gel formation in polymers resulting from simultaneous crosslinking and scission; *J. Chem. Phys.*, **38**, 2999 (1963).
[35] SAITO O., H. Y. KANG AND M. DOLE; Theory of gel-dose curves for polymers undergoing simultaneous crosslinking and scission; *J. Chem. Phys.*, **46**, 3607 (1967).
[36] BRAY J. C. AND MERRILL E. W.; Poly (vinyl alcohol) hydrogels. Formation by electron beam irradiation of aqueous solutions and subsequent crystallization; *J. Appl. Polym. Sci.*, **17**, 3779 (1973).
[37] PEPPAS N. A. AND E. W. MERRIL; PVA hydrogels: Reinforcement of radiation-crosslinked networks by crystallization; *J. Polym. Sci., Polym. Chem. Ed.*, **14**, 441 (1976).
[38] CHARLESBY A. AND R. FOLLAND; The use of pulsed NMR to follow radiation effects in long chain polymers; *Radiat. Phys. Chem.*, **15**, 393 (1980).
[39] CHARLESBY A.; Characterization of macromolecular morphology by pulsed NMR spectroscopy. In: *Radiation Effects on Polymers*, ACS Symposium Series 475, eds: R. L. Clough and S. W. Shalaby, ACS, Washington D.C., p. 193 (1990).

[40] SCHNABEL W.; *Polymer Degradation. Principles and Practical Applications*; Hanser, München (1981).
[41] LEMAIRE D. G. E.; Untersuchungen zum strahleninduzierten Strangbruch von Poly (U) in wäßriger Lösung; Thesis, Ruhr-University, Bochum, Germany (1984).
[42] ULANSKI P. AND J. M. ROSIAK; Preliminary studies on the radiation-induced changes in chitosan; *Radiat. Phys. Chem.*, **39**, 53 (1992).
[43] VON SONNTAG C.; *The Chemical Basis of Radiation Biology*; Taylor & Francis, London (1987).
[44] GöRLICH W. AND W. SCHNABEL Kinetic investigation on the dehydration of poly (vinyl alcohol) and model compounds in aqueous solution; *Eur. Polym. J.*, **8**, 1087 (1972).
[45] ULANSKI P., E. BOTHE, J. M. ROSIAK AND C. VON SONNTAG; OH-radical-induced crosslinking and strand breakage of poly (vinyl alcohol) in aqueous solution in the absence and presence of oxygen. A pulse radiolysis and product study; *Macromol. Chem. Phys.*, **195**, 1443 (1994).
[46] ULANSKI P., E. BOTHE, J. M. ROSIAK AND C. VON SONNTAG; Radiolysis of the poly (acrylic acid) model 2,4-dimethylglutaric acid: a pulse radiolysis and product study; *J. Chem. Soc., Perkin Trans. II*, **1996**, 3 (1996).
[47] CHARLESBY A., P. J. FYDELOR, P. M. KOPP, J. P. KEENE AND A. J. SWALLOW; Pulse radiolysis studies of aqueous polymer-thiourea systems. In: *Pulse Radiolysis*, eds. E. EBERT, J. P. KEENE, A. J. SWALLOW AND J. H. BAXENDALE, ACADEMIC PRESS, LONDON, p. 193 (1965).
[48] BORGWARDT U., W. SCHNABEL AND A. HENGLEIN; Pulsradiolytische Messung der Geschwindigkeitskonstanten der Kombination von Polyäthylenoxid-Radikalen in wäßriger Lösung; *Makromol. Chem.*, **127**, 176 (1969).
[49] BECK G., J. KIWI, D. LINDENAU AND W. SCHNABEL; On the kinetics of polymer degradation in solution. I. Laser flash photolysis and pulse radiolysis studies using the light scattering detection method; *Eur. Polym. J.*, **10**, 1069 (1974).
[50] LUBIS R., J. OLEJNICZAK, J. M. ROSIAK AND J. KROH; System for measurement of changes of LSI in polymer solution after electron pulse; *Radiat. Phys. Chem.*; **36**, 249 (1990).
[51] DEEBLE D. J., E. BOTHE, H.-P. SCHUCHMANN, B. J. PARSONS, G. O. PHILLIPS AND C. VON SONNTAG; The kinetics of hydroxyl-radical-induced strand breakage of hyaluronic acid. A pulse radiolysis study using conductometry and laser-light-scattering; *Z. Naturforsch.*, **45 c**, 1031 (1990).
[52] KUJAWA P., ULANSKI P. AND J. M. ROSIAK; Time-resolved investigations of pulse-initiated polymerization; *Radiat. Phys. Chem.*, **52**, 389 (1998).
[53] BOTHE E. AND E. JANATA; Instrumentation of kinetic spectroscopy. 13 a.c.-Conductivity measurements at different frequencies in kinetic experiment; *Radiat. Phys. Chem.*, **44**, 455 (1994).
[54] BOTHE E. AND D. SCHULTE-FROHLINDE; Release of K^+ and H^+ from poly U in aqueous solution upon γ and electron irradiation. Rate of strand break formation in poly U; *Z. Naturforsch.*, **37 c**, 1191 (1982).
[55] BOTHE E., G. A. QURESHI AND D. SCHULTE-FROHLINDE; Rate of OH radical induced strand break formation in single stranded DNA under anoxic conditions. An investigation in aqueous solutions using conductivity methods; *Z. Naturforsch.*, **38 c**, 1030 (1983).

[56] BOTHE E., G. BEHRENS, E. BöHM, B. SETHURAM AND D. SCHULTE-Frohlinde Hydroxyl radical-induced strand break formation of poly (U) in the presence of oxygen: comparison of the rates as determined by conductivity, e.s.r. and rapid-mix experiments with a thiol; *Int. J. Radiat. Biol*., **49**, 57 (1986).

[57] ROSIAK J. M., J. OLEJNICZAK AND W. PEKALA; Fast reactions of irradiated polymers. I. Crosslinking and degradation of polyvinylpyrrolidone; *Radiat. Phys. Chem.*, **36**, 747 (1990).

[58] ULANSKI P., E. BOTHE, K. HILDENBRAND, J. M. ROSIAK AND C. VON SONNTAG; Hydroxyl-radical-induced reactions of poly (acrylic acid); a pulse radiolysis, e.p.r. and product study. Part I. Deoxygenated aqueous solutions; *J. Chem. Soc., Perkin Trans. II*, **1996**, 13 (1996).

[59] ULANSKI P., E. BOTHE, K. HILDENBRAND, J. M. ROSIAK AND C. VON SONNTAG; Hydroxyl-radical-induced reactions of poly (acrylic acid); a pulse radiolysis, e.p.r. and product study. Part II. Oxygenated aqueous solutions; *J. Chem. Soc., Perkin Trans. II*, **1996**, 23 (1996).

[60] KUJAWA P. AND J. M. ROSIAK Pulse radiolysis of 2-[(methacryloyloxy)ethyl]trimethyl ammonium chloride in aqueous solution; *Radiat. Phys. Chem.*, (in print, 1999).

[61] KUJAWA P., N. MOHID, K. ZAMAN, W. MANSHOL, P.ULANSKI AND J. M. ROSIAK; Pulse radiolysis of butyl acrylate in aqueous solution; *Radiat. Phys. Chem.*, **53**, 403 (1998).

[62] TAKACS E. A. SAFRANY, L. WOJNAROVITS, F. MARTELLINI, M. YOSHIDA, R. KATAKAI, S. S. EMMI AND M. CARENZA; Pulse radiolysis of acryloyl-L-proline methyl ester in dilute aqueous solution; *J. Macromol. Sci. — Pure Appl. Sci.*, **A33**, 1609 (1996).

[63] TAKACS E. A. SAFRANY, L. WOJNAROVITS, F. MARTELLINI, M. YOSHIDA, R. KATAKAI, S. S. EMMI, G. BEGGIATO AND M. CARENZA; Pulse radiolysis of methacryloyl-L-proline methyl ester in dilute aqueous solution; *J. Macromol. Sci. — Pure Appl. Sci.*, **A35**, 21 (1998).

[64] BEHZADI, A., U. BORGWARDT, A. HENGLEIN, E. SCHAMBERG AND W. SCHNABEL; Pulsradiolytische Untersuchung der Kinetik diffusionskontrollierter Reaktionen des OH-Radikals mit Polymeren und Oligomeren in wäßriger Lösung; *Ber. Bunsenges. physik. Chem.*, **74**, 649 (1970).

[65] MATHESON M. S., A. MAMOU, J. SILVERMAN AND J. RABANI; Reaction of hydroxyl radicals with polyethylene oxide in aqueous solution; *J. Phys. Chem.*, **77**, 2420 (1973).

[66] ULANSKI P., ZAINUDDIN AND J. M. ROSIAK; Pulse radiolysis of poly (ethylene oxide) in aqueous solution. II. Decay of macroradicals. *Radiat. Phys. Chem.*, **46**, 917 (1995).

[67] ULANSKI P., I. JANIK AND J. M. ROSIAK; Radiation formation of polymeric nanogels; *Radiat. Phys. Chem.*, **52**, 289 (1998).

[68] ULANSKI P. AND J. M. ROSIAK; The use of radiation technique in the synthesis of polymeric nanogels; *Nucl. Instr. Meth. B*., **151**, 356 (1999).

[69] ULANSKI P. AND J. M. ROSIAK; Radiation-chemical synthesis of polymeric nanogels; 21th Miller Conference on Radiation Chemistry, Doorwerth, Holland, 24-29 April, (1999).

[70] VON SONNTAG C., E. BOTHE, P. ULANSKI AND A. ADHIKARY; *Radiat. Phys. Chem.*, **55**, 599–603 (1999).

[71] ASMUS K.-D., H. MöCKEL AND A. HENGLEIN; Pulse radiolytic study of the site of OH radical attack on aliphatic alcohols in aqueous solution; *J. Phys. Chem.*, **77**, 1218 (1973).
[72] ALEXANDER P. AND A. CHARLESBY; Effect of X-rays and γ-rays on synthetic polymers in aqueous solution; *J. Polym. Sci.*, **23**, 355 (1957).
[73] GRIFFITH CIMA L. AND S. T. LOPINA; Network structures of radiation-cross-linked star polymer gels; *Macromolecules*¸**28**, 6787 (1995).
[74] ULANSKI P., E. BOTHE, K. HILDENBRAND, J. M. ROSIAK AND C. VON SONNTAG; Radiolysis of poly (acrylic acid) in aqueous solution; *Radiat. Phys. Chem.*, **46**, 909 (1995).
[75] ULANSKI P., E. BOTHE, K. HILDENBRAND, C. VON SONNTAG C. AND J. M. ROSIAK; The influence of repulsive electrostatic forces on the lifetimes of poly (acrylic acid) radicals in aqueous solution. *Nukleonika*, **42**, 425 (1997).
[76] JANIK I., P. KUJAWA, P. ULANSKI AND J. M. ROSIAK; Pulse radiolysis of polymers in aqueous solution. Kinetic study; *J. Chim. Phys.*, **94**, 244 (1997).
[77] PLONKA A.; Developments in dispersive kinetics; *Prog. Reaction Kinetics*, **16**, 157 (1991).
[78] PLONKA A.; Dispersive kinetics; *Annu. Rep. Prog. Chem., Sec. C, Phys. Chem.*, **91**, 107 (1994).
[79] DYBALA A.; Master Thesis, Technical University of Lodz, Poland (1997).
[80] NISHIDE H., M. D. CHO, T. KAKU AND Y. OKAMOTO; Investigation of the reactions of the OH radical with poly (acrylic acid) and poly (methacrylic acid) in aqueous solution using the Tb^{3+} fluorescence probe; *Macromolecules*, **26**, 2377 (1993).
[81] BUCHANAN, K. J., B. HIRD AND T. M. LETCHER; Crosslinked poly (sodium acrylate) hydrogels; *Polym. Bull.*, **15**, 325 (1986).
[82] ULANSKI P., E. BOTHE AND C. VON SONNTAG; Some aspects of the radiolysis of poly (methacrylic acid) in oxygen-free aqueous solution*; Radiat. Phys. Chem.*, in the press (1999).
[83] ULANSKI P., E. BOTHE AND C. VON SONNTAG; OH radical induced depolymerization of poly (methacrylic acid); *Nucl. Instr. Meth. B*., **151**, 350 (1999).
[84] ROSIAK J. M. AND ULANSKI P.; Transformations of radiation-generated macroradicals of poly (acrylic acid) in deoxygenated aqueous solutions. Part II. Chain scission and crosslinking; *Polimery*, 42, 219-228 (in Polish) (1997).
[85] STASICA P., P. ULANSKI AND J. M. ROSIAK; Letter to the editor; *J. Pineal Res.*, **25**, 65 (1998).
[86] STASICA P., P. ULANSKI AND J. M. ROSIAK; Reaction of melatonin with radicals in deoxygenated aqueous solution; *J. Radioanal. Nucl. Chem.*, **232**, 107 (1998).
[87] STASICA P., P. PANETH AND J. M. ROSIAK; Hydroxyl radical addition to the melatonin molecule. A computational study; *J. Pineal Res.*, submitted (1999).
[88] POEGGELER B., R. J. REITER, D.-X. TAN, L.-D. CHEN AND L. C. MANCHESTER; Melatonin, hydroxyl radical-mediated oxidative damage, and aging: A hypothesis, *J. Pineal Res.*, **14**, 151 (1993).
[89] REITER R. J.; The pineal gland and melatonin in relation to aging: a summary of the theories and of the data; *Exp. Gerontology*, **30**, 199 (1995).
[90] PIERPAOLI W. AND REGELSON W.; *The melatonin miracle*; Simon & Schuster, New York, (1995).
[91] VON SONNTAG C. AND H.-P. SCHUCHMANN; The elucidation of peroxyl radical reactions on aqueous solution with the help of radiation-chemicals methods; *Angew. Chem. Int. Ed. Engl.*, 30, 1229 (1991).

[92] ROSIAK J. M., A. RUCINSKA-RYBUS AND W. PEKALA; *Method of Manufacturing of Hydrogel Dressings*; Polish Patent No. 151, 581, US Patent No. 4,871,490, Hungarian Patent No. 204,866, UK Patent No. 2,200, 643, German Patent No. 3, 744, 289 (1989).
[93] REMBAUM A., S. P. S. YEN, E. CHEONG, S. WALLACE, R. S. MOLDAY, I. L. GORDON AND W. J. DREYER; Functional polymeric microspheres based on 2-hydroxyetyl methacrylate for immunochemical studies; *Macromolecules*, **9**, 328 (1976).
[94] REMBAUM A., S. P. S. YEN AND W. VOLKSEN; Labelled cells; *Chemtech*, **3**/**78**, 182 (1978).
[95] YOSHIDA M., M. ASANO AND I. KAETSU; Preparation of microspheres for slow release drug by radiation-induced suspension polymerization and their properties; *Yakuzaigaku*, **42**, 137 (1982).
[96] KREUTER J.; Nanoparticulate systems in drug delivery and targeting; *Journal of Drug Targeting*, 3, 171 (1995).
[97] SAUNDERS B. R. AND B. VINCENT; Microgel particles as model colloids: theory, properties and applications; *Adv. Colloid Interface Sci.*, **80**, 1 (1999).
[98] KUMAKURA M. AND I. KAETSU; Preparation by radiation polymerization of functional microspheres with aldehyde groups; *Reactive Polymers*, **2**, 243 (1984).
[99] KAETSU I.; Recent progress on the immobilization of biocomponent by radiation polymerization and the application to biomedical uses; *Radiat. Phys. Chem.*, **25**, 517 (1985).
[100] SAFRANY A., S. KANO, M. YOSHIDA, H. OMICHI, R. KATAKAI AND M. SUZUKI; Functional polymeric microspheres by radiation polymerization; *Radiat. Phys. Chem.*, **46**, 203 (1995).
[101] SLOMKOWSKI S.; Controlled polymerization of ε–caprolactone — from macromolecules to microspheres; *Macromol. Symp.,* **103**, 213 (1996).
[102] ULANSKI P., S. KADLUBOWSKI AND J. M. ROSIAK; A new approach to the synthesis of polymeric nanogels; 5th International Conference on Frontiers of Polymers and Advanced Materilas, Poznan, Poland, 21-25 June 1999, book of abstracts, p. 244 (1999).
[103] ROSIAK J. M., P. ULANSKI, I. JANIK AND S. KADLUBOWSKI; Method of formation of polymeric nanogels; submitted to Polish Patent Office; P-331868 (1999).
[104] LANGNER R.; Drug delivery and targeting; *Nature,* **392** (suppl.), 5 (1998).
[105] AL.-ASSAF S., PHILLIPS G. O., DEEBLE D. J., PARSONS B., STARNES H. AND C. VON SONNTAG; The enhanced stability of the cross-linked hylan structure to the hydroxyl (OH) radicals compared with the uncross-linked hyaluronan; *Radiat. Phys. Chem.*, **46**, 207-217 (1995).
[106] ALLAN G. G., L. C. ALTMAN, R. E. BENSINGER, D. K. GHOSH, Y. HIRABAYASHI, A. N. NEGOI AND S. NEGOI; Biomedical applications of chitin and chitosan; In: *Chitin, chitosan and related enzymes*, J. P. Zikakis (ed.), Academic Press, Orlando, p. 119 (1984).
[107] ULANSKI P., A. WOJTASZ-PAJAK, J. M. ROSIAK AND C. VON SONNTAG; Radiolysis and sonolysis of chitosan — two convenient techniques for a controlled reduction of the molecular weight; *Adv. Chitin Sci.*, submitted (1999).
[108] ULANSKI P., J. M. ROSIAK, A. WOJTASZ-PAJAK AND H. STRUSZCZYK; Method of reduction of chitosan molecular weight; submitted to Polish Patent Office, P-331868 (1999).
[109] LAMBERT B. J. AND J. M. HANSEN ISO radiation sterilization standards; *Radiat. Phys. Chem.*; **52**, 11 (1998).

[110] YOSHII F., K. MAKUUCHI, D. DARWIS, T. IRIAWAN, M. T. RAZZAK AND J. M. ROSIAK; Heat resistance of poly (vinyl alcohol) hydrogel; *Radiat. Phys. Chem.*, **46**, 169 (1995).
[111] MAKUUCHI K. private communication (1997).
[112] GRAHAM, N. B.; Controlled drug delivery systems; *Chem. Ind.*, **15**, 482 (1990).
[113] ROSIAK J. M. AND J. OLEJNICZAK; Medical applications of radiation-formed hydrogels; *Radiat. Phys. Chem.*, **42**, 903 (1993).
[114] ROSIAK J. M., W. DEC AND A. KOWALSKI; IUMPA: Secretion of medroxiprogesterone acetate from a carrier according to a drug dose; *Med. Sci. Monit.*, **2** (suppl. 2), 78 (1996).
[115] ROSIAK J. M., A. KOWALSKI AND W. DEC; Drug delivery system for the treatment of endometrial carcinoma; *Radiat. Phys. Chem.*, **52**, 307 (1998).
[116] CZECHOWICZ-JANICKA A., S. GALANT AND J. M. ROSIAK; Polymer ocular inserts with controlled release of drugs. I. Evaluation of materials in animals; *Klin. Oczna* **94**, 41 (1992).
[117] DYBEK K., A. KUBIS AND J. M. ROSIAK; Studies on dressings for dental surgery use. Part 3. Effect of gamma-radiation and of formaldehyde on properties of gelatinous dental dressings; *Pharmazie*, **47**, 273 (1992).
[118] LINDEN L.-A.; Native and synthetic ionotropic hydrogels for use in dental care; Chemistry Forum V — International Symposium, 19-21 April 1999, Warsaw, Poland, p. 65 and references cited therein (1999).
[119] GUISTI P., N. LAZZERI, N. BARBANI, P. NARDUCCI, A. BONARETTI, M. PALLA AND L. LELLI; Hydrogels of poly (vinyl alcohol) and collagen as new bioartifical materials; *J. Mater. Sci., Mater. Med.*, **4**, 538 (1993).
[120] YOSHII F., K. MAKUUCHI, ZAINUDDIN, A. SURADJAT, D. DARWIS AND M. T. RAZZAK; Heat stability of radiation crosslinked poly (vinyl alcohol) hydrogel; *Jpn. J. Med. Instrum.*, **62**, 285 (1992).
[121] HOFFMAN A. S.; Adsorption and immobilization of proteins on gas discharge-treated surfaces; *J. Appl. Polym. Sci. Polym. Symp.*, **46**, 341 (1990).
[122] GEN S.; Intraocular lens of polyvinyl alcohol hydrogel. *Sci. Technol. Jpn.*, **8**, 62 (1989).
[123] STASICA P., M. CIACH, M. RADEK AND J. M. ROSIAK; Approach to construct hydrogel intervertebral disc implants — experimental and numerical investigations, in preparation (1999).
[124] CARENZA M.; Recent achievements in the use of radiation polymerization and grafting for biomedical applications; *Radiat. Phys. Chem.*, **39**, 485 (1992).
[125] CHEN G. AND A. S. HOFFMAN; Graft co-polymers that exhibit temperature-induced phase transitions over a wide range of pH; *Nature*, **374**, 240 (1995).
[126] VAKKALANKA S. K. AND N. A. PEPPAS; Swelling behaviour of temperature- and pH-sensitive block co-polymers for drug delivery; *Polym. Bull.*, **36**, 221 (1996).
[127] JANIK I., P. ULANSKI AND J. M. ROSIAK; Pulse radiolysis of poly (methyl vinyl ether) in aqueous solution. Formation and structure of primary radicals; *Nucl. Instr. Meth. B*., **151**, 318 (1999).
[128] BELL C. L. AND N. A. PEPPAS; Water, solute and protein diffusion in physiologically responsive hydrogels of poly (methacrylic acid-g-ethylene glycol); *Biomaterials*, **17**, 1203 (1996).
[129] BELL C. L. AND N. A. PEPPAS; Modulation of drug permeation through interpolymer complexed hydrogels for drug delivery applications; *J. Controlled Release*, **39**, 201 (1996).

[130] GUDEMAN L. F. AND N. A. PEPPAS; pH-Sensitive membranes from poly (vinyl alcohol)/poly (acrylic acid) interpenetrating networks; *J. Membrane Sci.*, **107**, 239 (1995).
[131] PEPPAS N. A. AND S. L. WRIGHT; Drug diffusion and binding in ionizable interpenetrating networks from poly (vinyl alcohol) and poly (acrylic acid); *Eur. J. Pharm. Biopharm.*, 46, 15 (1998).
[132] INOUE K., T. FUJISATO, Y. GU, K. BURCZAK, S. SUMI, M. KOGIRE, T. TOBE, K. UCHIDA, I. NAKAI, S. MAETANI AND Y. IKADA; Experimental hybrid islet transplantation: applications of polyvinyl alcohol membrane for entrapment of islets; *Pancreas*, **7**, 562 (1992).
[133] KUJAWA P., M. LEMIESZ, M. KOLODZIEJCZYK, J. NAREBSKI, L. A. PAJEWSKI AND J. M. ROSIAK Encapsulation of living cells in alginate and alginate/PVAL hydrogels; Proc. of the Int. Workshop "Bioencapsulation VI", Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain, 30 August–1 September 1997, talk 2.4 (1997).
[134] KOZICKI M., P. KUJAWA, L. PAJEWSKI, M. KOLODZIEJCZYK, J. NAREBSKI AND J. M. ROSIAK; Encapsulation of living cells towards artificial, hybrid organs, *Engineering of Biomaterials*, 7–8, 11 (1999).
[135] KOZICKI M., P. KUJAWA AND J. M. ROSIAK Encapsulation of living cells towards artificial, hybrid organs; 5th International Conference on Frontiers of Polymers and Advanced Materials, Poznan, Poland, 21-25 June 1999, book of abstracts, p. 225 (1999).

THE USE OF RADIATION PROCESSING TO PREPARE BIOMATERIALS FOR APPLICATIONS IN MEDICINE

I. Kaetsu

Kinki University,
Faculty of Science and Technology,
Osaka, Japan

Abstract. Research and development of biofunctional materials by radiation techniques for biomedical uses by the author's group during Research Cooridination Program was reviewed. New injectable drug delivery systems(DDS) were developed using poly (ethylene glycol) and sol-gel transition polymers for prolonged anesthesia. New medical applications of DDS such as the efficient preparation of sick model animals and the durable nutrient supply for post-operation patients were developed. Sol-gel transition polymer solution and stimuli-responsive polyelectrolyte hydrogel were studied basically. Then, intelligent release and permeation devices were constructed using radiation prepared micro-porous films and chips, and stimuli-responsive gels for the intelligent channel gates. The intelligent functions of the prepared biomembranes and biochips were successfully proved. Integration systems of intelligent devices were also constructed and proved the signal transfer releases. The programmed control of intelligent system was studied and proved. On the other hand, the neuron cell culture and neural network formation were studied using multi-electrode cultivator to learn the ideal intelligent functions in brain-nervous system. Harmonization of frequency cicle of electric signal in the electrode was observed with the proceeding of network formation.

1. INTRODUCTION

The authors studied the radiation-polymerization of supercooled monomers at low temperatures in 1966–1975, and found this polymerization was very advantageous for the synthesis of polymer composite materials [1].

In 1973–1983, the authors studied the application of polymerization in the supercooled state to the immobilization of various biofunctional materials such as enzymes, proteins, microbial cells, organella and tissue cells [2]. Since 1976, the authors studied the immobilization for controlled release of drugs and hormones(drug delivery systems) also by means of supercooled state polyerization [3]. This kind of research results on drug delivery was practically used in the clinical stage for local chemotherapy and hormone therapy [4]. Since 1989, the authors advanced to the research on the intelligent controlled release and drug delivery. This kind of research included the basic study on the stimuli responsive hydrogels and the design of intelligent biomembranes and biochips as the application systems [5]. Radiation lithography and hole fabrication techniques could be used effectively for the construction of intelligent devices. Computer programmed control of intelligent releases was also investigated [6]. It was also found that intelligent biomembranes and biochips could be prepared efficiently by the conventional processing of radiation coating and curing with a conveyer system [7]. On the other hand, the brain nervous system was the focus of interest fy the authors as an ideal model for integrated intelligent system [8]. The neuron cell culture and the neuron-network formation were studied using the multi-electrode cell cultivator, which was prepared by lithography and spattering techniques [9]. Based on those research works as the background, the authors reviewed the main research results in recent years under the framework of the Research Co-ordination Programme.

2. RESULTS AND DISCUSSION

2.1. Research on drug delivery systems

2.1.1. Development of injectable drug delivery systems

The authors have developed the polymer-drug composites with various solid forms such as disk, needle, and sphere and applied to the implantable drug delivery systems. However, recently medical doctors wanted a liquid from polymer-drug mixture that can be injectable conveniently, but solidified in the body after injection for the continuous sustained release.

For this purpose, the authors studied and proposed two kinds of matrices for the drug. The one was poly (ethylene glycol) having a melting temperature just above the body temperature. Another one was sol-gel transition type polymer which turned into solid particles from an aqueous solution by phase transition at a critical temperature just above body temperature. The injectable poly (ethylene glycol)-morphine mixtures were applied to the prolongation of anesthesia effect for the patients after operation. The results proved the remarkable prolonged efficacy of anesthesia (Fig. 1) [10]. The poly (N-isopropyl acrylamide, as a sol-gel transition polymer) -anticancer(5-FU) mixtures were applied to the local chemotherapy of tumor tissue by injection and the followed solidification, aiming an enlargiment of necrosis effect. The results proved the remarkable enlarging of necrosis area around the administration site on the tissue of rats and mice [11].

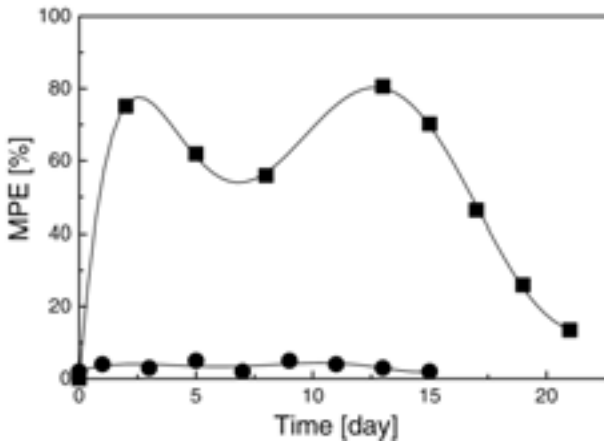


FIG. 1. Analgesia duration effect by controlled release of morphine from DDS sample containing 7.5mg morphine: —■— 7.5mg Morphine, —●— Control.

2.1.2. New application targets of drug delivery systems

The authors have applied the implantable DDS (drug delivery system) to some new targets, which were strongly intended by medical researchers. The one is the application to the efficient preparation of sick model expermental animals for pathological researches by continuos release technique. For this purpose, lipopolysaccharide-polymer composites were implanted in rats for sepsis model animal preparation and L-ethionin-polymer composite were implanted for chronic pancreastitis model animals [12]. The results showed the considerable time shortening effect of sick model animals by controlled release of sick promoting substances. Another application of DDS was studied on geutamine-polymer composite for efficient continuous supply of glutamine as a neutrient for the patients after serious operation. The results on animal experiment showed promising improvement effect [13].

3. RESEARCH OF STIMULI SENSITIVE AND RESPONSIVE POLYMERS

3.1. Sol-gel transition polymers

Research interest in stimuli-sensitive and responsive polymers has increased more and more among the researchers of biomaterials. The radiation can contribute to this region variously as the means of synthesis and fabrication. The authors have studied this kind of polymers since 1985 [14] and especially applied it to the intelligent drug delivery since 1988 [15]. Principle of stimuli-responsive volume changes of polymers between shrinkage and expansion can mainly be attributed to the two mechanisms, phase transition and ionic interaction. The typical phase transition type polymer is the poly (N-isopropyl acrylamide) having hydrophilic-hydrophobic pendant chains. The typical ionic interaction type polymer is the polyelectrolyte having a dissociative group chain. The volume changes can be attributed to the conformation change of polymer between extended and coiled molecular structures. A linear polymer shows sol-gel phase changes in an aqueous solution in response to a stimulation. On the other hand, the crosslinked hydrogel shows the stimuli-responsive volume changes of shrinking and expanding. The authors studied the thermo-responsive sol-gel transition co-polymers of N-isopropyl acrylamide for DDS applications. The phase transition temperature, LCST(Lower critical solution temperature) can be varied extensively with the kind and composition of co-monomers. LCST also change by the kind and concentration of inorganic salts such K salts and Na salts in the solution(Fig. 2) [16]. Other phase transition polymer and co-polymers such as poly (dimethly acrylamide) were also investigated in relation to LCST.

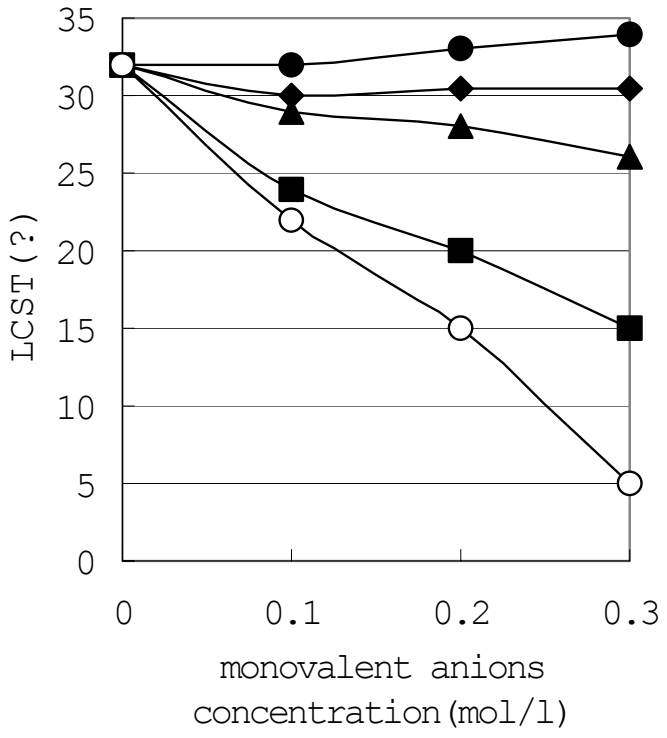


FIG. 2. Effect of various anions (sodium salts) on the LCST of aqueous solutions of poly (NIPAAM) —●—NaSCN, —◆—NaI, —▲—NaCl, —■—tartaric acid Na, —○—citric acid Na.

3.2. Synthetic stimuli-responsive hydrogels-polyelectrolyte

The authors have studied polyelectrolytes such as acrylic and it's sodium salt since 1988 [17] as the ionic interaction type stimuli-responsive polymers showing pH, ionic and electric responsivenesses. The first paper by the authors was reported on poly (acrylic acid) hydrogel(poly anion) in 1992 (Fig. 3) [18]. The IPN(Inter-penetrating networked polymer) effect to increase the responsiveness has been studied on poly (acrylic acid) (polyanion) and poly (methacryloyloxyethyl trimethyl ammonium chloride) (poly cation) hydrogels since 1987(Fig. 4) [6]. It was found that the double crosslinking with enough hydrophilic polymer such as poly (acrylamid) as the second matrix was very effective for the improved responsiveness. This kind of penetrating crosslinking can be studied advantageously using radiation technique.

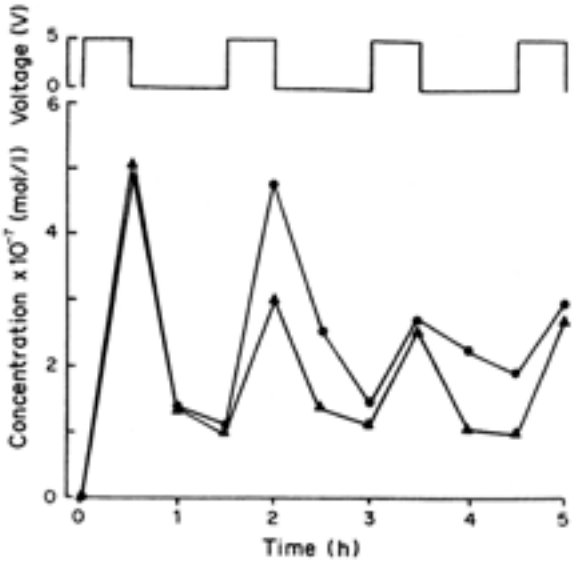


FIG. 3. Release profile of methylene blue from sodium acrylate-ethyleneglycol dimethacrylate(2G) co-polymer gel under on-off switching of electric field: —●—AAcNa: 2G = 1: 0.2 in molar ratio; —▲—AAcNa: 2G = 1: 0.3; monomer: solvent (H_2O-CH_3OH mixture, H_2O:CH_3OH=2:3)=1:5.

3.3. Natural stimuli-responsive hydrogels polyelectrolytes

Natural polymers include a lot of various polyelectrolytes such as polysaccharides and proteins. In general, natural polyelectrolytes have better biocompatibility than the synthetic polymers. Moreover, some of them have the biodegradability. Those would be advantageous for the future *in vivo* and *ex vivo* uses of intelligent devices.

However, it is not always to give a crosslinked structure to form a stable gel to natural polyelectrolytes. Irradiation tends to induce degradation easily in many polysaccharides. Therefore, the authors studied and proposed two methods for the gelation of natural polysaccharide that are applicable to all kinds of natural polymers. The one is the entrapping of natural polysaccharide inside the synthetic hydrogel by polymerization of aqueous mixture of vinyl monomer, crosslinker and natural polymer (Fig. 5) [19]. Another one is the entrapping of natural polysaccharide inside the network of crosslinked gel of other natural polymers such as agarose, chitosan and starch (Table I) [20]. Both methods gave the stable hydrogel including natural polyelectrolytes and those hydrogels showed the pH and electro-responsivenesses.

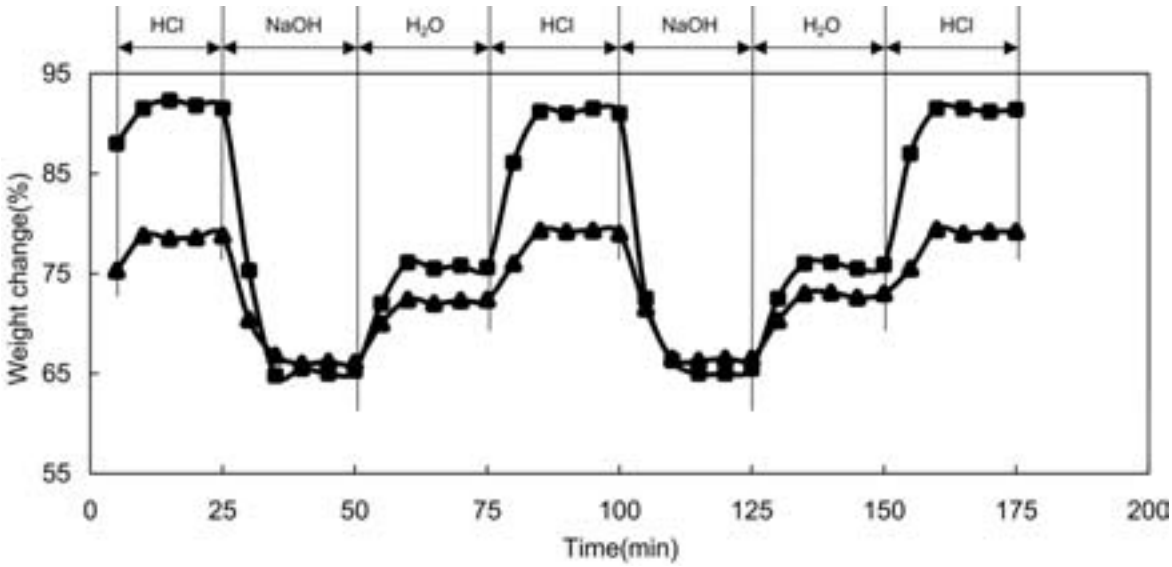


FIG. 4. Comparison of pH responsiveness in IPN gel and non-IPN gel Non-IPN gel: polymethacryloxyehyl trimethylammonium chloride IPN gel: 2nd monomer system consisting of acrylamide-polyethleneglycol #400 dimethacrylate-distilled water (100-2–800 wt ratio) was absorbed and polymerizd inside the non-IPN gel: —▲—Non-IPN gel, —■—IPN gel.

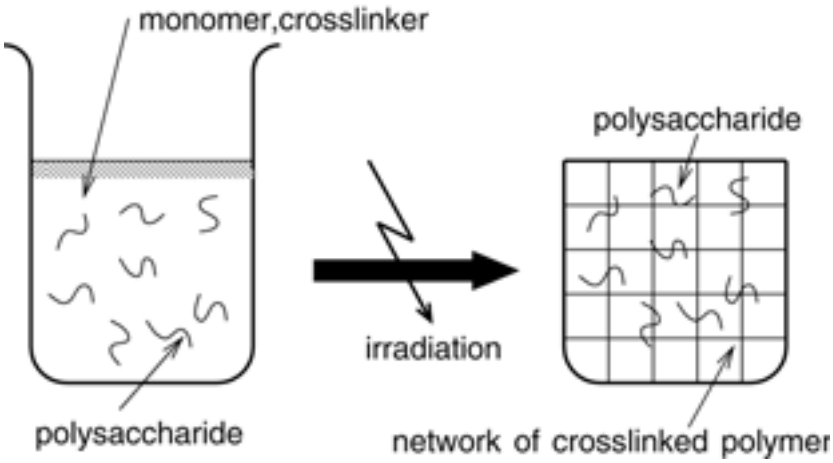


FIG. 5. Model scheme of entrapped crosslinking of polysaccharide-vinyl monomer system.

TABLE I. GELATION OF POLYSACCHARIDE WITH HYALURONIC ACID

polysaccharide	gelation	gelation of polysaccharide with hyaluronic acid
Agarose	○	○
Arabic gum	×	×
λ -Carrageenan	×	×
Dextrose	×	×
Gelatin	○	○
Gluco mannan (from konjac)	○	○
Sodium Alginate	×	×
Xanthan gum	×	×

○ : ok × : not ok

4. RESEARCH OF INTELLIGENT RELEASE SYSTEMS WITH BIOMEMBRANES AND BIOCHIPS

4.1. Intelligent biomembranes

The authors have studied the design and construction of intelligent controlled release and permeation system since 1992 (Fig. 6) [21] based on the research results of the stimuli-responsive hydrogels. The intelligent system can be defined as the one whose function is realized with on-off switching according to the intermittent inputs of environmental signals. It consists of sensor part to catch the signal, actuator (transducer) part to convert the signal to mechanical energy to initiate or stop the release and permeation function and micro-computer part to treat the first signal from the sensor and transfer the second signal to the actuator according to a program. This system should be as compact as possible and designed to recieve multiple signals and feedback multiple releases, from and to the environment. In order to fill those requests, the multi-channel gates structure was fabricated on the device. The radiation techniques such as ion-beam lithography and exima-laser irradiation can be very useful for this kind of micro-fine fabrication. Stimuli-sensitive hydrogels were used for the sensor and actuator parts. The monomer was coated on a radiation prepared micro-porous film and polymerized by U.V. irradiation to from the intelligent layer. Enzymes were often immobilized in the layer by entrapping to give the substrate (glucose, acetylcoline, glutamin, asaparagin etc.) responsive functions as a biomembrane. All intelligent release and permeation functions were proved clearly using those biomembranes (Figs 7, 8, 9) [22].

4.2. Intelligent biochips

The similar micro-fabrication was investigated on the silicon wafer using lithography technique. Then, the stimuli-responsive polymer was coated and polymerized in the presence or absence of enzyme, to form the intelligent layer as the channel gates. Those biochips proved the intelligent release functions of model drug clearly, as in the intelligent biomembranes (Fig. 10) [23].

4.3. Integration system of intelligent devices

The intelligent device can have the strong analogy with the neuron-synapse in the structural and functional properties, though the former is far simple and imperfect than the latter. Both have the signal responsive channel gates which open and close due to the signal responsive conformation changes of polymers, stimuli-sensitive polyelectrolyte and receptor protein. Therefore, the integrated system of intelligent devices can be the simplest model of neural network system. The authors have begun a trial for a simple integration of devices in series and in parallel (Figs 11, 12) [24]. The integrated system consisting of four devices in series showed the intelligent releases due to the signal transfer throughout the connected devices. The integrated system consisting of devices connected in 4 stages in parallel was tested and proved the positive or negative signal transfers and the resulting releases. Further study would be done in the future for the design of artificial neural network.

4.4. Computer programming system of intelligent device

Computer programming is most important and necessary for the design of multi-signals responsive multi-components feedback release systems. The authors have studied this area for the one-signal responsive one-release system first and then the two-signals two-components release system. It was proved that the drug releases occurred with the on-off switching according to the threshold values settled in the program (Figs 13, 14) [6].

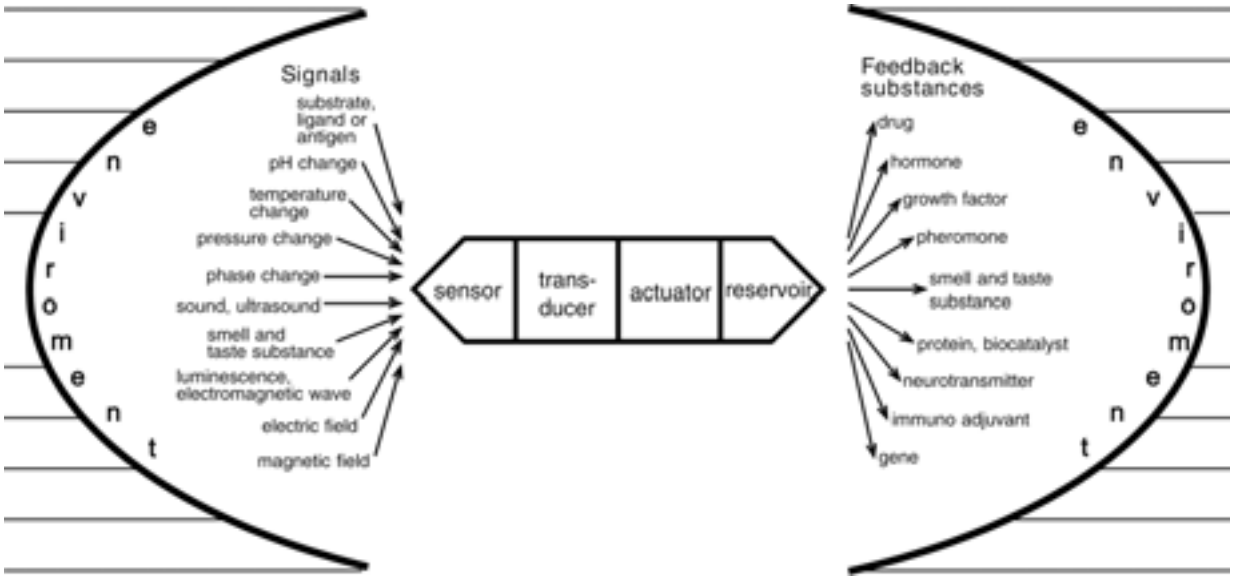


FIG. 6. Model scheme of signal responsive chemical release system.

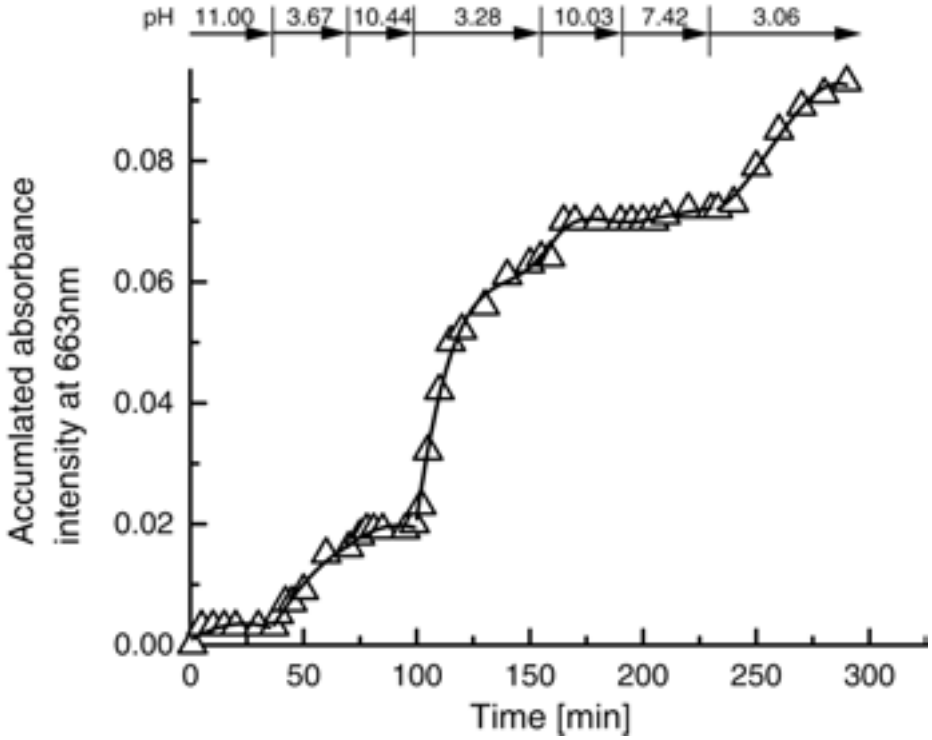


FIG. 7. pH responsive on-off swithching release of methylene blue from PET film having polyacrylic acid sensor gate.

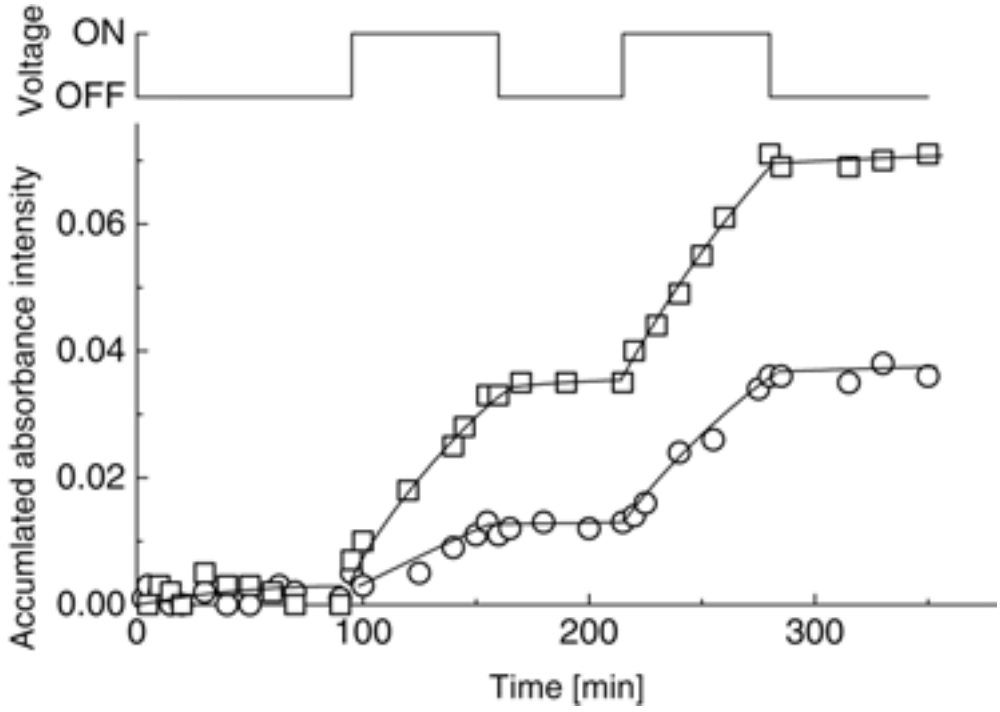


FIG. 8 Electric field responsive release of metylene blue from polyacrylic acid gate with electrode zone: Electric field ○ :12V, □ :3V.

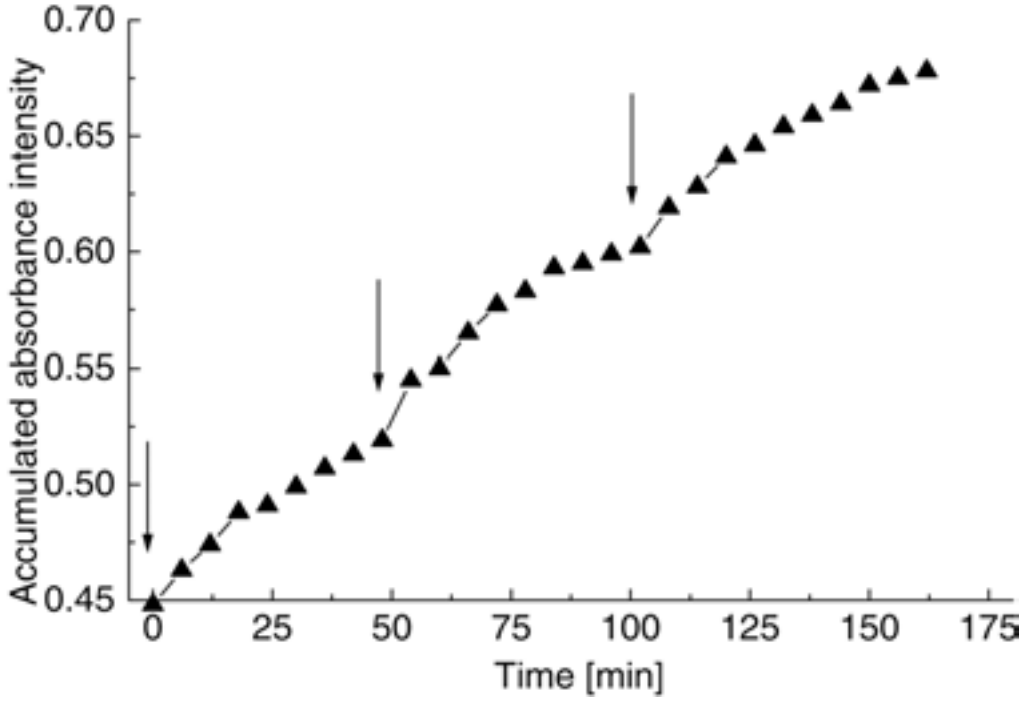


FIG. 9. Acetylcholine responsive release of methylene blue(5.0g/l) from silicon chip through polyacrylic acid gate with immobilized acetylcholine esterase. Gate size: 500 μm ϕ; ↓ addition of acetylcholine (0.05g).

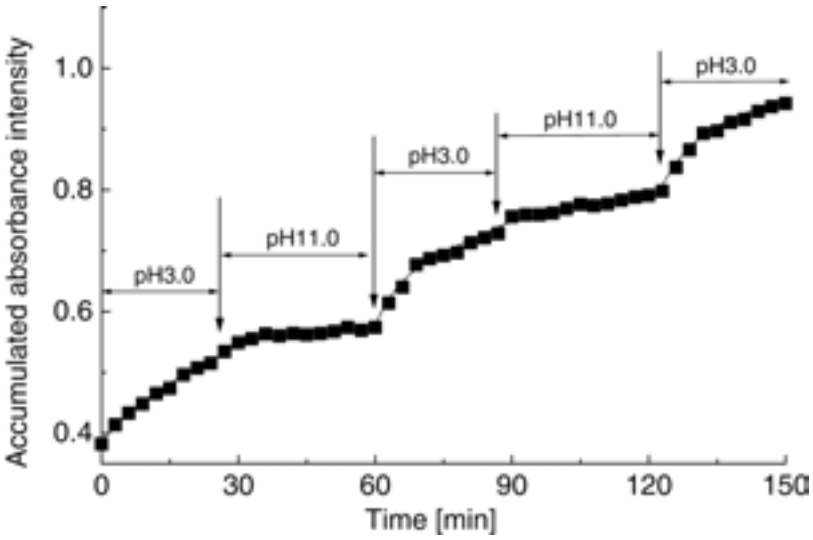


FIG. 10. pH-responsive release of methylene blue(5.0g/l) from Silicon chip through polyacrilic gate, gate size: 500 μm ϕ.

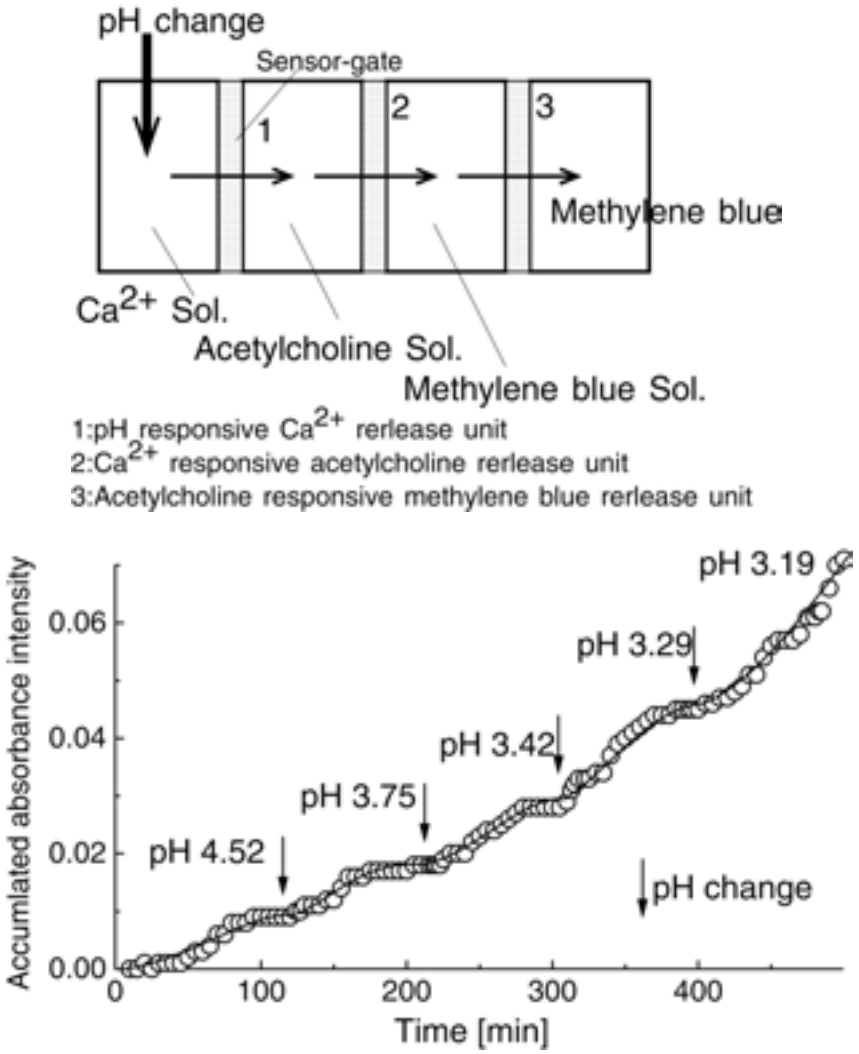
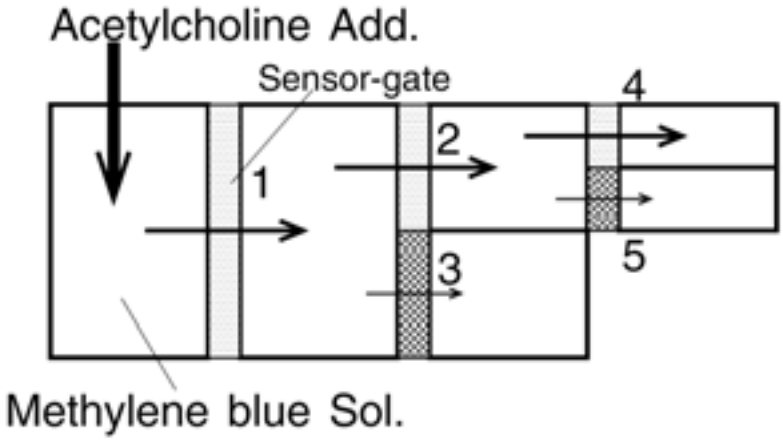


FIG. 11. Model scheme of integrated signal transfer-responsive release system in series and the result of release at the end unit (No. 3).



1:Acetylcholine responsive methylene blue rerlease unit
2:Acetylcholine responsive methylene blue rerlease unit
3:pH responsive methylene blue rerlease unit
4:Acetylcholine responsive methylene blue rerlease unit
5:pH responsive methylene blue rerlease unit

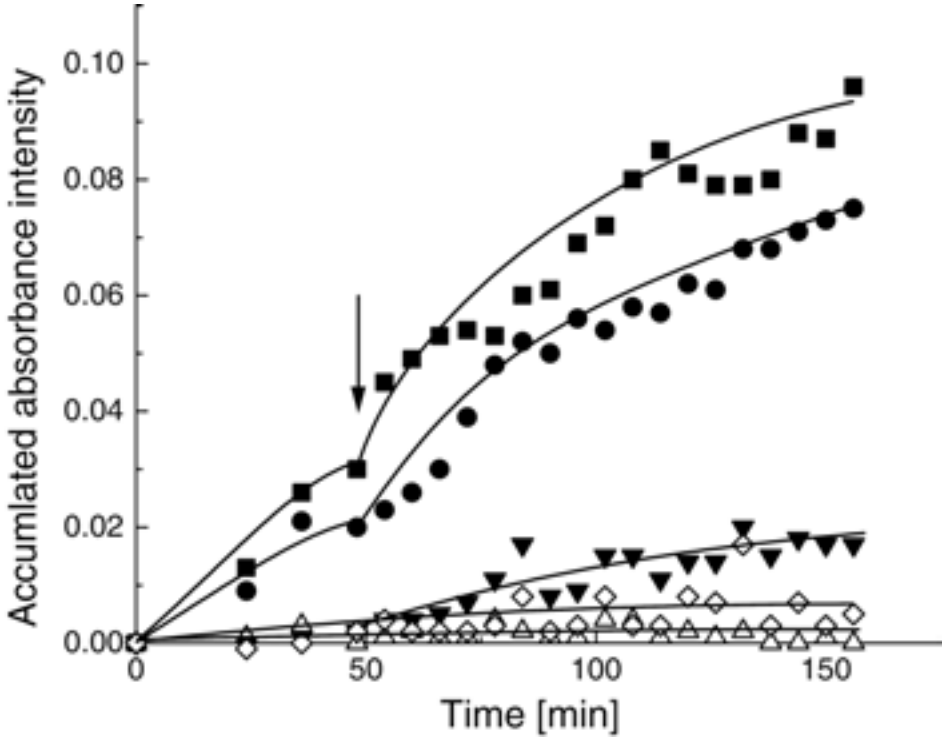


FIG. 12. Model scheme of integrated signal transfer responsive release system in series and parallel and the results of releases in each unit: ↓ Addition of Acetylcholine; ■ No. 1 (positive); ● No. 2 (positive); □ No. 3 (negative); ▼ No. 4 (positive); No. 5 (negative).

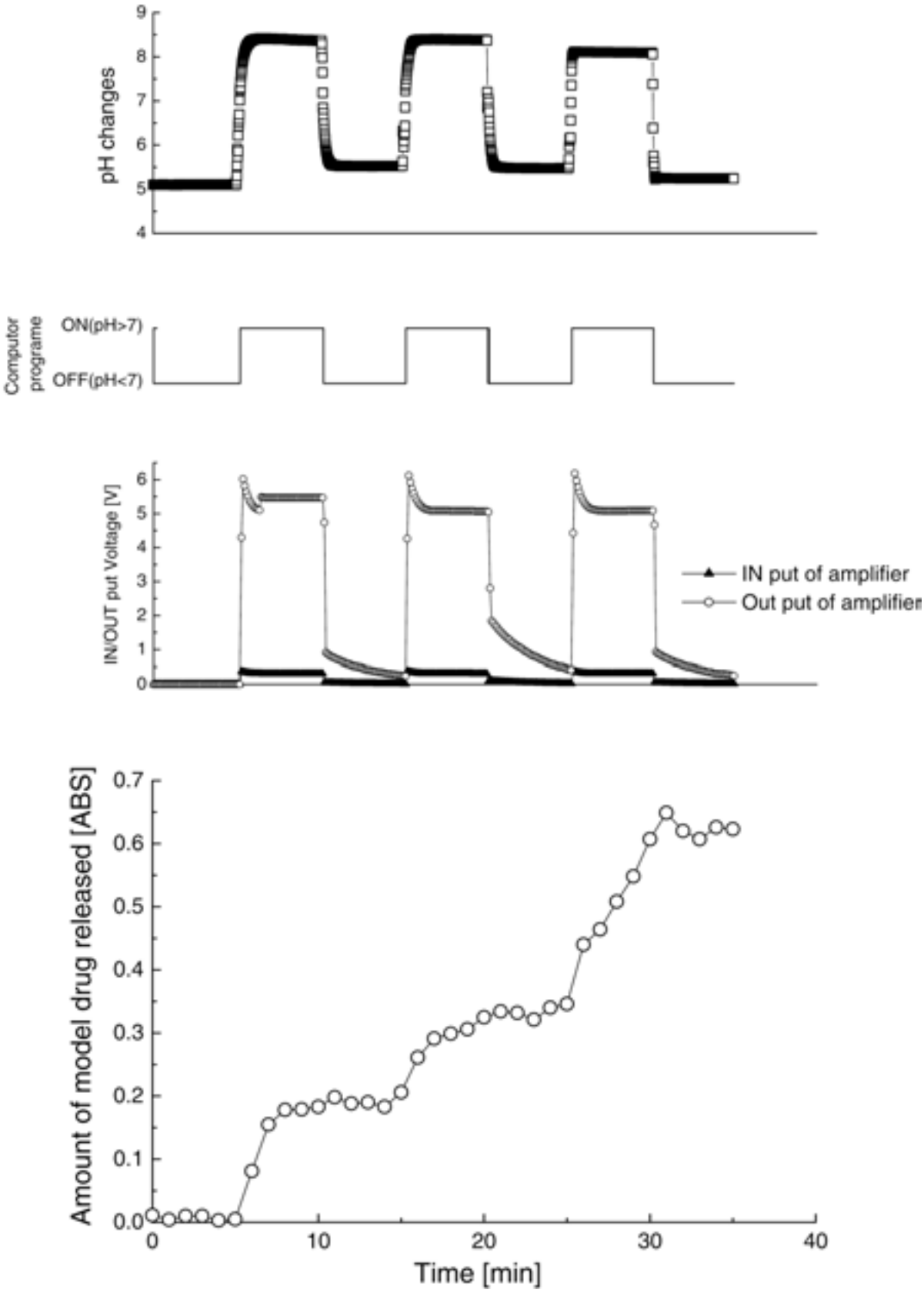


FIG. 13. Diagram and the results of pH responsive computer programmed drug release system.

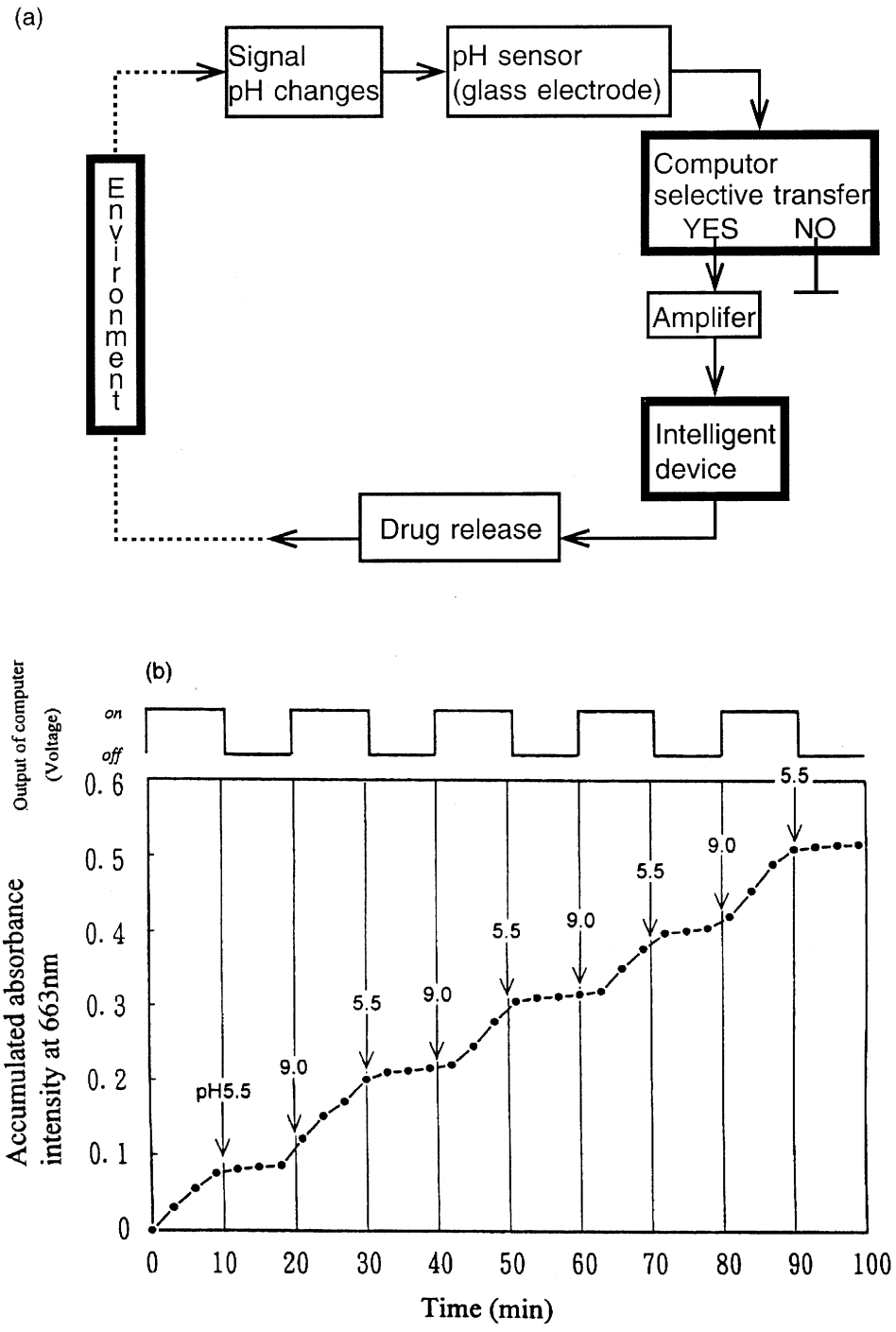


FIG. 14. Flow diagram for pH responsive computer programmed drug release (a) and the result (b); Polymer hydrogel: polyacrylic acid sodium salt-polyetyleneglycol #400 dimethacrylaye (1:1 molar ratio), programmed threshold value: pH=7.

4.5. Coating and curing processing of intelligent chips and membranes

The intelligent hydrogel layer on a base film or chip can be prepared efficiently by means of conventional radiation coating and curing processing. Model processing system with U.V. irradiator and a conveyer was tested and proved the efficient production of biomembranes and biochips by the U.V. curing system (Fig. 15) [7].

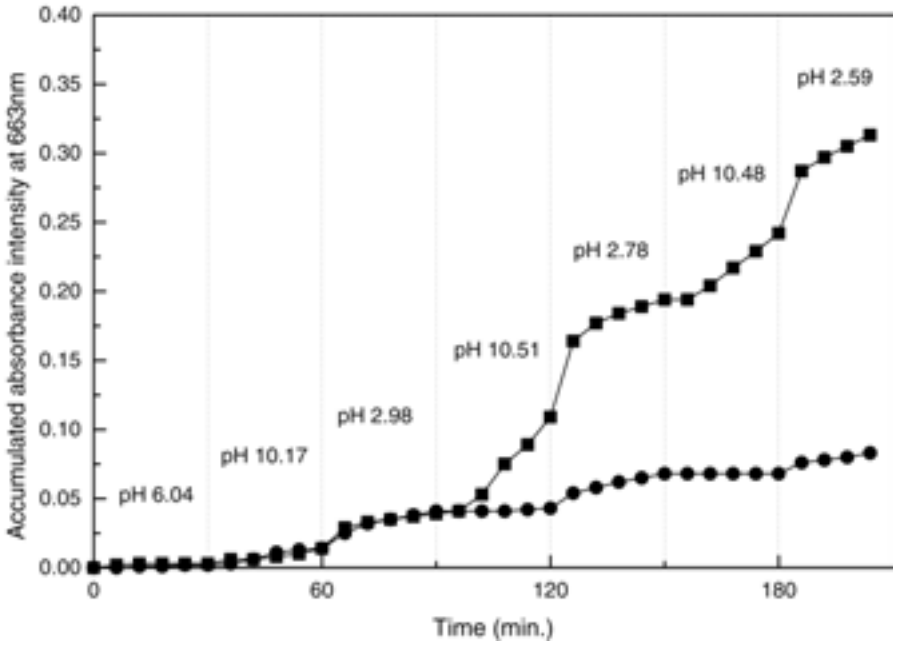


FIG. 15 pH-responsive permeation of model drug (M.B.=0.1g/l) through radiation cured biomembrane. Composition of coating: AAc(25%), 4G(75%) -on porous polycarbonate base film. Pore size: —■—10 μm, —●—2.0 μm.

5. CONCLUSIONS

(1) New injectable drug delivery systems (DDS) which can be solidified inside the body to realize sustained release were developed. Those would be useful for medical doctors for the implantable uses of DDS.

(2) Implantable DDS have been applied to some new medical purposes and targets promisingly. The uses of implantable DDS would be extended with the new ideas and needs of applications by medical researchers.

(3) Research of stimuli-sensitive and responsive polymers and hydrogels has been carried out basically especially on sol-gel transition polymers and synthetic and natural polyelectrolytes. Application of sol-gel transition polymer to an injectable DDS and the entrapping gelation of natural polyelectrolyte are the techniques of new ideas by the authors.

(4) Intelligent devices and systems with biomembranes and biochips have been developed using radiation fabrication techniques and stimuli-responsive hydrogels. This kind of devices would be further advanced in the near future using micro- and nano-fabrication techniques with radiations, for the micro- and neno-patterned super-fine structures.

(5) The integration of intelligent devices for signal transfer releases and the program control for multi-signals responsive muti-drug releases were studied using combined intelligent devices. Those areas would be important for the design of networked intelligent feedback release systems.

(6) Neuron cell growth and neural network formation were studied using multi-electrode cultivator. This kind of research would be useful for brain-nervous mimetic design of intelligent devices and systems in the future.

REFERENCES

[1] KAETSU I., OKUBO H., ITO A., HAYASHI K.,: J. Polymer Sci., A-1, 10,2203(1972)
[2] KAETSU I., KUMAKURA M., YOSHIDA M.,: Biotechnol. Bioeng., 21, 847(1979)
[3] YOSHIDA M., KUMAKURA M., KAETSU I.,: J. Pharmaceu. Sci., 68, 86(1979)
[4] KAETSU I.,: J. Bioactive and Biocompatible Polym., 3, 164(1988)
[5] KAETSU I.: Radiat. Phys. Chem., 46(2), 247(1995)
[6] KAETSU I., UCHIDA K., SUTANI K., SAKATA S.,: Radiat. Phys. Chem., 57, 465(2000)
[7] KAETSU I., UCHIDA K., NAKAYAMA.,: Radiat. Phys. Chem., in press
[8] KAETSU I., SHINDO H., UCHIDA K., SUTANI K.,: Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 23, 695(1996)
[9] KAETSU I., TANAKA K., UCHIDA K., SUZUKI Y., SUTANI K.,: Artif Organ, 26(3)767(1993)
[10] KAETSU I., UCHIDA K., USETA S., SUTANI K.,Drug Delivery System, 11(6)399(1996)
[11] KAETSU I., UCHIDA K., SUTANI K., TAMORI H., OYANAGI H.,: Drug Delivery System, in press
[12] KAETSU I., UCHIDA K., SUTANI K., SHIOMI T., MIYAMOTO M., HOKI M., OYANAGI H.,: Arfif. Organ, 26(2)534(1997)
[13] KAETSU I., UCHIDA K., KAKINOKI S., SUTANI K., HOKI M., OYANAGI H.,Drug Delivery System, in press
[14] MORITA Y., KAETSU I.,: Radiat. Phys. Chem., 39(6), 473(1992)
[15] KAETSU I., UCHIDA K., SUTANI K., USETA S., SHINDO H., DOI K.,: Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 22, 754(1995)
[16] KAETSU I., KAKINOKI S., UCHIDA K., SUTANI K., unpublished data
[17] KAETSU I.,: Polymer Fabrication, 44, 318(1995)
[18] KAETSU I., UCHIDA K., MORITA Y., OKUBO M.,: Radiat. Phys. Chem., 40(2), 157(1992)
[19] KAETSU I.,Polysaccharides in Medical Applications Dumitrin S. Ed. pp243(1996)
[20] SUTANI K., KAETSU I., MATSUMURA T., UCHIDA K.,: J. Jap. Soc. for Biomaterials, in press
[21] KAETSU I., SHINDO H., UCHIDA K., SUTANI K., Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 23, 471(1996)
[22] KAETSU I., UCHIDA K., SHINDO H., GOMI S., SUTANI K.,: Radiat. Phys. Chem., 55, 193(1999)
[23] KAETSU I., UCHIDA K., USETA S., SUTANI K., DOI K.,: Drug Delivery System, 11(5)317(1996)
[24] KAETSU I., UCHIDA K., SUTANI K., USETA S., SHINDO H., DOI K., Proceed. Intern. Symp. Control. Rel. Bioact. Mater., 23, 695(1996)

RADIATION SYNTHESIS OF HYDROGELS WITH DIPROTIC ACID MOIETIES AND THEIR USE IN THE ADSORPTION OF BIOMOLECULES

M. Sen

Hacettepe University, Department of Chemistry,
Beytepe, Ankara, Turkey

Abstract. Radiation synthesis of diprotic acid moieties containing poly (N-vinyl 2- pyrrolidone) and polyacrylamide hydrogels and their use in the adsorption of biomolecules such as enzymes, proteins and drugs have been investigated. Hydrogels with varying cross-linked densities and ionic moieties were prepared from the ternary systems of N-vinyl 2-pyrrolidone/itaconic acid/water and acrylamide/maleic acid/water by irradiating with γ rays at ambient temperature. For the characterization of network structure of hydrogels new equations were derived. Determination of average molecular weight between cross-links of hydrogels sensitive to pH changes of the swelling medium was investigated. In order to explain the influence of other external stimuli such as temperature and ionic strength of the swelling medium and the type of the buffer solution on the equilibrium swelling properties were investigated. The effect of these external stimuli on the biomolecule adsorption capacity of hydrogels were investigated for bovine serum albumin, α-amylase, invertase, model and commercial drugs. The results show that the hydrogels prepared in this study can be considered as potential carriers for the biomolecules and the drug delivery systems.

1. INTRODUCTION

Hydrophilic polymers when cross-linked chemically or physically forming three dimension networks swell but do not dissolve in water. They are termed hydrogels when the amount of water retained is between 20–100% of the total weight, and when water content exceeds 100% these hydrogels are called superadsorbent hydrogels. A hydrogel can be considered as a container of water made of a three dimensional mesh. Many materials, both naturally occurring and synthetic fit the definition of hydrogels. Dextrans, starch, alginates, and collagens are examples of natural polymers that can be cross-linked to form hydrogels. Hydrogels based on synthetic polymers include poly (hydroxy alkyl methacrylates), poly (acrylamide), poly (ethylene oxide), poly (N- vinyl 2-pyrrolidone) and poly (vinyl alcohol). Wichterle and Lim [1] were the first to suggest that a hydrogel based on poly (2-hydroxy ethyl methacrylate) could be a synthetic biocompatible material.

Recently hydrogels have found a wide range of biomedical applications including controlled drug delivery systems, replacement blood vessels, wound dressing, soft tissue substitution, contact lenses and a variety of other related and potential uses. Hydrogels are generally found to be very well tolerated when implanted *in vivo* and can be easily tailored to suit the many functions of prosthetics in contact with blood or tissues. The success of hydrogels as biomaterials lies in their resemblance to living tissue because of their relatively high water content which minimizes the frictional irritation of surrounding tissue. Additional advantages of hydrogels are their non-toxicity, non-antigenicity, non-irritability, and chemical stability. The relatively high water content of hydrogels made them also permeable to small molecules like oxygen, nutrients, and metabolites. The high solute permeability of hydrogels made them ideal materials of choice as devices for the controlled release of drugs and other active agents. Much of the research on hydrogels has been focused on the application in controlled drug delivery. By proper design of hydrogels it is possible to control the kinetics of delivery of active ingredients.

Hydrogels are commonly used as burn and wound dressing materials [2]. They prevent microbial contamination of the wound, inhibit the loss of body fluids and provide free flow of oxygen to the wound and generally accelerate the healing process.

The most characteristic property of hydrogels is their ability to swell in the presence of water and to shrink in the absence of it. The two most important factors controlling the extent of swelling are the hydrophilicity of polymer chains and the cross-link density. By incorporating some stimuli-responsive co-monomers either into the backbone of the network structure or as pendant groups it is possible to prepare hydrogels with responsive properties. These hydrogels possess the ability to swell, shrink, bend or even degrade in response to a signal. These stimuli-responsive hydrogels are also called intelligent hydrogels. They reversibly swell and shrink with small changes in the environmental conditions. The most common environmental factors that causes an abrupt volume changes in hydrogels are pH, temperature, electric field, ionic strength and type of salt. In addition to wide range of applications of these hydrogels in biomedical applications, they are also used in the separation and purification processes.

Hydrogels are typically synthesized by one of the two well established procedures: a) polymerization and simultaneous or postpolymerization cross-linking of hydrophilic monomers, and b) modification of hydrophilization of existing polymers with potential hydrogel properties. A comprehensive review of the chemistry and various synthetic approaches used in hydrogel preparation can be found in the compilation of Peppas [3]. A more recent review by Mathur, et al. [4] provides an in depth discussion on the methods of hydrogel synthesis. The inherent advantages of using high energy radiation in the synthesis of hydrogels for biomedical applications have been reviewed by Carenza [5]. The preparation of hydrogels by radiation treatment of aqueous solutions of hydrophilic monomers or polymers carries some advantages over the conventional techniques. It does not require initiators, cross-linkers and can be used practically with any vinyl monomer and both polymerization and cross-linking reactions can be initiated at ambient or sub-ambient temperatures.

Responsive behaviour of hydrogels makes them also very attractive materials for some specific applications in adsorption, enrichment and separation processes. Sites showing selectivity for proteins, enzymes, biomolecules or dyes, pigments or metal ions can be easily incorporated into network structure by radiation induced polymerization. During the last decade a number of papers published from this laboratory showed clearly the advantages of irradiating aqueous monomer solutions to synthesize co-polymeric hydrogels. In this context the use of even very small quantities of diprotic acid-containing monomers proved to impart fascinating properties to the hydrogels of starting monomers proved to impart fascinating properties to the hydrogels of starting homopolymers. In this project, radiation synthesis of N-vinyl 2-pyrrolidone and acrylamide based diprotic acid containing hydrogels and their use in the adsorption, enrichment of the protein molecules and transtermal drug delivery systems have been investigated and some of selected results are explained in details below.

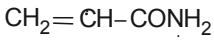
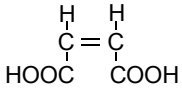
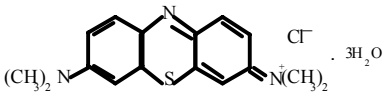
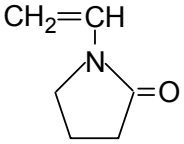
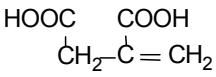
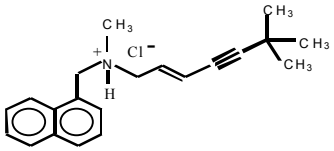
2. EXPERIMENTAL

2.1. Chemicals

The monomers used in this study namely, acrylamide(AAm), maleic acid(MA), and N-vinyl 2-pyrrolidone(VP), Itaconic acid(IA) were obtained from BDH and Aldrich, respectively. KH_2PO_4, K_2HPO_4 and H_3PO_4 used to prepare phosphate buffers and tri-sodium

citrate, sodium dihydrogen citrate and citric acid used to prepare citrate buffers were obtained from BDH. The model drug Mehtylene Blue was obtained from Ficher and BSA (Fraction V) and α-amylase and invertase from Aldrich. Pure terbinafine hydrocloride was used after purification of the coomertial drug Lamisil of Novarsis Company. The chemical formulea of monomers and drugs are given in Scheme 1.

Scheme 1.

Monomer 1	Monomer 2	Drugs
Acrylamide	Maleic acid	Methylene blue
		
n-vinyl-2 pyrrolidone	Itaconic acid	Terbinafine hydrocloride
		

2.2. Preparation of hydrogels

For the preparation of Acrylamide/maleic acid hydrogels, acrylamide (AAm) weighted 1g was dissolved in 1ml aqueous solution of 0, 20, 30, 40, 50, 60mg maleic acid respectively. For the preparation of poly (n-vinyl-2 pyrrolidone/itaconic acid) hydrogels, 2ml n-vinyl-2 pyrrolidone was dissolved 1ml aqueous solution of 0, 60, 120, 180, 240 mg itaconic acid. In order to increase the amount of IA in the VP monomer and to be prepare different network properties having hydrogels, 2ml n-vinyl-2 pyrrolidone was dissolved 1ml aqueous solution of 60, 120, 180 and 240 mg itaconic acid and 0.25% (v/v, EGDMA/VP) cross-linking agent EGDMA added in the mixture. These solutions were placed in PVC straws of 3mm diameter and irradiated at different doses in air at ambient temperature in a ^{60}Co Gammacell 220 type γ irradiator.

Hydrogels obtained in long cylindrical shapes were cut into pieces of 2–3 mm long. Hydrogels were dried in vacuum oven at 315K to constant weight and subjected to soxhlet extraction with water as solvent Uncross-linked polymer and/or residual monomer were removed with this extraction from the gel structure. Extracted gels were dried again in vacuum oven at 315K to constant weight. Percentage gelation i.e. percentage conversion of monomers into insoluble network was based on the total weight of the VP or AAm and diprotic acid in the initial mixture. The amount of uncross-linked acid was determined by titration of extract against NaOH(0.05N) to phenolphthalein end point.

2.3. Swelling studies

Dried hydrogels were left to swell in a solution of desired pH (2–9), ionic strength I(0.01–0.20M), and temperature (4.0–65.0^{o}C). Swollen gels removed from the swelling media at regular intervals were dried superficially with filter paper, weighed and placed in the same bath. The measurements were continued until a constant weight was reached for each sample. The weight fraction of polymer in swollen gel w, was used to calculate the volume fraction ν_{2m} and equilibrium degree of swelling (EDS), Q, of the gel sample equilibrated in the buffer solution.

$$\nu_{2m} = \left[1 + \rho / \rho_w (w^{-1} - 1)\right]^{-1} \quad (1)$$

where ρ and ρ_w are the densities of swollen gel and water. The equilibrium degree of swelling (EDS) was defined as $Q = 1 / \nu_{2m}$.

The percentage mass swelling of hydrogels was calculated from the following relation;

$$S\%(m) = [\,(m_t - m_o)] \times 100 \quad (2)$$

where m_o is the mass of dry gel at the time 0 and m_t is the mass of swollen gel at time t.

2.4. Adsorption studies

2.4.1. Adsorption of proteins onto AAm/MA hydrogels

For the adsorption of bovine serum albumin (BSA) Fibrinogene and γ-globulin onto AAm/MA hydrogels, 0.1g of dry gel samples were first swollen to equilibrium and then placed in 5 mL solution of 1.0–8.0 mg/mL protein in phosphate buffer (pH2.0–8.0) and allowed to equilibrate for 12 h at different temperatures The amount of adsorbed protein was determined by Commassie Brilliant Blue binding method (Scopes, 1984).

2.4.2. Adsorption of enzymes onto P(AAm/MA) hydrogels

For the adsorption of enzymes onto P(AAm/MA) hydrogels, 0.1g of the dry gel was placed in 20 mL solution of 100 mgdL^{-1} α-amylase or invertase in phosphate buffer (10 mM, pH7.0) and allowed to equilibrate for 48 h at 25°C. The enzymatic activities of free and adsorbed α-amylase and invertase were determined by an enzyme reaction using starch as substrate and starch-iodine and Folin — Wu methods respectively (Yoo, et al., 1987).

2.4.3. Adsorption of drugs onto P(AAm/MA) and P(VP/IA) hydrogels

For the investigation of drug release behaviour of P(AAm/MA) and P(VP/IA) hydrogels prepared in this study, Methylene Blue(MB) and Terbinafine hydrocloride(TER-HCl) were used as model drugs. Dry polymer discs (2 mm thickness, 4 mm diameter) were loaded with MB by immersion into aqueous solution of MB (0.5 g/dL) at ambient temperature at pH7 for 2 days. The drug loaded hydrogels were stored for later evaluations in MB solution at ambient temperature.

In order to obtain pure TER-HCl firstly, 250 mg Lamisil was dissolved in 20 ml distilled water. After removing undissolved part the solution was dried in a vacuum oven at 315 K to constant weight. For the investigation TER-HCl adsorption capacities of hydrogels, dry polymer discs(2mm thickness, 4 mm diameter) were loaded with TER-HCl by immersion into aqueous solution of TER-HCl (0.16–3.75 mg TER-HCl/mL) at 4 °C for 2 days.

2.4.4. Controlled release of methylene blue and terbinafin hydrocloride from P(AAm/MA) and P(VP/IA) hydrogels

The controlled release of MB and TER-HCl from hydrogel matrices was measured after a MB or TER-HCl loaded, swollen gel was placed in a vessel containing 50 mL of phosphate buffer solution (0.1 mole/L) at 25°C and at a constant shaking rate. At various times aliquots of 3 mL were drawn from the medium to follow the MB and TER-HCl release and placed again into the same vessel so that the liquid volume was kept constant. MB release was determined spectrophotometrically using a Unicam 8715 spectrophotometer at 641 nm. and TER-HCl at 222 nm. The controlled release of non-specifically adsorbed MB and TER-HCl were followed at pH7 and pH6.0–2.0 were used for the controlled release of specifically bonded MB and TER-HCl from hydrogels. After release at pH2, the hydrogels were immersed in 0.1 mole/L HCl for 2 days to remove any remaining MB and TER-HCl in the gel system.

3. RESULTS AND DISCUSSION

3.1. Preparation of hydrogels

When pure acrylamide and N-vinyl 2-pyrrolidone monomer are irradiated with γ rays, polymerizaiton and cross-linked reactions take place, simultaneously. Total dose required for 100% gelation of AAm/MA hydrogels has been found to be 7 kGy when MA used in the range of 0.0–3.5 mol% in the initial mixture. Total dose required for the onset gelation was determined to be 3 kGy for pure VP and the sensitizing effect of water for the gelation of VP was very well demonstrated in our previous study [6]. In this study for the preparation of mechanically stable hydrogels the ternary mixtures of AAm/MA/H_2O and VP/IA/H_2O were irradiated to 25 kGy with gamma rays. Mole percentages of MA in P(AAm/MA)-1, P(AAm/MA)-2, P(AAm/MA)-3, P(AAm/MA)-4 hydrogels are 1.1, 2.2, 3.1 and 4.5 respectively and 100% gelation were observed for all systems at 25 kGy dose. Mole percentages of IA in the P(VP/IA)-1, P(VP/IA)-2, P(VP/IA)-3 hydrogels are 2.0, 3.0, 3.2 and percentage gelation are 87.7, 85.4 and 82.7% respectively. These results show that increasing mole percentage of MA and IA in the initial mixture increases the amount of MA and IA in the gel system but increases the amount of IA in the initial mixture causes a decrease in the extent of gelation from monomer to gel. These results indicate that IA acts as an effective chain transfer agent in the co-polymerization of VP.

In order to follow the effect of cross-linker on the gelation of VP/IA/H_2O mixtures and conversion of IA from monomer to gel, VP/IA/EGDMA/H_2O mixtures irradiated upto 32 kGy dose with gamma rays. Increase the amount of IA in the initial mixture increases the amount of IA in the gel system, but degrease in the gelation from momomer to gel. IA also show the chain transfer agent properties in the presence of EGDMA in the irradiation mixture. The mole percentages of IA in the 25 kGy irradiated P(VP/IA/EGDMA)-1, P(VP/IA/EGDMA)-2, P(VP/IA/EGDMA)-3 and P(VP/IA/EGDMA)-4 are 3.0, 6.0, 10.0 and 14% and mole percentages are 3.0, 6.0, 10, 14 respectively.

3.2. Characterizaiton of hydrogels

3.2.1. pH responsive characteristics

With the introduction of mono or diprotic acidic groups into the main chain, pH of the solution becomes even more important factor determining the swelling kinetics and equilibrium swelling value. The analysis of swelling for non-ionic hydrogels is mostly based on the theories of Flory and Rehner [7]. Their equations have been widely used to characterize the polymeric networks prepared either from polymers or mixture of monomers. The Flory Rehner model considers a situation where cross-links are randomly introduced in the dry stage. Peppas and Merrill [8] derived a model that accounts for the introduction of cross-links in the relaxed state as in the case of solution polymerization. Both of these models deal with neutral polymer networks. When the chains forming the network contain ionizable groups, the forces influencing swelling are greatly increased due to localization of charges within the hydrogel. The equilibrium swelling ratios attained are often an order of magnitude larger than those of neutral networks, as intermolecular coulombic, hydrogen bonding and polar forces are significantly present. By including the influence of ionic contribution to forces arising from mixing and elastic-retraction of chains, Brannon-Peppas and Peppas [9] have predicted the theoretical swelling behaviour of ionic hydrogels. In a recent article we have published equations to predict the swelling of hydrogel, with diprotic acid moieties as a function of polymer, solvent and solution based parameters [10]. The derivation of predictive equation were based on the approach of Peppas with the introduction of phantom network model of Erman and Flory since in the highly swollen state a real network exhibits properties closer to those of the phantom network model. The equation describing the total free energy change for an ionic network placed in a swelling agent is given as:

$$\Delta G = \Delta G_{mix} + \Delta G_{el} + \Delta G_{ion} \tag{3}$$

Which can be written in terms of chemical potentials by taking the derivative of each term respect to the number of molecules of swelling agent in the system. For the ΔG_{mix} term, Flory -Huggins relationship: for ΔG_{el}, respective equation valid for a phantom network is substituted. The ionic contribution is calculated by using the equation derived by Brannon-Peppas and Peppas (1991) by considering the primary (K_{a1}) and secondary (K_{a2}) of diprotic acids, the weight fraction of ionizable polymer in the network (X), the ionic strength of the medium (I) and the degree of ionization (i). Following is the final form of the equation used in the prediction of swelling behaviour of diprotic acid containing hydrogels [10]:

$$\left(\frac{\left[2K_{a1}K_{a2}+10^{-pH}K_{a1}\right]}{2\left[(10^{-pH})^2+10^{-pH}K_{a1}+K_{a1}K_{a2}\right]}\right)^2\left(\frac{V_1\nu_{2m}^2X^2}{4I\bar{v}^{-2}}\right)=\ln(1-\nu_{2m})+\nu_{2m}+\chi\nu_{2m}^2+ \tag{4}$$

$$\frac{(1-2/\phi)V_1\nu_{2r}^{2/3}\nu_{2m}^{1/3}}{\bar{v}\overline{M}_c}$$

where, V_1 is the molar volume of swelling agent, ν_{2m} is the polymer volume fraction in the equilibrium-swollen system, $\bar{v}$ is the specific volume of the polymer, ν_{2r} is the polymer volume fraction in the relaxed state (that means after cross-linking but before swelling), χ is

the Flory-Huggins polymer-solvent interaction parameter, ϕ is the functionality at the cross-linking site, $\overline{M}_c$ is the number average molecular weight between consecutive cross-links.

The above equation has enabled us to theoretically predict swelling behaviour of hydrogels containing diprotic acid moieties under a number of polymer-based parameters, solution property parameters and polymer-solvent combination type parameters. By rearranging equation 2 it was also possible to determine simultaneously the average molecular weight between cross-links, and the polymer-solvent interaction parameter from the equilibrium swelling data. Figs 1–2 represent the equilibrium degree of swelling (EDS) of VP/IA and AAm/MA hydrogels at 25 ^{o}C in phosphate buffer solution from pH = 2 to 9 at fixed ionic strength of I = 0.1M. Consistent with poly-electrolytic behaviour swelling of hydrogels was found to increase with pH. The solid curves in these figures represent the theoretical swelling curves of hydrogels. The construction of theoretical swelling curves are explained in detail in our previous work[10]. The experimental data points and theoretical curves are in very good accordance as it is seen from these figures. In all compositions maximum extent of swelling were reached at pH = 7, this being due to the complete dissociation of acidic groups of itaconic acid and maleic acid at this pH value. The first and second dissociation constants of IA and MA are pK_{a1} = 3.85, pK_{a2} = 5.44 and pK_{a1} = 1.85, pK_{a2} = 6.06 respectively [11]. Since the two dissociation constants for IA are rather close, the consecutive swellings at around these pH values overlap and only single-step swelling versus pH curves are observed in Fig. 1. For MA containing hydrogels however, due to large difference in pK_a values, swelling takes place in stepwise manner as shown in Fig. 2. The swelling shows sudden increases at the pH values around corresponding pK_a values.

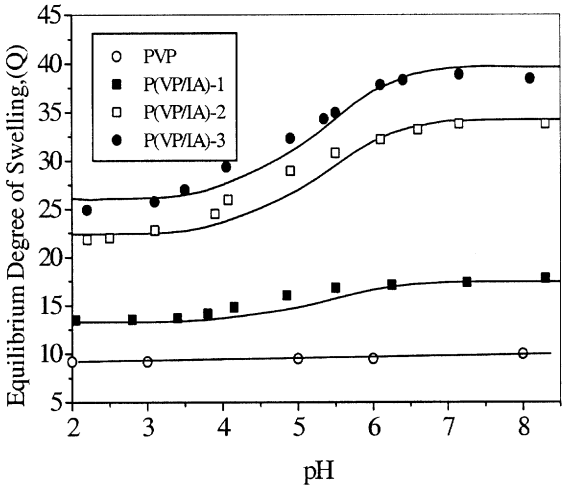


FIG. 1. Effect of pH on the EDS values of P(VP/IA) hydrogels.

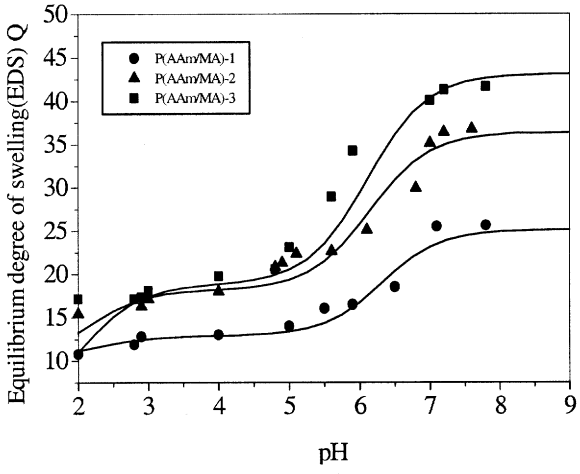


FIG. 2. Effect of pH on the EDS values of P(AAm/MA) hydrogels.

The change in equilibrium degree of swelling with pH has also been evaluated for the determination of average molecular weight between cross-links and the polymer-solvent interaction parameter [12].

3.2.2. Ionic strength-responsive characteristics

The effect of external ionic strength on the equilibrium degree of swelling of VP/IA co-polymeric hydrogels at 25°C is given in Fig. 3. Solution pH was fixed at 3.0 and 8.0 to obtain minimum and maximum swelling for each gel, thereby allowing ionic strength effects to be

observed most clearly. An increase in ionic strength generally decreased the swelling because the difference in concentration of mobile ions between the gel and solution is reduced causing a decrease in the osmotic swelling pressure of these mobile ions inside the gel. It is seen from Fig. 3 that with increasing ionic strength of the swelling solution, the EDS values show a continuous decrease and this effect becomes more pronounced at pH8 than pH3.

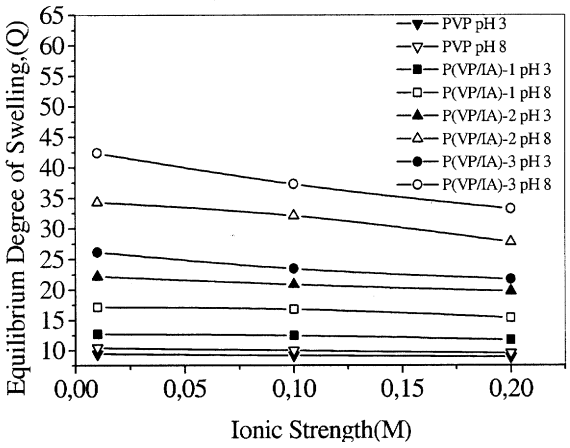


FIG. 3. Effect of ionic strength on the EDS value of P(VP/IA) hydrogels.

The effect of relative amounts of IA in the gel system can be clearly seen by comparing the ionic strength dependence of the equilibrium degree of swelling of P(VP/IA)-3. The changes were observed most clearly at fully ionized state (pH8). When the ionic strength of the medium was increased from 0.01 to 0.20M the equilibrium degree of swelling was observed to decrease by 10% for P(VP/IA)-1 and 27% for P(VP/IA)-3 at pH8.

3.2.3. Temperature-responsive characteristics

Figs 4–5 represent the influence of temperature on the equilibrium degree of swelling of P(VP/IA) hydrogels in phosphate buffer solution at pH3 and pH8 respectively. Equilibrium degree of swelling of non-ionic PVP hydrogels in pH3 and pH8 phosphate buffers is also included in Figs 4–5 for comparison. As can be seen from these figures P(VP/IA) hydrogels exhibit large continuous changes in water content as a function of temperature. These changes are due to volume collapse upon warming. It has been shown that a number of hydrogels demonstrate nearly continuous volume transition and associated phase transition from a low temperature, highly swollen gel network to a collapsed, high temperature phase near their critical points [15]. The phase transition is analogous though fundamentally distinct from the lower critical solution phase transition (LCST). Güner and Ataman [16] have found that uncross-linked PVP exhibits LCST between 35–40°C depending on the salt type in the aqueous solution, thus the polymeric gels rich in VP monomer can be expected to exhibit volume collapse upon warming.

However, due to slight decrease of EDS of PVP hydrogel a sharp volume transition was not observed at both pH values in the temperature range of 4–65°C. The EDS value of PVP hydrogels are 10.1 and 11.0 at 0°C in the pH3 and pH8 buffer solutions respectively. These values decreased slightly with a rise in temperature up to 65°C to 8.9, and 9.3 at pH3 and 8 respectively. In this temperature range the desorption of water from the hydrogel becomes generally difficult, suggesting that water remaining in the hydrogel mostly consists of the binding water, referred to as uncontrollable water. However, water adsorbed in the P(VP/IA)

hydrogels is of both controllable and uncontrollable water which, controllable water had a characteristic swelling-deswelling ability with change in temperature closely related to the IA content in the gel system. From a comparison of Figs 4–5 it has been observed that the temperature range where the volume change is greatest (the transition region) is slightly shifted to higher temperatures and is broadened due to more continuous transition as the gel ionization increases at higher pH. The slight shift may be attributed to the lower transition temperature of poly (itaconic) acid as compared to that of PVP.

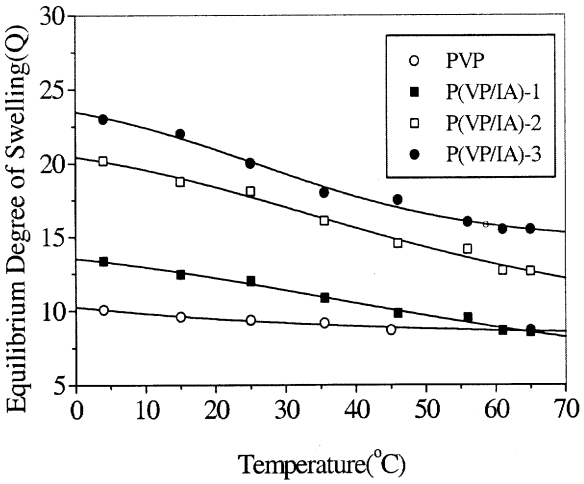


FIG. 4. Effect of temperature on the EDS value of P(VP/IA) hydrogels at pH3.

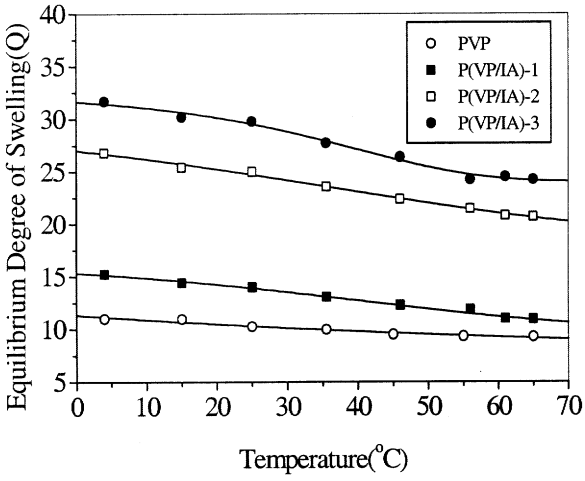


FIG. 5. Effect of temperature on the EDS value of P(VP/IA) hydrogels at pH8.

Other external stimulis such as the type of the buffer and the presence of metal ions on the swelling behaviour of diprotic acid containing hydrogels are explained in detail in our previous studies [17–18].

3.3. Adsorption of biomolecules onto diprotic acid containing hydrogels

Environment sensitivity or control of swelling ability of poly-electrolyte hydrogels under a variety of external conditions make them ideal adsorbents for removal, adsorption on enrichment of some water soluble agents such as proteins, biomolecules. Novel applications of hydrogels in bioseparation or as intelligent artificial systems have been widely presented in the recent literature [19]. The hydrogels based on poly-electrolyte structures and synthesized via irradiation of aqueous solutions of acrylamide and N-vinyl 2- pyrrolidone with small quantities of maleic or itaconic acids are regarded as systems with potential immobilization, chelating and adsorptive for various bioapplications. Investigation of protein adsorption behaviours of polymers is very important for the identification of biocompatibility of material. Generally, high albumin and lower fibrinogen and globulin adsorption is preferred for the high biocompatibility of biomaterials. The factors effecting on the protein absorptive properties of these hydrogels has been presented below some selected model proteins and enzymes.

3.3.1. Adsorption of bovine serum albumin (BSA) onto P(AAm/MA) hydrogels

3.1.1.1. Effect of pH and Temperature

Irrespective of the pH of the medium, temperature and ionic strength, it has been observed that practically no BSA was adsorbed on pure PAAm hydrogels. It is the presence of MA moieties which make these gels potential adsorbents for BSA. The effect of pH on BSA

adsorption onto P(AAm/MA) hydrogels was investigated by changing the pH of the solution between 2 and 8 by using phosphate buffer. The ionic strength of the buffer solution was kept at 0.01M and the initial concentration of BSA was 4.0 mg/mL The variation of adsorbed BSA per gram dry hydrogel systems is given in Fig. 6. It is generally observed that the maximum adsorption of BSA to non-ionic systems is around at the isoelectric point of the protein, which is approximately pH5.0. However, the maximum adsorption for P(AAm/MA) was observed slightly below isoelectric point(around pH4.7) This shift was attributed to the increase of possible electrostatic interactions between positively charged BSA and negatively charged polymer chains. It is well known that the protein molecules are charged positively below the isoelectric point and the percentage total ionization of MA is approximately 50% at pH5. The first and second dissociation constants of MA are pK_{a1} = 1.85 and pK_{a2} = 6.06 respectively. Although, the dissociation of MA increases at high pH values, variation of the charge on protein molecules and conformational changes cause a decrease in the amount of adsorbed BSA.

For the investigation of effect of temperature on the BSA adsorption onto P(AAm/MA) hydrogels, adsorption measurements were carried out at 4, 25, 37 and 45 °C. As can be seen from Fig. 7 adsorption of BSA generally is not sensitive to the temperature changes above 25°C. The increases in the amount of adsorbed BSA with the temperature increase from 4 to 25 °C can be attributed to the increase of the chain mobility of protein molecules and increase in the dissociation of MA in the gel systems at 25 °C. pH4.7 and 25 °C are selected as working temperature and pH for the investigation of other external stimuli to BSA adsorption onto hydrogels.

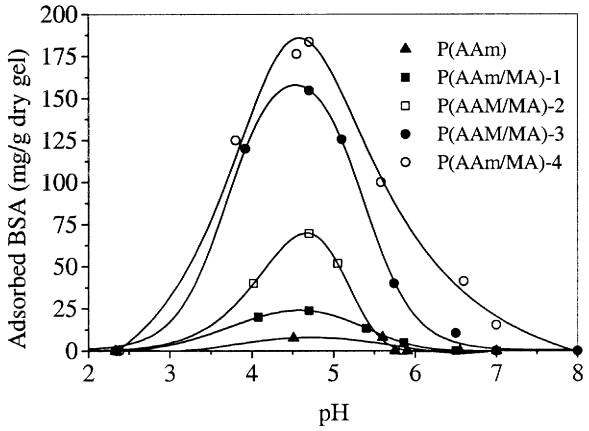


FIG. 6. Effect of pH on the BSA adsorption on the BSA capacity of P(AAM/MA) hydrogels.

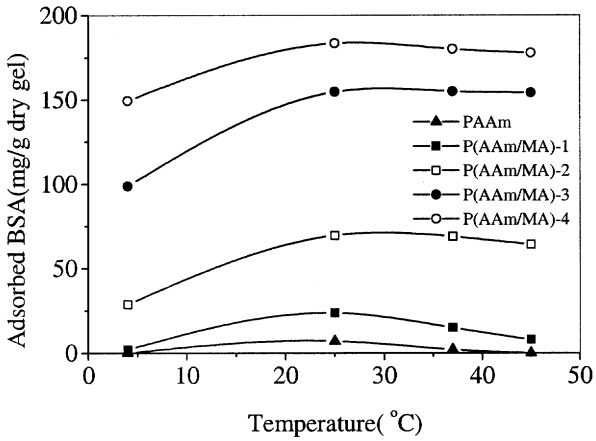


FIG. 7. Effect of temperature adsorption capacity of P(AAM/MA)hydrogels.

3.3.1.2. Effect of protein concentration and ionic strength on the adsorption

The effect of initial concentration of BSA on the adsorption capacity of P(AAm/MA) hydrogels at 25 ºC and 0.01 M phosphate buffer solution is given in Fig. 8. The mass of adsorbate per unit mass of adsorbent q_e, can be calculated from the following equation:

$$q_e = ((C_i - C)/m) \times V_t \quad (5)$$

where q_e is in mg adsorbate per gram dry adsorbent, C_i and C are the initial and equilibrium concentration of solution of adsorbate in mg/mL, V_t is volume of solution treated in mL and m is the mass of dry adsorbent in g [20-21]. As can be seen from Fig. 8 increase in the content of ionic co-monomer MA in the gel system increased q_e values at all initial BSA concentrations due to the specific interactions between the ionized polymer and protein molecules. Fig. 8 also shows that the BSA adsorption capacity of all hydrogel systems first increase with an increase in the initial BSA concentration and then reaches a plateau value (at about 4.0 mg/mL initial BSA concentration). The effect of external ionic strength on the BSA adsorption was evaluated by repeating the adsorption experiments described above by using 0.05 and 0.10 M phosphate buffer. The variation of adsorbed BSA with the C_i at 0.05 M phosphate buffer is given in Fig. 9. As can be seen from a comparison of Figs 8 and 9 an increase in the ionic strength decreased the q_e values. This may be explained by the adsorption of more ions to the BSA molecules at higher ionic strength. These bindings that causes conformational change in the protein structure, also increase the solubility which then lead to less adsorption.

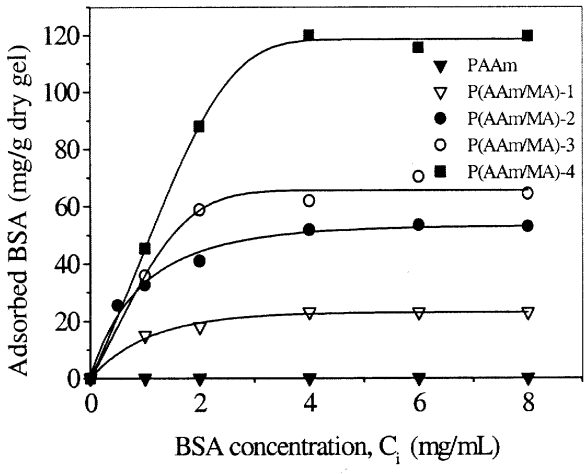


FIG. 8. Effect of initial concentration of BSA on its adsorption at 0.01 M ionic strength, pH4.7 and 25 °C. strength, pH4.7 and 25 °C.

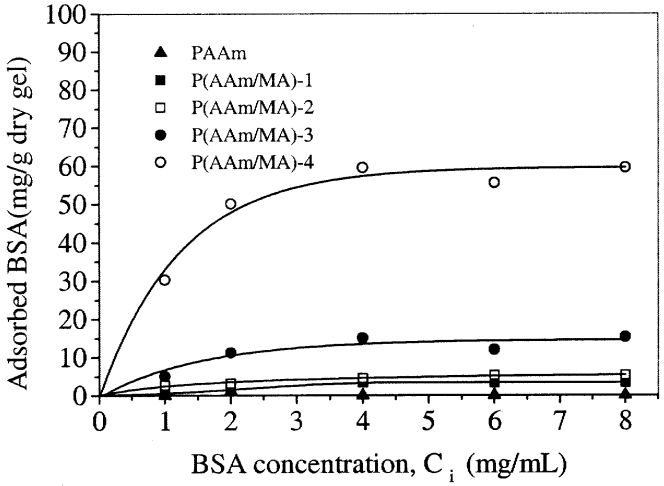


FIG. 9. Effect of initial concentration of BSA on its adsorption at 0.05 M ionic strength, pH4.7/ 25°C.

3.3.1.3. Effect of cations on the BSA adsorption

In order to investigate the effect of some cations on BSA adsorption in the gel systems prescribed, the adsorption experiments were followed in the presence of 0.01 M and 0.1 M NaCl and $CaCl_2$ solutions at pH4.7 and 25 ºC. The extent of the adsorbed BSA is given in Table I. Zero absorption was observed at both 0.1 M NaCl and $CaCl_2$ solutions. Comparison of Table I with Fig. 8 has shown that, the adsorption of BSA onto P(AAm/MA) hydrogels is very sensitive to the external ions in the adsorption medium. This can be explained by the adsorption of more ions to BSA molecules at high concentrations. The higher BSA adsorption capacities were obtained with $Na^{+,}$ relative to those observed with Ca^{2+}. This may be due to higher binding affinity between the BSA molecules and Ca^{2+} ions. It has been shown that the binding of cations and anions may cause conformational changes in the albumin molecules which lead to less adsorption to the solid surfaces [22].

TABLE I. THE EFFECT OF NACL AND $CACL_2$ ON BSA ADSORPTION

Gel system	Adsorbed BSA (mg/g dry gel)	
	NaCl (0.01 M)	$CaCl_2$ (0.01 M)
P(AAm/MA)-1	10.6	0.00
P(AAm/MA)-2	62.7	0.28
P(AAm/MA)-3	86.2	14.9
P(AAm/MA)-4	116.2	53.4

3.3.2. Adsorption of fibrinogen and γ-globulin onto P(AAm/MA) hydrogels

For the investigation of fibrinogen and γ-globulin adsorption onto P(AAm/MA) hyrogels, adsorption studies were followed at different pH values, temperatures and environmental conditions irrespective of the pH of the medium, temperature and ionic strength it has been observed that no fibrinogen and γ-globulin were adsorbed onto AAm and P(AAm/MA) hydrogels in our detection limits. If there is any adsorption on the hydrogels surface the extent of this value must be in ng level per gram gel. These abrupt changes towards protein molecules was attributed the variation of hydrophilicity and molecular weight of protein molecules. As known fibrinogen and γ-globulin are more hydrophilic molecules than albumin. In many studies is has been observed that hydrophobic polymers shows very high affinity fibrinogen and globulin than hydrophilic polymers[23]. Higher molecular weight of fibrinogen and γ-globulin than albumin molecules is another diminishing factor for protein absorption due to decrease of diffusion into hydrogel structure. Representative cross-section Scanning Electron Microscope pictures of P(AAM/MA) hydrogels are given in Fig. 10. As can be seen hydrogels have very uniform and small pore size distribution which most probably these small pores not allow the permeation of higher molecular weight protein molecules into hydrogel structure. High albumin and low fibrinogen and globulin adsorption is very well behaviour for P(AAm/MA) hydrogels when they want to be use as biomaterial in biomedical applications.

3.3.3 Adsorption of α- amylase onto P(AAm/MA) hydrogels

For the investigation of enzyme adsorption behaviour of diprotic acid containing hydrogels prepared in this project, α- amylase and invertase were used as a model enzymes The experimental results of amylase adsorption are given below. The results of invertase adsorption will be published separately. The total amount of adsorbed enzyme depends on the

mole percentage of MA in the gel systems as shown Fig. 11. Increasing the amount of MA from 1.1 to 4.5 mol-% causes a 2- fold increase in the adsorbed enzyme content. The reason of this increase was attributed to the increase in specific interactions between enzyme molecules and ionized MA in the hydrogel and free volume available for diffusion. Various binding capacities and preserved activities are given in literature for α-amylase immobilized systems. When adsorption was achieved chemically onto polystyrene and silica based support, coupling capacities and preserved activities are reported as 3 and 29 mg/g support and 7 and 40%, respectively; on dextran and cellulosic supports these values are given as 25 and 90 mg/g support and 25 and 67%, respectively [24]. As shown from Fig. 14 coupling capacity of P(AAm/MA) hydrogels is lower than most of the data reported in the literature However, preserved activity of adsorbed enzyme on the P(AAm/MA) hydrogels was found to be much higher than respective values.

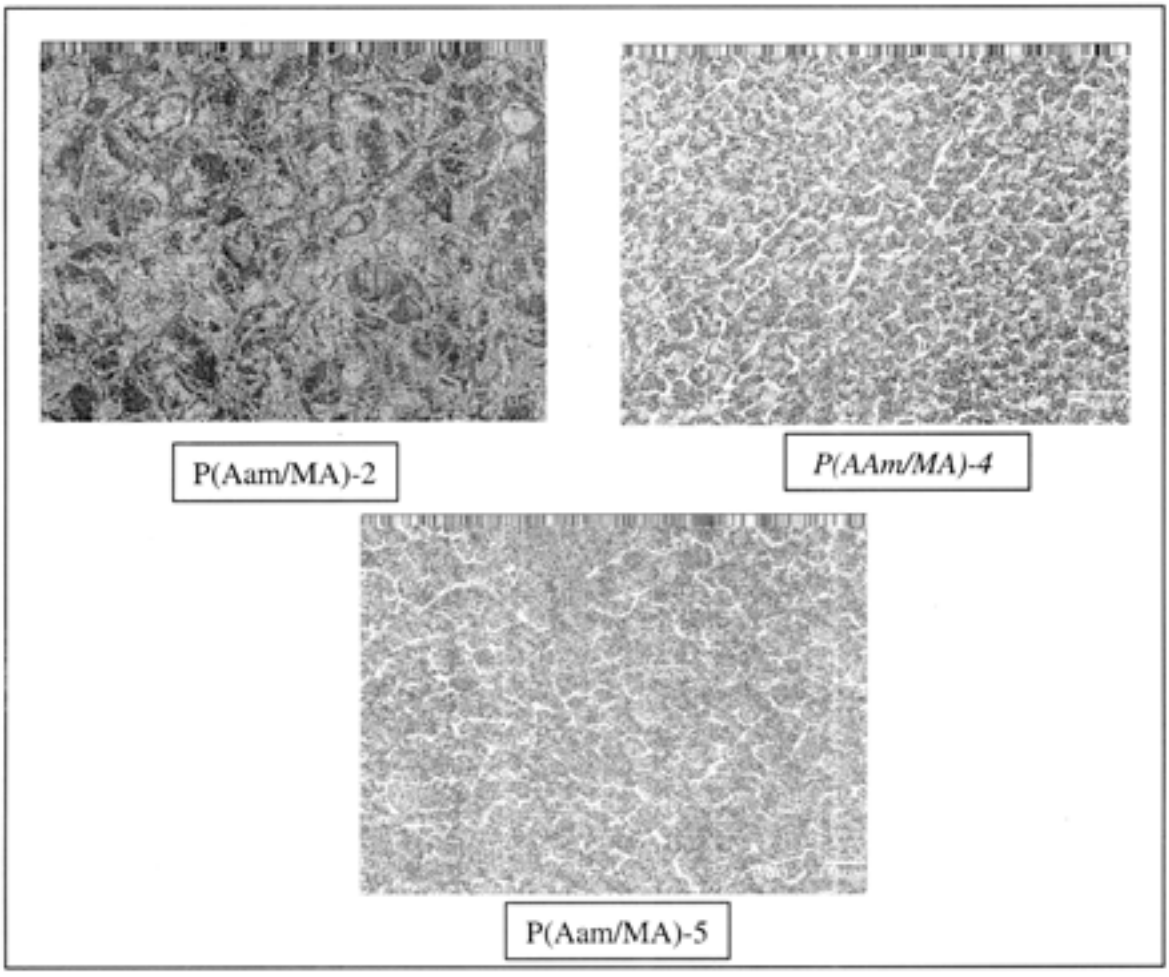


FIG. 10. Scanning Electron Microscope photographs of P(AAm/MA) hydrogels.

3.3.3.1 Parameters effecting enzyme activity

The activities of free and adsorbed α amylase were determined by measuring the absorbance of starch solution at 600 nm when the reaction was carried out at various pH, temperature, substrate concentration and storage times. The pH for maximum substrate conversion was found to be 7.5 for free α-amylase and 6.0 for adsorbed α-amylase irrespective of the MA content in hydrogel. The relative changes of substrate conversion with

pH of the solution is given in Fig. 12 for the free and adsorbed enzyme systems. The trends of the curves are similar for both cases. The shift in the optimum pH toward a lower value upon adsorption might be due to the difference in the hydronium ion concentration in the vicinity and in the bulk solution. It is known that poly-ionic matrices cause partitioning of proton between the bulk phase and the enzyme microenvironment [25]. The effect of temperature on the activity of free and adsorbed enzyme is given in Fig. 13. As can be seen from the figure, while the optimum temperature is 40°C for the free enzyme this value shifts to 50°C for adsorbed systems, the optimum temperature of the adsorbed systems is not however changed significantly with increasing MA content in the gel system. This can be explained by the creation of conformational limitations on the enzyme moment as a result of formation of interaction between the enzyme and the matrix. The increase in the optimum temperature may arise from the change of the conformational integrity of the enzyme structure upon binding to the material [25].

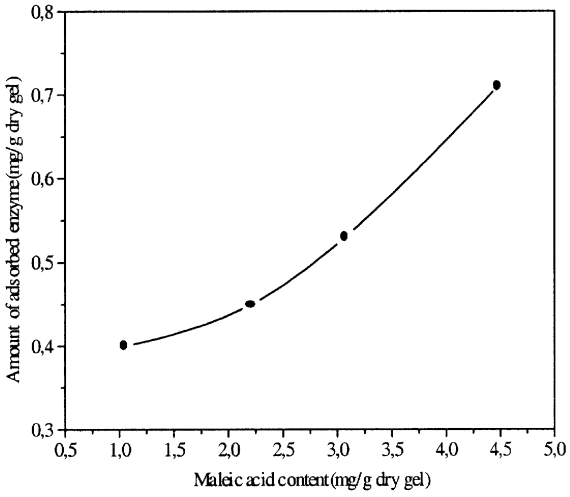


FIG. 11. The variation of amount of adsorbed enzyme with MA content in the gel systems.

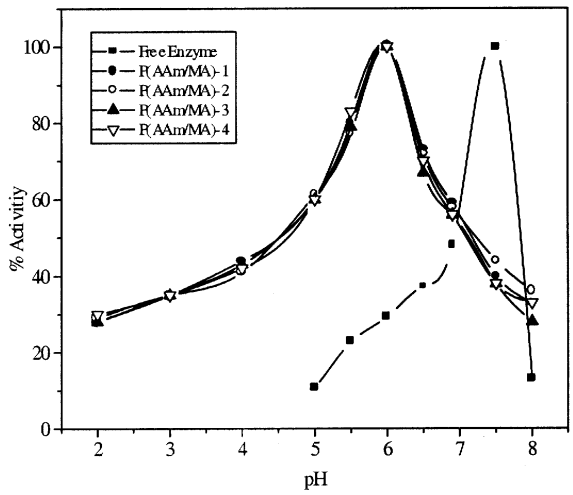


FIG. 12. Effect of pH on free and adsorbed enzyme activity.

3.3.3.2 Storage stability of free and adsorbed α amylase

Generally, enzymes are not stable in solutions and during storage their activities decrease gradually with time. When α-amylase solution was stored at 4°C its activity was lost almost completely even after a storage time of 20 days. However, adsorbed enzyme retained 47–59% of original activity of the free enzyme depending on the amount of MA in the hydrogels, Fig. 14. With increasing MA content, stability of adsorbed enzyme showed significantly increase especially at longer storage periods. This behaviour was attributed to the increase of specific interactions between enzyme molecules and ionized hydrogel.

3.3.3.3 Kinetic parameters of free and adsorbed α amylase

The activities of free and adsorbed enzyme for various substrate concentrations are plotted in the form of Lineweaver-Burk plot for the determination of kinetic parameters, K_m and V_m. As known V_{max} defines the highest possible velocity when all the enzyme is saturated

with substrate, therefore, this parameter reflects the intrinsic characteristics of immobilized enzyme, but may be affected by diffusional constraints. K_m is defined as the substrate concentration that gives a reaction velocity of ½ V_{max}. This parameter reflects the effective characteristics of the enzyme and depends upon both partitioning and diffusional effects [26] V_{max} values were calculated as 1.67×10^{-3} g $dm^{-3}.min^{-1}$ for free enzyme and increased from

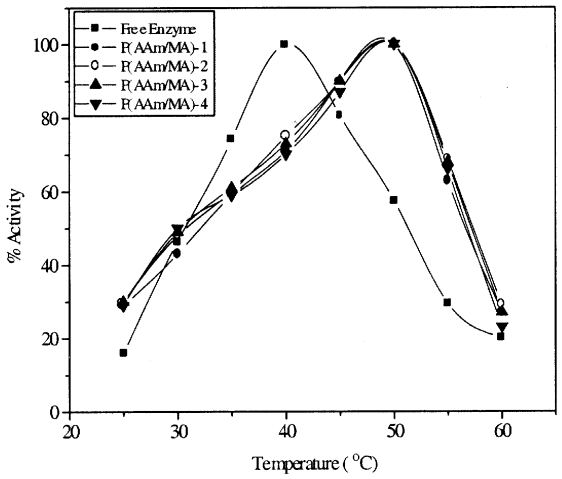


FIG. 13. Effect of temperature on free and adsorbed enzyme activity.

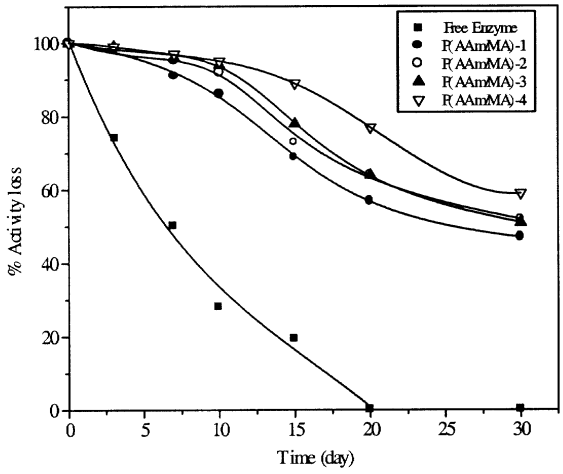


FIG. 14. Storage stability of free and adsorbed α-amylase at 4 °C.

1.63×10^{-3} to 1.96×10^{-3} for adsorbed enzyme with increasing amount of MA in the gel system. K_m value for free enzyme was found to be 2.51 g dm^{-3}. However, this value for adsorbed enzyme was increased from 12.3 to 12.9 with increasing MA content in the gel system. As can be seen from K_m data there is approximately 5-fold increase in this value with adsorption. This can be explained by the diffusional limitations of the substrate or due to an interaction between the substrate and the support or the conformational changes of the enzyme resulting in a lower possibility to form a substrate-enzyme complex.

All these results show that diprotic acid containing hydrogels can be use for the isolation and enrichment of enzymes as well as albumin.

3.4. Diprotic acid containing hydrogels and drug delivery systems

During the last decade many different kind of polymeric systems are proposed as drug carrier systems. One of these systems is poly-electrolyte polymers. Yoshida, et al. [27] synthesized the thermo and pH responsive acryloyl-L- proline ether ester(A-ProOEt) co-polymers with methacryloyl-glycine(MA-Gly) and methacryllic acid to be design a novel biofunctional gel for application in colon delivery systems. Recently, Nakamae, et al. [28] synthesized phosphate group containing metacryloyl-oxyethyl dihydrogen phosphate/N-isopropyl acrylamide co-polymeric hydrogels for the delivery of positively charged enzymes. They recommended that this type of pH sensitive hydrogels should be ideal for delivering drugs to the small intestines, while avoiding release in the stomach. For the investigation of cationic drug adsorption and release behaviour of diprotic acid containing hydrogels prepared in this study, firstly MB was used as the model drug. The second part of the release studies has been performed with commercial drug terbinafine hydrochloride(TER-HCl). The experimental results are explained in details below.

3.4.1. Adsorption and controlled release behaviour of methylene blue

The amounts of total (specific and non-specific) MB uptake into one gram of dry PVP and P(VP/IA) hydrogels are given in Fig. 15. As can be seen from the figure the amount of total MB taken increased rapidly after an IA content of 2.0 (mole %). The reason of this increase was attributed to the increase of free volume available for diffusion and specific bonding of positively charged drug to completely ionized hydrogel.

In order to determine the amount of non-specific adsorbed MB, hydrogels are placed in pH7 phosphate buffer solution. Fig. 16 shows the release kinetics of non-specific adsorbed MB from PVP and P(VP/IA) hydrogels. While 99% of MB was released from PVP hydrogels this value decreased to 42.0% with increasing IA content in the gel system. The percentage release of MB at pH7 was calculated from the following equation

$$\% \text{ Release} = \frac{w_t}{w_{total}} x100 \qquad (6)$$

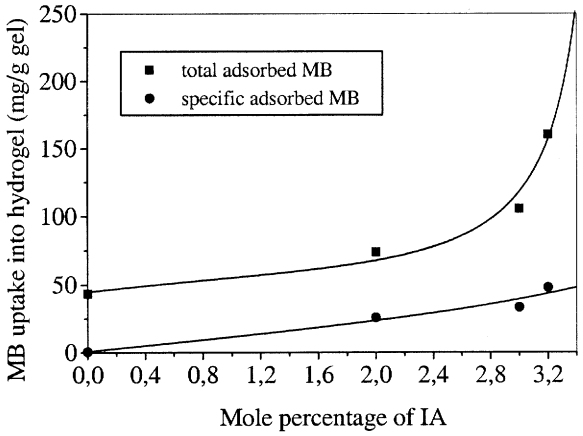


FIG. 15. The variation of total and specific adsorbed adsorbed MB with IA content in the gel system.

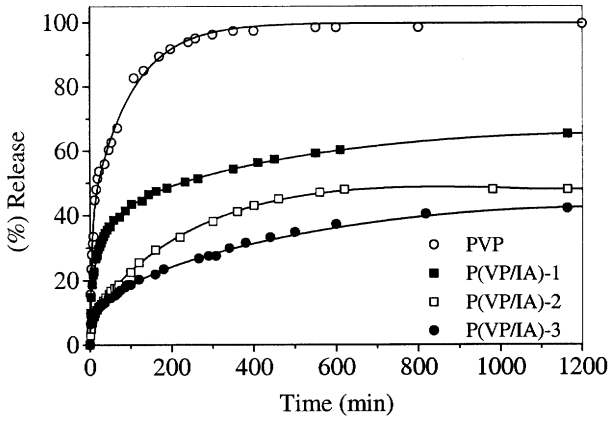


FIG. 16. Release of non-specific MB from P(VP/IA) hydrogels at pH7.

where, w_t is the weight of released MB at time t and w_{total} is the total weight of specific and non-specific adsorbed MB in the gel system. The incomplete release of MB from P(PV/IA) hydrogels at pH7 was expected to be due to binding of the cationic MB to the polymer. The difference between the total and non-specific adsorbed MB is therefore taken to be equal to the amount of specific adsorbed MB in the hydrogel. The variations of specific adsorbed MB with IA concentration are plotted in Fig. 15.

Fig. 16 also shows that the release rate was higher for pure PVP hydrogel than P(VP/IA) hydrogels and the release rate decreased with the increase of IA content in the gel system This can be explained by the increase in the diffusional path due to high swelling of P(VP/IA) hydrogels.

The controlled release of specific adsorbed MB from P(VP/IA) hydrogels was investigated primarily at pH5.5. The drug release was followed until equilibrium and then hydrogel was transferred into MB free buffer at pH4 and after reaching new equilibrium to pH2. The percentage of release of MB with time at each hydrogel system is given in Figs 17–

19. The percentage releases of specific adsorbed MB at pH5.5, 4.0 and 2.0 were calculated from the following equation

$$\% \text{ Release of specific adsorbed MB} = \frac{w_t}{w_{sp.}} x100 \quad (7)$$

where, w_t is the weight of released MB at time t and $w_{sp.}$ is the total weight of specific adsorbed MB in the gel system. As can be seen from Figs 17–19 the release rate decreased at pH5.5 and pH4 with increasing IA content in the gel system due to an increase of the diffusion path. Approximately 10.0% and 5.5% differences in the drug release were observed at 400 min between P(VP/IA)-1 and P(VP/IA)-3 hydrogels at pH5.5 and pH4.0, respectively. However, the release of MB at pH2 was opposite in trend to the release at pH5.5 and pH4.0. The release rate increased with increase of IA content in the gel system. The percentage of released MB from P(VP/IA)-3 hydrogel is approximately 14.0%, higher than that of P(VP/IA)-1 hydrogel at 400 min of release. As is known [29] the release of the drug may be influenced for two reasons. The first is the diffusion path of the drug in the network. As discussed previously a decrease on the cross-linking density causes to increase the swelling capacity and diffusion path. The second can be explained by the driving force concept for drug diffusion. The drug concentration in the gel defines the driving force for drug diffusion, which is due to the release rate increasing with the drug loading. The amount of drug concentration in P(PV/IA)-3 hydrogel is approximately 2.6 fold higher than that in P(VP/IA)-1 hydrogel when the release was completed at pH4.0. The Hydrogels were placed in pH2.0 buffer solution, hence, the release of MB from P(PV/IA)-3 hydrogel is much faster than P(VP/IA)-1 hydrogel.

Figs 17–19 also indicated that, the percentage releases of specific adsorbed MB for each hydrogel at individual pH values were approximately of the same magnitude. This could be explained by the same extent of ionization or protonization of hydrogel at each pH value. Mathematically, it is known that, being independent of the diprotic acid concentration in the gel system, the percentage ionization is constant for each hydrogel at a certain pH. Variation of the specific adsorbed MB (%) and ionization with pH for P(VP/IA) hydrogels are given in Fig. 20. It was noticed that the adsorption of the drug depends on the pH and percentage of ionization obviously and compared with the theoretically plotted ionization curve of IA there is no drug release in pH 7 buffer which is due to the physically bonding of the positively charged MB to completely ionized hydrogel. On the other hand, 100% release was not observed at pH 2 as expected from theoretically plotted ionization curve (dashed line). The complete release of MB was observed at pH 1. The theoretical ionization versus pH curve in Fig. 20 is plotted by using the K_{a1} and K_{a2} values of pure itaconic acid. As can be seen from Fig. 20 there is a significant difference between theoretically plotted ionization curve and adsorbed MB (%) versus pH curves. This change may be due to the changes of the dissociation constants of IA in the gel/drug-phosphate buffer system.

In this study the preparation of P(VP/IA) hydrogels and their drug release behaviours have been investigated. It has been found that the specific and non-specific adsorption capacity of hydrogels both increase with increasing IA content in the gel system. This has been explained due to the incorporation of more specific acidic groups into the network and consequent higher swelling capacity of gels. The release studies show that one of the basic parameters affecting the drug release behaviour of P(VP/IA) hydrogels is the pH of the solution. To conclude, the hydrogels prepared in this study can be considered as potential

carriers for the drug delivery systems and may be used as especially local therapeutic applications of cationic drugs.

4.4 Adsorption and controlled release behaviour of terbinafine hydrochloride

Terbifine or terbinafine hydrochloride(TER-HCl) is a topically and orally active allyl-amine antifungal agent which appears to act by preventing fungal ergesterol biosynthesis via specific and selective inhibition of fungal squale oxidase[30]. In standard *in vitro* susceptibility tests terbinafine has demonstrated activity against a wide range of dermotophyte filamentous, dimorphic and dematiaceous fungi as well as yeasts. Topical terbinafine has been effective in approximately 80% of patients with cutaneous candidiasis with a mycrological cure rate of 93%. Oral terbinafine is ineffective in the treatment of pityriasis versicolor, but topical therapy with the drug produced clinical and mycological cure in approximately 80% of patient.

The usual duration of treatment for fungal or yeast skin infection has been 2 to 4 weeks (topical therapy) or 3 to 6 weeks (oral therapy) but shorter courses of topical terbinafine (1 to 2 weeks) were as effective as standard- duration therapy in dermatomycoses [31].

Due to higher efficiency of topical terbinafine than other topical antifungal drugs for many fungal or yeast skin infection and lower drug requirement are the main advantages of this drug for using transdermal drug delivery systems (TDDS).

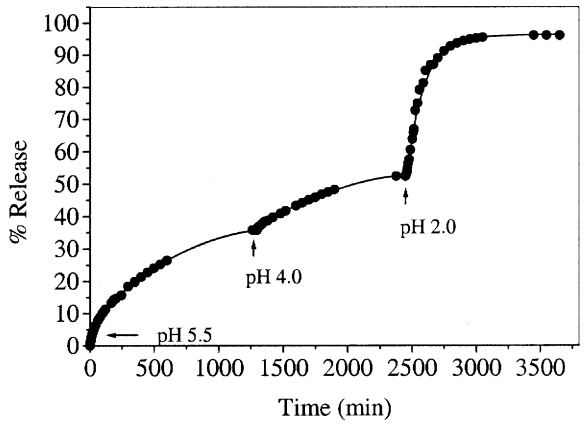


FIG. 17. Release of specific adsorbed MB from P(VP/IA)-1 hydrogels.

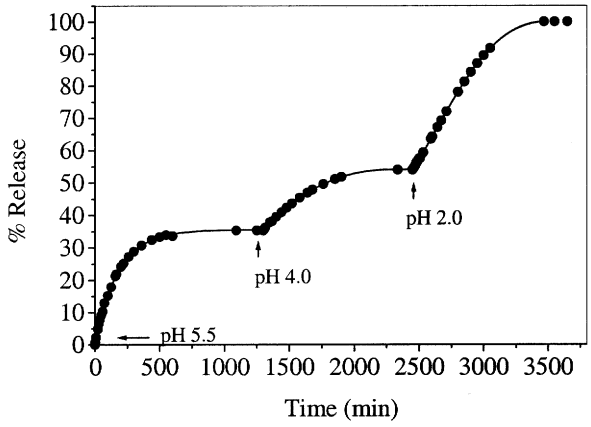


FIG. 18. Release of specific adsorbed MB from P(VP/IA)-2 hydrogels.

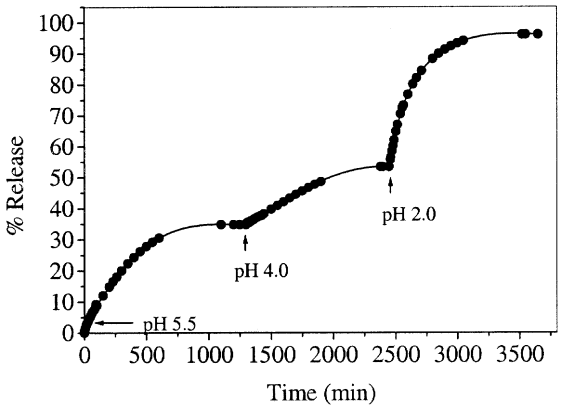


FIG. 19. Release of specific adsorbed MB from P(VP/IA)-3 hydrogels.

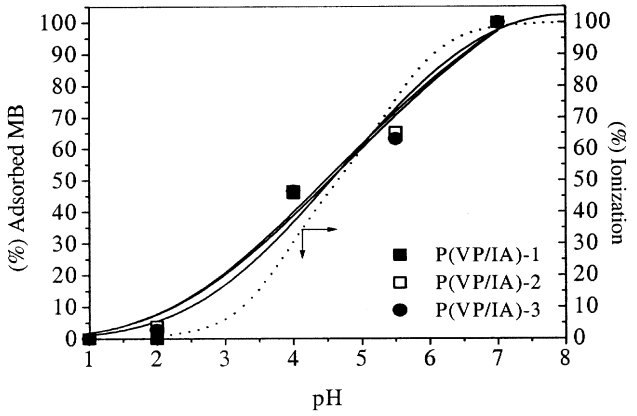


FIG. 20. Variation of specific adsorbed MB (%) and ionization with pH for P(VP/IA) hydrogels.

For the investigation of cationic drug adsorption behaviour of PAAm and P(AAm/MA) hydrogels prepared in this study, hydrogels were firstly swelled in TER-HCl solution at pH4.0 in concentration range 0.16–0.80 mg/mL. The consideration for selecting the particular drug concentration and pH is the solubility of TER-HCl in aqueous solution. The maximum solubility of TER-HCl was found to be 0.80 mg/mL water and the pH of this solution was 4.0. So the drug loading into hydrogels were investigated in this concentration range.

The amount of total (specific and non-specific) adsorbed TER-Cl into one gram of dry gel at different drug concentrations are given in Fig. 21. As can be seen from the figure the amount of total TER-HCl taken increased with increasing MA content and initial drug concentration. The reason of this increase was attributed to the increase of free volume available for diffusion and specific bonding of positively charged drug to partially ionized hydrogel.

The effect of initial concentration of TER-Cl solution on the adsorption capacities of hydrogels is also shown in Fig. 21; increase in the drug concentration in the swelling medium increased the amount of adsorbed drug as observed in many adsorption studies [32]. In order to obtain adsorption isotherms of hydrogels the mass of adsorbate per unit mass of adsorbent (q_e) was plotted versus equilibrium concentration of drug(C). q_e values can be calculated from the equation 5. As can be seen from Fig. 22 increase in the content of ionic co-monomer MA in the gel system increased q_e values at all initial drug concentrations due to the specific interactions between the ionized polymer and drug molecules and also increase of swelling value. The L and S type curve in PAAm and P(AAm/MA) hydrogels respectively, indicate that the type of the isotherm change from monomolecular to multimoleculer adsorption[32].

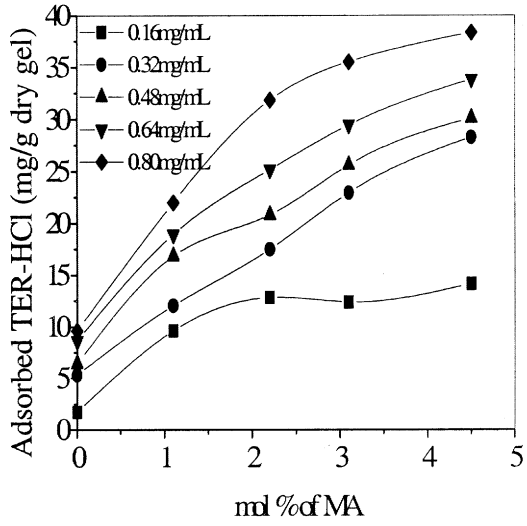


FIG. 21. Effect of MA content and drug concen tration on the adsorption capacities of PAAm and P(AAm/MA) hydrogels.

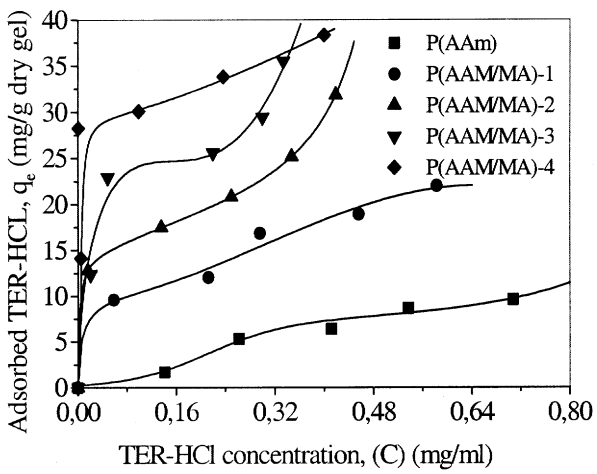


FIG. 22. TER-HCl adsorption isoterms of PAAm and P(AAm/MA)) hydrogels.

For the investigation of cationic drug TER-HCl adsorption behaviour of P(VP/IA) hydrogels prepared in this project, hydrogels were swelled in TER-HCl solution in the concentration range 0.50 –3.75 mg/mL HCl solution at pH4.0. The solubility of TER-HCl can be increased from 0.80 to 3.75 mg/mL when TER-HCl dissolved 10^{-4} M HCl solution. The amount of total (specific and non-specific) adsorbed TER-HCl into one gram of dry gel in

different drug concentrations are given in Fig. 23. In the notation used for the identification of samples, the number preceding the abbreviation denotes the percentage composition by weight. However XXVP is the total weight fraction of VP and EGDMA. As can be seen from the figure the amount of total TER-HCl taken increased with increasing IA content and initial drug concentration and the adsorption capacity of P(VP/IA) hydrogels were higher than P(AAm/MA) hydrogels. The reason of this increase was attributed to the increase of drug concentration and higher equilibrium degree of swelling value of P(VP/IA) hydrogels than P(AAm/MA).

For the investigation of drug release behaviour of AAm and P(AAm/MA) hydrogels, firstly loading experiments were conducted in 0.32 and 0.80 mg/mL TER-HCl solutions. In order to determine the amount of non-spesific adsorbed TER-HCL, hydrogels are placed pH7.0 phosphate buffer solution. Fig. 24 shows the percentage release non-specific adsorbed TER- HCl from AAm and P(AAm/MA) hydrogels. While 15.5% of drug was released from Aam hydrogels this value decreased to 4.9 with increasing MA content in the gel when initial drug concentration 0.32 mg/mL. As can be seen from the figure increasing drug concentration also decreased the extent of non-specific release of TER-HCl from the gel systems.

The percentage release of TER-HCl at pH7 was calculated from equation 6. The incomplete release of TER-HCl from hydrogels at pH7 was expected to be due to binding of the cationic TER-HCl to the polymer. The difference between the total and non-specific adsorbed TER-HCL is therefore taken to be equal to the amount of specific adsorbed TER-HCl in the hydrogel.

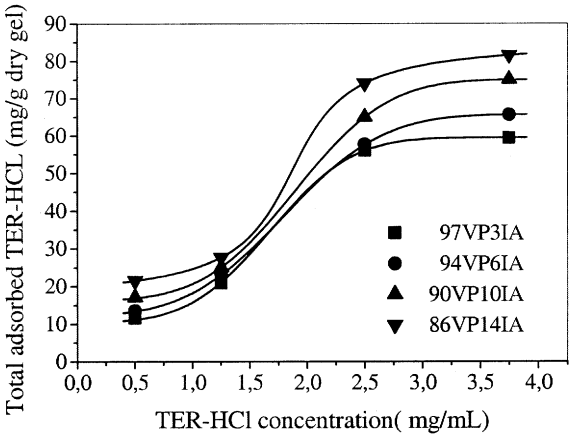


FIG. 23. Effect of IA content and drug concentration on the adsorption capacities of P(VP/IA) hydrogels.

The controlled release of specific adsorbed TER-HCl from P(AAm/MA) hydrogels was investigated primarily at pH6.1. The drug release was followed until equilibrium and then hydrogel was transferred into TER-HCl free buffer at pH5.5 and after reaching new equilibrium to pH4.4. The percentage of release of TER-HCl with time for AAm, P(AAm/MA)-2 and P(AAm/MA)-4 hydrogel systems are given in Fig 25. The percentage release of specific adsorbed TER-HCl at pH6.1, 5.5 and 4.4 were calculated from equation 7. As can be seen from the figure the release rate and percentage release decreased at pH6.1 with increasing MA content in the gel system due to increase of specific bonding of drug to hydrogel structure. Approximately 40, 15 and 9.0% drug release was observed in equilibrium

release of PAAm, P(AAm/MA)-1 and P(AAm/MA)-4 hydrogels at pH6.1 respectively. While 100% of TER-HCl was released from AAm hydrogels at pH5.5 only 40 and 30% of drug released from P(AAm/MA)-1 and P(AAm/MA)-4 hydrogels when the system reached to equilibrium respectively. The release of TER-HCl from P(AAM/MA)-4 hydrogels was opposite in trend than P(AAm/MA)-1 at pH5.5 and 4.4. Very fast release rates was observed for P(AAm/MA) hydrogels at this pH values may be due to higher drug content of hydrogel.

The overall (specific and non-specific) cumulative release of drug from all hydrogels systems depend on the pH of the solution, see Figs 26–27. As can be seen from the figures, while all adsorbed drug was released at pH5.5 for P(AAm) hydrogels in 0.32 and 0.80 mg/mL initial drug concentrations, this pH shifted to 4.5 with increasing MA content in the gel system. This can be explained again increase of specific interactions between drug and hydrogel with increasing drug adsorption and may be due to changes of the dissociation constant of MA in the gel-drug-phosphate buffer system.

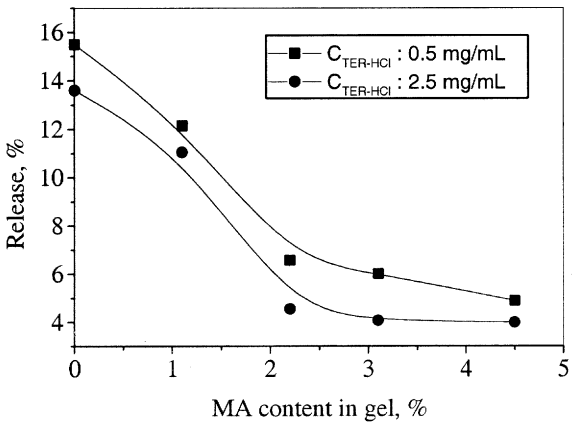


FIG. 24. Effect of MA content on the percentage release of non-specific absorbed TER-HCL.

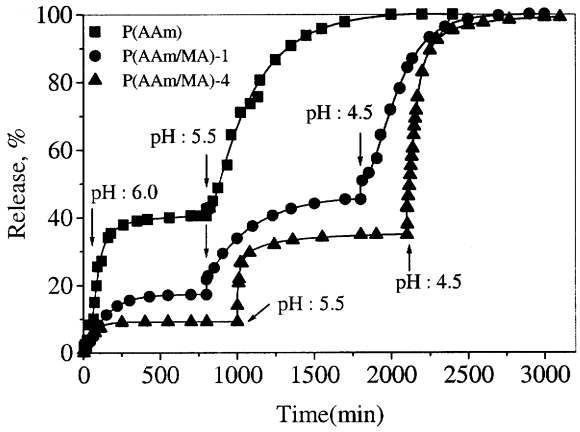


FIG. 25.Release of specific adsorbed TER-HCl from PAAm and P(AAm/MA) hydrogels.

In order to determine theamount of non-specific adsorbed TER-HCl, hydrogels are placed pH7.1 For the investigation of drug release behaviour of P(VP/IA) hydrogels and in order to phosphate buffer solution. Fig. 28 shows the released non-specific adsorbed TER-HCl from P(VP/IA) hydrogels. As can be seen from the figure increase in the content of ionic co-monomer IA in the gel system increased non-specific release value at all initial drug concentrations, may be due to completion of specific bonding to hydrogel in this concentration range.

Figs 29 and 30 show the overall (specific and non-specific) cumulative release of drug from all hydrogel systems that depend on the pH of the solution for 0.50 and 3.75 initial drug concentrations. As can be seen from the figures, when all adsorbed drug was released at pH6.1 for each hydrogel systems that the adsorption carried out in 0.50 mg/mL initial drug solution, this pH shifted to 5.0–5.5 with increasing initial drug concentration. This observations are very similar in P(AAm/MA) systems results and the reason of this changes was explained above.

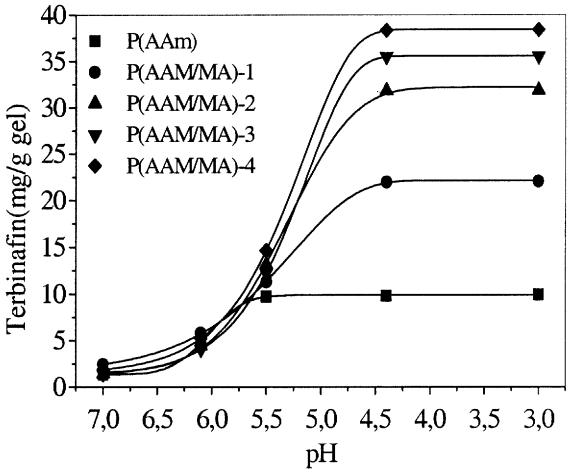


FIG. 26. Effect of pH on the cumulative release of TER-HCl from PAAm and P(AAm/MA) hydrogels when the adsorption achieved from 0.32 mg/mL TER-HCl solution.

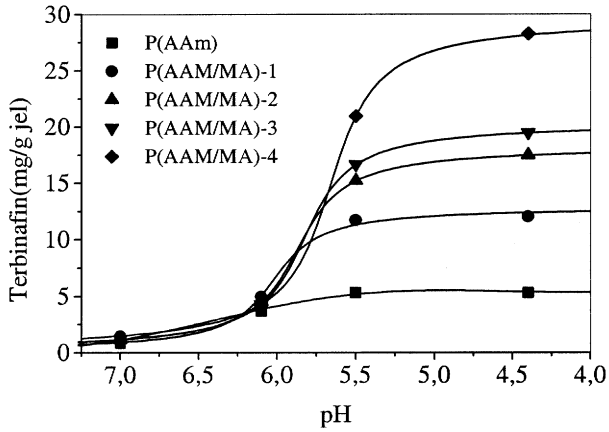


FIG. 27. Effect of pH on the cumulative release of TER-HCl from PAAm and P(AAm/MA)hydrogels when the adsorption achieved from 0.8 mg/mL TER-HCl solution.

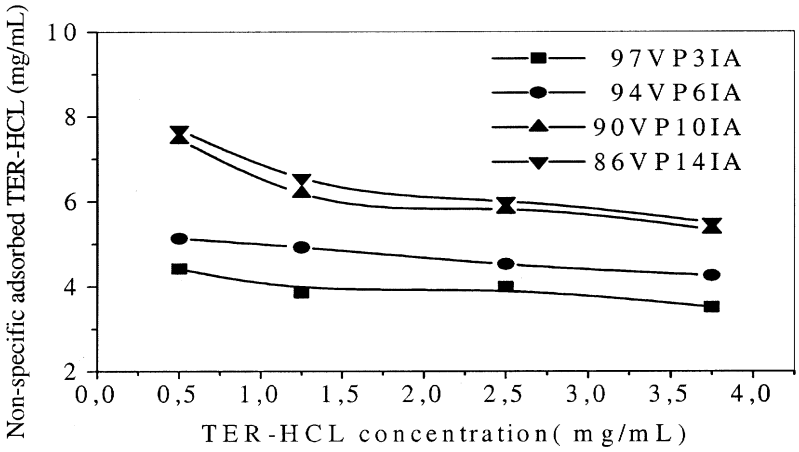


FIG. 28. Effect of TER-HCl concentration and IA content on the release of non-specific adsorbed TER-HCl. from P(VP/IA) hydrogels.

Figs 29–30 also indicate that the percentage releases of specific absorbed TER-HCL for each hydrogel at individual pH values were approximately of the same magnitude. Variation of the specific adsorbed TER-HCl (%) and ionization with pH for P(VP/IA) hydrogels are given in Fig. 31 and 32. for two initial drug concentration. As can be seen from the figures there is a significant difference between theoretically plotted ionization curve and adsorbed TER-HCL% versus pH curves. As explained above this changes may be due to the change of the dissociation constant of IA in the gel/TER-HCl/phosphate buffer system.

In this part of our project, adsorption of TER-HCl onto P(AAm/MA) and P(VP/IA) hydrogels and their release behaviours have been investigated. It has been found that the specific adsorption capacity of hydrogels both increase with increasing MA and IA content in the gel system. This has been explained due to the incorporation of more specific acidic groups into the network and a littler higher swelling capacity of gels. The release studies show that one of the basic parameters affecting the drug release behaviour of P(AAm/MA) and P(VP/IA) hydrogels is the pH of the solution.

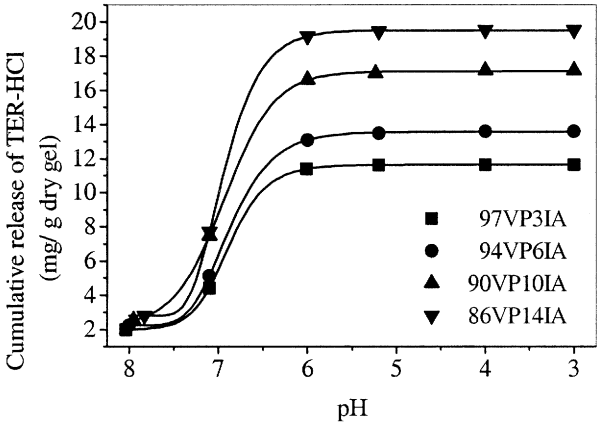


FIG. 29. Effect of pH on the cumulative release of TER-HCl from P(VP/IA) hydrogels when the adsorption achieved from 0.50 mg/mL TER-HCl solution.

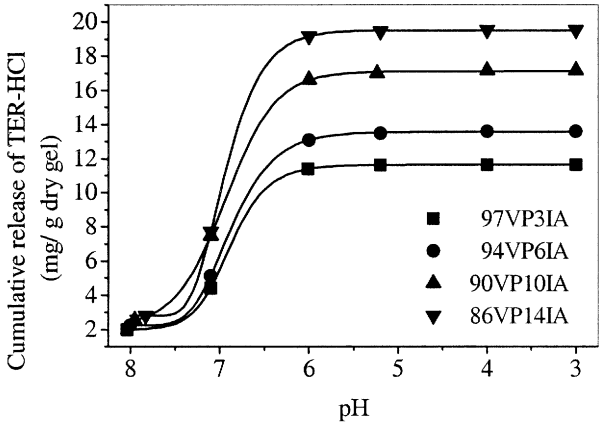


FIG. 30. Effect of pH on the cumulative release of TER-HCl from P(VP/IA) hydrogels when the adsorption achieved from 3.75 mg/ml TER-HCl solution.

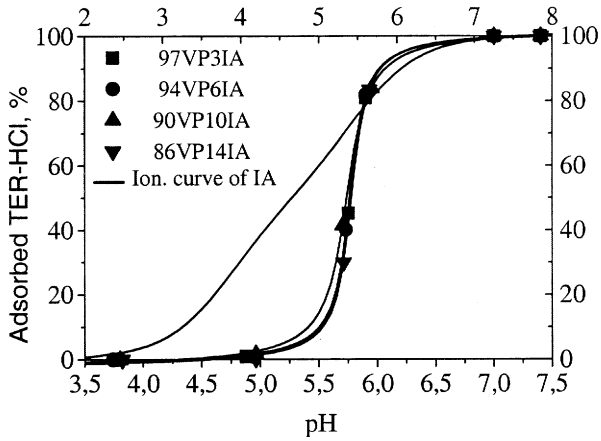


FIG. 31. Variation of specific adsorbed TER-HCl (%) and ionization with pH for P(VP/IA) hydrogels when the adsorption achieved from 0.50 mg/mL TER-HCl solution.

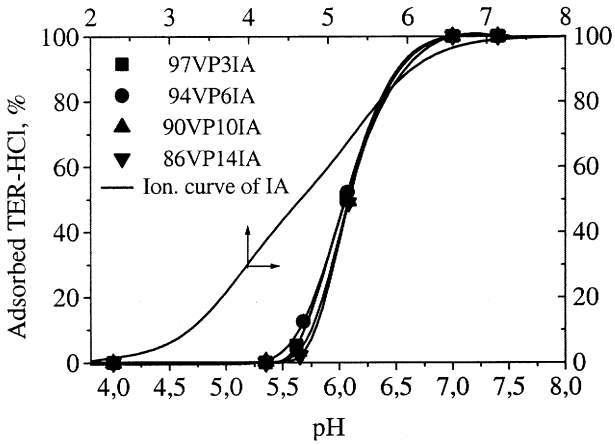


FIG. 32. Variation of specific adsorbed TER-HCl (%) and ionization with pH for P(VP/IA) hydrogels when adsorption achieved from 3.75 mg/mL TER-HCl solution.

3.5. Deswelling behaviour of hydrogels

When a hydrogels was used as trans dermal delivery system or wound dressing, one of the most important properties is deswelling behaviour in air and living tissue. For the investigation of deswelling behaviour of P(VP/IA) hydrogels, 50 mg dry gel was swelled in water and TER-HCl solution and placed in an oven at different temperatures or on human skin. Swolled gels removed from oven or human skin at regular intervals and weighed and placed in the same place. The measurements were continued until a constant weight was reached for each sample. Representative deswelling curves of P(VP/IA) hydrogels at different temperatures are given in Figs 33–34. In order to compare water loss rates of hydrogels, water loss versus time curves are plotted. Figs 35–36. Very similar deswelling curves were obtained for other hydrogel systems.

In order to determine deswelling order and rate constant, deswelling data fitted different kinetic equations, and best fit was observed with variable order decay. For all hydrogel systems water loss decay order was found to be 0.50–0.70.

The variation of water loss decay constant with temperature and IA content are given in Figs 37–38. Figures show the release rate decrease with increasing of IA content in the gel system and release of water was faster for TER-HCl adsorbed P(VP/IA) hydrogels than only water adsorbed hydrogels. This can be explained by the increase in the diffusional path due to increase of swelling capacity with IA content and decrease with TER-HCl.

Water loss release studies were achieved on the same place of skin and experiments were followed one week with different P(VP/IA) hydrogels. End of the deswelling studies any irritation was not observed on the skin surfaces. Deswelling behaviour of hydrogels prepared in this project will be explained in details in our recent publish paper [34].

To conclude, according to the experimental results of adsorption studies with blood proteins and deswelling studies, the hydrogels prepared in this study can be considered as potential carriers for the biomolecules and drug delivery systems and may be used as especially local therapeutic trans dermal delivery applications of cationic drugs.

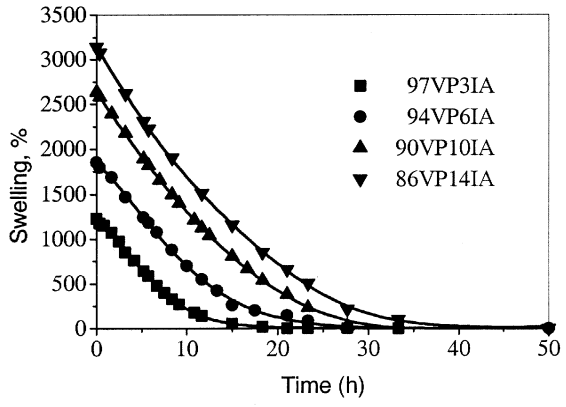


FIG. 33. Deswelling curves of P(VP/IA) hydrogels at 25°C.

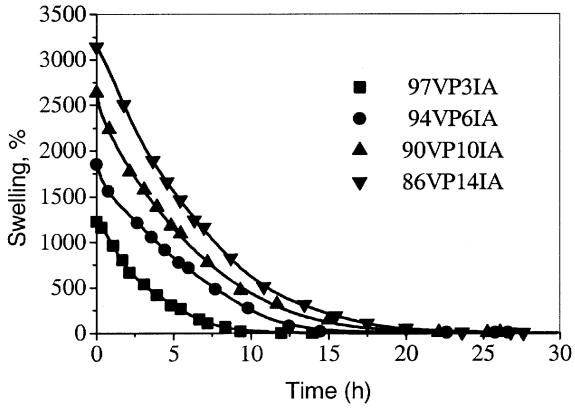


FIG. 34. Deswelling curves of P(VP/IA) hydrogels at 37°C.

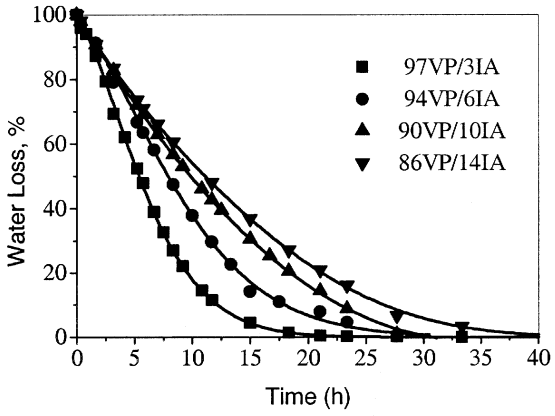


FIG. 35. Water loss curves of P(VP/IA) hydrogels at 25°C.

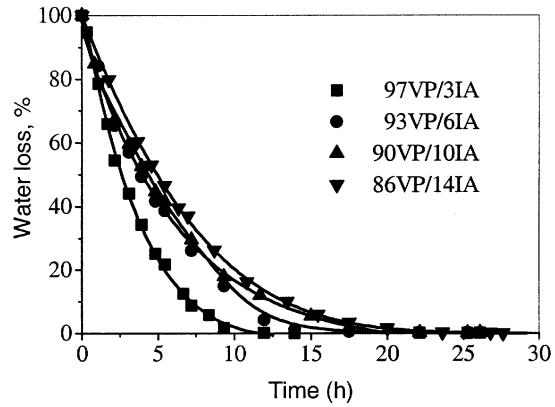


FIG. 36. Water loss curves of P(VP/IA) hydrogels at 37°C.

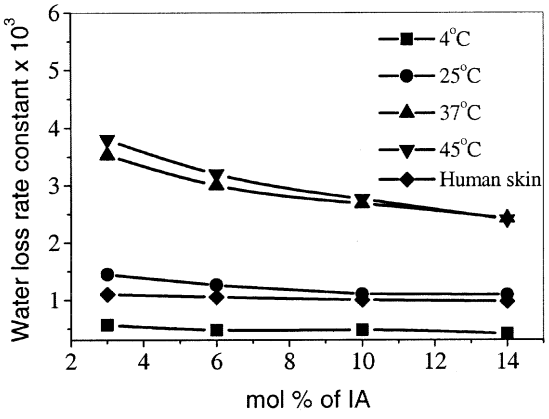


FIG. 37. Variation of water loss rate constant with IA content and temperature for P(VP/IA) hydrogels swollen in water.

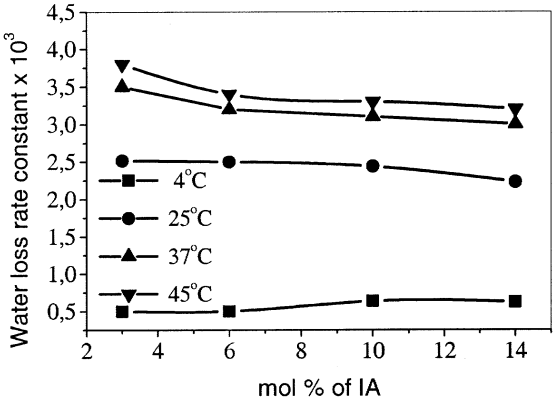


FIG. 38. Variation of water loss rate constant with IA content and temperature for P(VP/IA) hydrogels swollen in 3.75mg/mL TER-HCl solution.

REFERENCES

[1] WICTERLE, O., LIM., D., Hydrophilic gels for biological use, Nature **185** (1960) 117.

[2] ROSIAK, J. M., ULANSKI, P. RZEZNICKI, A., Hydrogels for biomedical purposes. Nuclear Instrument and Methods in Physics Research B **105** (1995) 335.

[3] PEPPAS, N. A. in Hydrogels in Medicine and Pharmacy, CRC Press, Boca Raton, Vol. 3, (1987) p. 1.

[4] MATHUR, M. A., SHAILENDER, K. M., SCRANTON, A., Methods for synthesis of hydrogel networks: A review, J., Macromol. Sci., Rev. Macromol. Chem. Phys. **C36** (1996) 405.

[5] CARENZA, M. Recent achievements in the use of radiation polymerization and grafting for biomedical applications. Radiat. Phys. Chem. **39** (1992) 485.

[6] SCOPOS, K. R. Protein purification principles and practice Springer, New York (1984).

[7] YOO, Y. J., HANG, J. HATCH, R. T., Comparison of amylase activities from different assay methods. Biotechnol. Bioeng. **30** (1987) 147.

[8] GÜVEN, O., SEN, M., Preparation and characterization of poly (N-vinyl 2- pyrrodinode hydrogels, Polymer **32**, (1991) 2491.

[9] FLORY, P. J., REHNER, R.,. Statistical mechanism of crosslinked polymer networks II. swelling. J. Chem. Phys. **11** (1943) 512.

[10] PEPPAS, N. A., MERRILL, E. W., Poly (vinyl alcohol) hydrogels: reinforcement of radiation-crosslinked network by crystallization. J. Polym. Sci., Polym. Chem. Ed. **14** (1976) 441.

[11] BRANNON-PEPPAS, L., PAPPAS, N. A., Equilibrium swelling behaviour of pH sensitive hydrogels. Chem. Eng. Sci. **46** (1991) 715.

[12] ŞEN, M., GÜVEN, O.,. Prediction of swelling behaviour of hydrogels containing diprotic acid moieties. Polymer **39** (1998) 1165.

[13] WEAST, R. C., Handbook of Chemistry and Physics. 53rd ed. The Chemical Rubber Co., Ohio. 1972.

[14] ŞEN, M., YAKAR, A., GÜVEN, O., Determination of average molecular weight between cross-links from swelling behaviours of diprotic acid-containing hydrogels. Polymer **40**(1999) 2696.

[15] HIROTSU, S., HIROKAWA, Y. AND TANAKA, T. Volume-phase transition of ionized N-isopropylacrylamide gels J. Chem. Phys., **87** (1987) 1392.

[16] GÜNER, A. AND ATAMAN, M., Effects of inorganic salts on the properties of aqueous poly (vinyl pyrrolidone) solutions Colloid and Polym. Sci., **272** (1994) 175.
[17] ŞEN, M. KANTOĞLU, Ö., GÜVEN, O. The effect of external stimuli on the equilibrium swelling properties of poly (N-vinyl 2-pyrrolidone/itaconic acid) poly-electrolyte hydrogels. Polymer **40** (1999) 913.
[18] KANTOĞLU, Ö., ŞEN, M., GÜVEN, O., The effect of external stimuli on the uranyl ions uptake capacity of poly (N-vinyl 2-pyrrolidone/itaconic acid) hydrogels prepared by gamma rays, Nuclear Inst. and Methods in Physics Research B **151** (1999) 218.
[19] OTTONBRITE, R. M., HUANG, S. J., PARK, K., (eds.) Hydrogels and biodegradable polymers for bioapplications ACS Symposium Series, 627, Washington DC. 1996.
[20] SARAYDIN, D., KARADAĞ, E., ÖZTOP, H. N., GÜVEN, O., Adsorption of BSA onto acrylamide-maleic acid hydrogels. Biomaterials **15** (1994) 917.
[21] AKKAŞ, P., SARI, M., ŞEN, M., GÜVEN, O., The effect of external stimuli on the bovine serum albumin adsorption capacity of poly (acrylamide/maleic acid) hydrogels prepared by gamma rays. Radiat. Phys. and Chem. 55 (1999) 717.
[22] DENİZLİ, A., TUNCEL, A., KOZLUCA, A., ECEVİT, K. AND PİŞKİN, E. Cibacron Blue F3G-A Attached Poly (Vinyl Alcohol) Particles for Specific Albumin Adsorption. Separation Sci. and Tech. **32** (1997) 1003.
[23] KAYIRHAN DENIZLI, F., Ph.D. Thesis, Hacettepe University, 1999.
[24] WYKES, R. J., DUNNILL, P. M. AND LILLY, D. Immobilization of α-amylase by attachment to soluble support materials. Biochim. Biophys. Acta **250** (1971) 522.
[25] ARICA, M. Y., HASIRCI, V.N. AND ALAEDDİNOĞLU, G., Covalent Immobilization of α-amylase onto pHEMA microspheres: preparation and application to fixed bed reactor. *Biomaterials* **16** (1995) 761.
[26] OHTSUKA, Y. KAWAGUCHI, H. YAMAMATO, T. Immobilization of α-amylase on polymeric carriers having different structures. J. Appl. Polym. Sci. **29** (1984) 3295.
[27] YOSHIDA, M., NEGISHI, M., ASANO, M., SUWA, T., MIYAJIMA, M., KATAKAI, R., Thermo and pH- responsive gels for application in colon delivery systems, Radiat. Phys. and Chem. 55 (1999) 677.
[28] NAKAMAE, K., NISHINO, T., FURUKAWA, M., NIZUKA, T., YAMANAKA, A., KANZAI, Y., HOFFMAN, A.S., pH sensitive enzyme release from polymer gel with phosphate groups, Fifth Word Biomaterials Congress, Toronto, Canada, June, 1996.
[29] DENİZLİ A., KİREMİTCİ M. AND PİSKİN E. Subcutaneous polymeric matrix system p(HEMA-BGA) for controlled release of an anticancer drug (5-fluorouracil). *Biomaterials* **9** (1988) 363.
[30] RYDER, N. S., Effect of allylamine antimycotic agents on fungal sterol biosynthesis measured by sterol side-chain methylation. J. of General Microbiology **131** (1985) 1595.
[31] BALFOUR, J. A., FAULDS, D., Terbinafine: A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses, Drug, **43** (1992) 259.
[32] ŞEN, M., GÜVEN, O., Radiation synthesis of poly (N-vinyl 2-pyrrolidone/itaconic acid) hydrogels and their controlled release behaviours. Radiat. Phys. and Chem. **55** (1999) 113.
[33] GILES, C. H., MACEWAN, T. H., NAKHWA, S. N. SMITH, D. Studies in adsorption part XI. A system of Classification of solution adsorption isotherms, and its use in diagnosis of adsorption mechanisms and in measurements of specific surface areas of solids. J. Chem. Society (1960) 3973.
[34] ŞEN, M., GUVEN, O. Dynamic deswelling kinetic of diprotic acid containing hydrogels(in preparation).

RADIATION SYNTHESIS OF SUPPORTED HYDROGELS FOR BIOMEDICAL AND BIOTECHNOLOGICAL PURPOSES

E.A. Hegazy

Polymer Chemistry Department,
National Center for Radiation Research and Technology,
NCRRT, Atomic Energy Authority,
Nasr City, Cairo, Egypt

Abstract. Since the commencement of this IAEA Research Project in 1997, attempts have been made to synthesize and characterize different hydrogels by using gamma irradiation as initiator. The factors affecting the preparation and homogeneity of prepared hydrogels were thoroughly investigated. Different polymeric materials and monomers were used for the preparation of these hydrogels. Characterization and possibility of their practicable uses were studied. Biomedical and histological studies on some grafted co-polymers showed that the grafted materials seem to be inert. Consequently, it might be used as biocompatible materials. The hemodialysis application was studied to find that the prepared membranes by radiation grafting method possessed good properties and they are of great interest in the field of separation of toxic materials from blood. Smart hydrogels were prepared for drug delivery systems. Butyl acrylate and methacrylic acid co-polymer gels showed a good sensitivity to the pH change for possible use in the field of drug delivery systems specifically for colon. Temperature and pH- sensitive terpolymer for modulated delivery of drugs was also investigated. Terpolymer hydrogels composed of PVA, NIPAAm and different pH-sensitive polymers such as acrylic acid, methacrylic acid and N-N dimethyl aminoethyl methacryate were prepared. The equilibrium swelling for the prepared different terpolymers was thoroughly investigated at various pH's. The hydrophilicity of NIPAAm and other pH-sensitive co-monomer greatly influences the critical collapse pH- of the terpolymer. Immobilization of invertase by radiation-induced polymerization of poly (vinyl alcohol) solution and acrylamide was also studied. The effect of crosslinking agent on the activity of enzyme was studied to show that as the content of crosslinking agent increases the relative activity of the enzyme decreases. The pH effect on the activity of the immobilized invertase was investigated to find that the optimum acting at pH 4.5 which is similar to the free one. This means that the matrix does not affect the optimum pH dependence of enzyme.The suitable temperature for maximum activity of immobilized enzyme was shifted to 55 $^{\circ}$C. Details of these investigations are presented in this report. Three papers have been published in International Scientific Journals on the work done under the contract of this project and are attached herewith.

1. INTRODUCTION

The application of polymers to medicine has become one of the principal challenges facing the polymer scientist. The scale and level of studies on the employment of radiation chemical methods for the preparation of polymeric biomedical designed for application in medicine and biotechnology has expanded significantly in recent years[1-3]. The use of ionizing radiation for the preparation of polymeric biomaterials is one of the examples of the application of atomic energy for the benefit of humanity. Radiation grafting for various biomedical applications remains an extremely active field of development[4]. Demand is growing for biomaterial usage in dialysis and immobilization of enzymes and as artificial cells, organs, and prostheses, tissue adhesives and cements, plasma expanders and controlled drugs released[5-10].

Radiation-induced modification of various polymers for prolonging contact with blood & tissue; immobilization of various biologically active substances (BAS) (enzymes, medical

drugs, hormones, etc.)[11-13] and radiation-induced cross-linking of polymers in order to obtain mechanically strong hydrogels (biological active substance carriers, dressing, eye lens, etc.)[14-17].

Synthetic membranes are being used increasingly in medicine to process blood for variety of therapeutic purposes. Such procedures are characterized by extracorporeal circulation and mass transfer across synthetic membranes in direct contact with blood. The most common of these procudures is hemodialysis, which is used for the treatment of acute or chronic renal failure and drug detoxification[18-21]. For improvement, new hemodialysis membrane should meet three basic criteria; compatibility with blood, improvement of permeability and selectivity and strength

During recent years, radiation-induced polymerization has been used for the immobilization of various biological active substrate (BAS) for reuse in order to achieve economics in their employment, in order to stabilize the (BAS) in relation to thermal influence, solvents and pH, in order to prepare polymeric articles of different shapes, for controlled liberation of (BAS) under the conditions of specific application, and for the employment of effects associated with polymeric matrix (strength, Rheology)[22-32].

“Responsive” polymer gels are materials whose properties, change in response to specific chemical environmental stimuli including temperature, pH, electric field, solvent quality, light intensity and wave length, pressure, ionic strength, ion identity and specific environmental tiggers like glucose. The properties that often change most dramatically are the swollen volume, the unique properties of responsive gels have resulted in substantial application research, especially over the past decade. In general, this application can be classified as mechanical devices, controlled solute delivery, devices, or chemical separation techniques[33-36].

Surface modification of polymers has received a great attention during the last decade since it could bring about specific surface properties including non-thrombogenicity. Since the major problems for application of biomaterials that contact occur by blood and/or tissue material surface, much research has focused in recent years on the creation of biocompatible surface. Radiation-induced grafting of monomers makes it possible to introduce a wide variety of properties on the surface[37-39]. These applications will naturally centre around the properties which include the ability to (a) be synthesized (polymerized) in situ as it is required by bone grouts, dental restoratives and tissue adhesive, (b) readily modify their structure and control their permeability to specific molecules, which is essential for oxygenator and dialysis membrane as well as for controlled drug release, (c) modify their surface charge characteristics which are responses in aqueous media such as blood and extra cellular fluids, (d) control their phsicomechanical properties, especially the elastomeric and resilience characteristics which are important for artificial heart valves and pumps as well as in plastic and reconstructive surgery. It is quite natural that although many polymers are currently used in biomedical applications, the vast majority are used in the same formulations developed for non-medical, industrial applications.

Among the many different immunoassays available for the quantitative determination of various analyses, the enzyme-labeled immunosorbent assay (ELISA) is now probably the most commonly used technique[40]. The ELISA is based on adsorption of antibody (or antigen) to the walls of a microtitration tray. For many applications, the ultimate lower detection level

(often incorrectly indicated as sensitivity) of the assay is of great importance. Many diagnostic testes and assays use submicron-size uniform latex particles, or microspheres, as substrate or supports for immunologically based reactions[40-42]. Before microspheres can be used in any test or assay, they must be prepared for binding and coated with ligand (usually a protein). A basic requirement of successful assay is a strong bonding of this removal during subsequent washing and reaction steps. Also low non-specific adsorption, good reproducibility and long-term stability is necessary.

2. RESULTS AND DISCUSSION

The research work was dealing with the preparation of different hydrogels for biomedical and technological purposes. The research studies and results obtained can be reported in the following items:

- Preparation and Characterization of Supported Hydrogels as Biocompatible Materials Interfaced with Blood and Tissue.
- Use of Radiation Grafted Membrane in the Dialysis of Low Molecular Weight Metabolites.
- Stimuli-Responsive Hydrogels for Possible Use in Drug Delivery Systems.
- Hydrophilic/Hydrophobic Materials for Protein Adsorption.
- Immobilization of Enzyme by Radiation Grafted Hydrogels.

The results obtained for the above items can be summarized, as follows.

2.1. Preparation and characterization of supported hydrogels

The graft co-polymers were prepared by direct radiation grafting of Sty/MAn binary monomers system onto PE films using Co-60 γ-rays. Attention was focused on the selection of the reaction parameters suitable for the commercial production of such supported hydrogels. The factors which affect the preparation process and grafting yield are; type of solvent, dose and dose rate, co-monomer composition and their concentrations in the diluents. The structure and composition of the grafted chains were also investigated

2.1.1. Effect of solvent

Table I shows the influence of different solvents on the graft co-polymerization of Sty/MAn binary monomers onto HDPE and LDPE. It can be seen that the higher degrees of grafting of such binary monomers/solvent-mixture are obtained in presence of acetone and ethyl methyl ketone as compared with those obtained in other solvents investigated here. It is expected that appropriate solvent that swells the surface grafted layer initially formed, enhance the diffusivity of Sty/MAn into the interior regions of the polymer substrate.

2.1.2. Effect of co-monomer composition

Some attention on enhancing grafting efficiencies has involved the use of mixed monomers systems, particularly with regard to synergistic effects leading to more efficient grafting processes. Therefore, the grafting of Sty/MAn binary system of various relative compositions is investigated at overall co-monomer concentration 20 mol%, at a dose rate of 0.7 and 1.38 Gy/s. The obtained results are illustrated in (Figs 1, 2). It is clear that the grafting yield initially increases with increasing the styrene content in the co-monomer feed solution to

reach a maximum degree of grafting at (Sty/MAn) composition of (70/30 mol%) and (60/40 mol%) for HDPE and LDPE, respectively. Synergism could be taken place for the grafting of MAn in all co-monomer compositions. However, such synergism was observed only in styrene-rich co-monomer composition. It was also observed that the grafting process is dependent on the dose rate; the higher the dose rate, the lower the degree of grafting is obtained when the MAn-rich co-monomer feed solution is used. However, using a styrene-rich co-monomer feed solution, it resulted in increasing the degree of grafting as the dose rate decreases.

TABLE I. EFFECT OF DIFFERENT SOLVENTS ON THE GRAFTING PROCESS OF THE STY/MAN BINARY MONOMERS (50/50 MOL%) SYSTEM UNTO LDPE AND HDPE FILMS. TOTAL IRRADIATION DOSE; 10 K GY, DOSE RATE; 1.4 GY S^{-1}, CO-MONOMER CONCENTRATION; 20 MOL%

Solvent	Degree of Grafting (%)	
	LDPE	HDPE
Acetone	404.25	120
Butanone	97.9	75
Ethyl acetate	122.8	55
Dioxane	14	5
Benzene	73	45
DMF	70.5	20

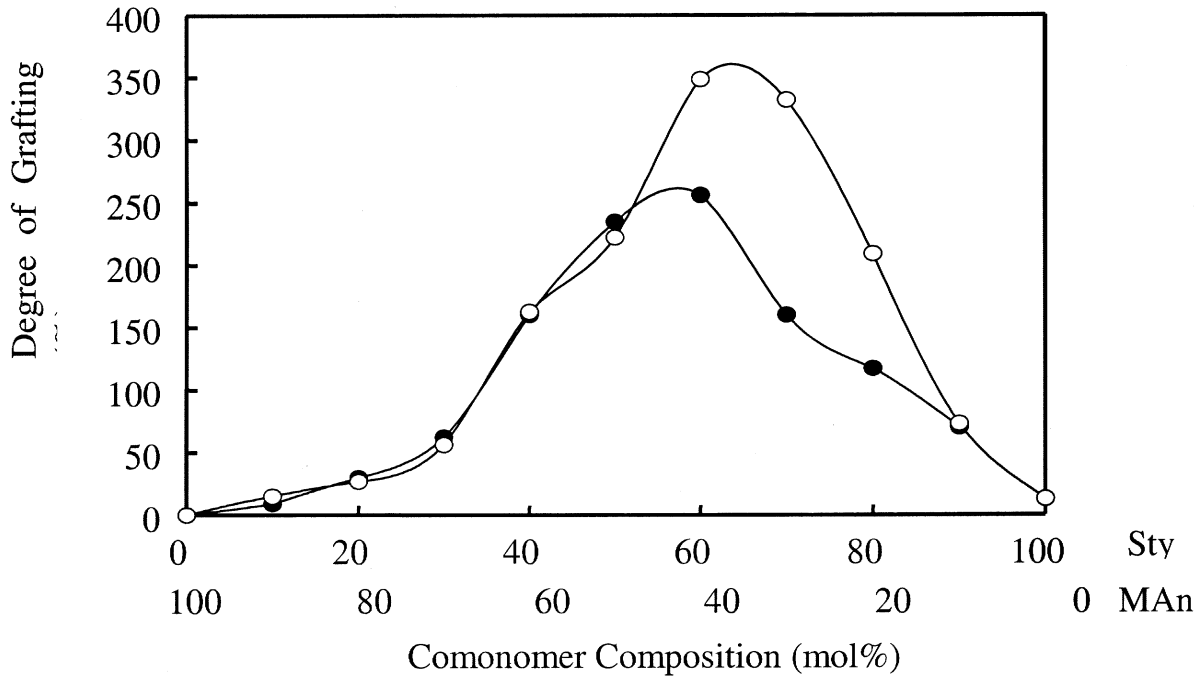


FIG. 1. Effect of Sty/Man co-monomer composition on the degree of grafting onto HDPE films at different irradiation dose rate (Gy/s); (●) 1.4 and (○) 0.8. co-monomer concentration in acetone; (20 mol%) and irradiation dose ; 7.5 k Gy.

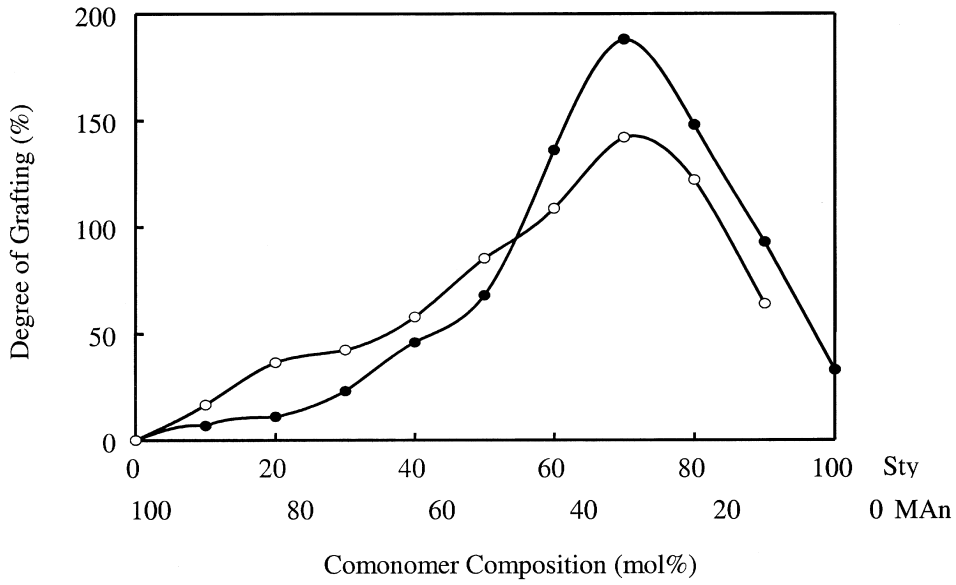


FIG. 2. Effect of Sty/Man co-monomer composition on the degree of grafting onto LDPE films at different irradiation dose rate (Gy/s); (●) 1.4 and (○) 0.8. co-monomer concentration in acetone; (20 mol%) and irradiation dose ; 7.5 k Gy.

Results suggested that the relative participation of charge transfer complex in the graft co-polymer depends on the composition of co-monomer feed solution and the concentration of CTC in feed solution reach its maximum when Sty-rich co-monomer composition of 70 mol% was used. Also the molar ratio of Sty/MAn on the graft co-polymer support such assumption that the CTC participated in graft co-polymerization processing. The molar ratio of Sty/MAn in all graft co-polymer chains of different co-monomer compositions, specially these containing excess Sty which give high percent graft, was formed to be 1: 1 in the first stage of grafting [Table II].

TABLE II. EFFECT OF CO-MONOMER COMPOSITION ON THE MOLAR RATIO OF GRAFTED P-STY AND P-MAN GRAFT CHAINS IN THE OVERALL GRAFT CO-POLYMER AT DOSE RATE OF 1.38 GY/S AND 0.8 GY/S., USING UV SPECTROPHOTOMETRIC AND TITRATION METHOD AT DIFFERENT DEGREES OF GRAFTING

Degree of Grafting	(Sty/MAn) Compn.	Dose Rate	(Sty/MAn) Molar Ratio using UV	(Sty/MAn) Molar Ratio using Titration
(%)	(mol%)	Gy/s	(%)	(%)
14.9	(10/90)		1.05	1.01
26	(20/80)		1.03	0.98
32	(80/20)		1.12	1.17
39	(70/30)	1.38	1.08	1.13
46.9	(30/70)		0.98	1.03
53	(50/50)		1.02	0.99
55	(60/40)		1.1	1.13
64.2	(40/60)		1.03	1.08
13	(10/90)		1.1	1.07
21	(20/80)		1.0	1.01
55	(30/70)	0.7	1.13	1.03
70	(90/10)		1.45	1.37

2.1.3 Thermal and mechanical properties

It is very important that the grafted films must show a good tensile strength, especially for the use in biomaterials. The change in tensile strength and percent elongation at break with degree of grafting was determined and shown in (Figs 3 and 4), respectively. It can be seen that the tensile strength increases gradually with degree of grafting, however, the percent elongation decreases as the degree of grafting increases.

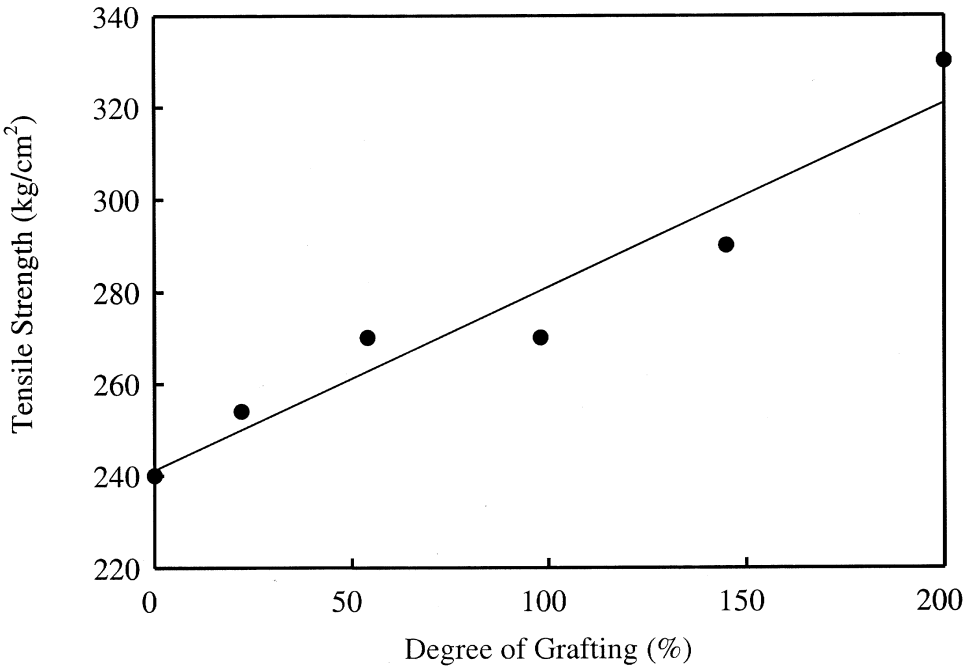


FIG. 3. Change in tensile strength with degree of grafting for LDPE-g-P(Sty/MAn) films.

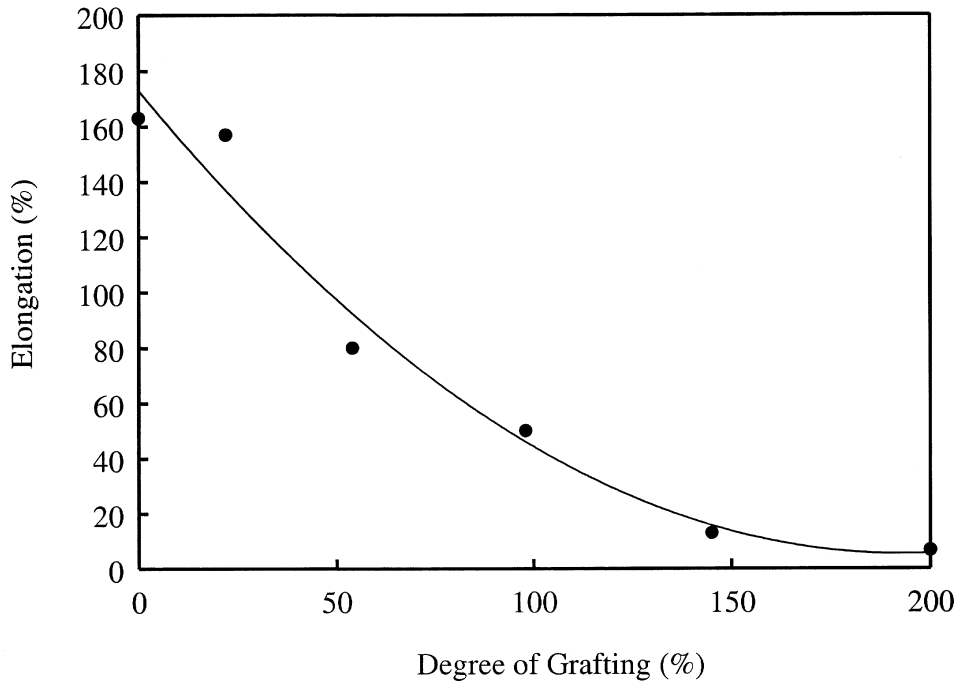


FIG. 4. Change in elongation percent with degree of grafting for LDPE-g-P(Sty/MAn) films.

The knowledge on the changes in thermal properties and crystallinity of LDPE-g (Sty/MAn) system is important for its application. Diffusion is generally limited to the amorphous regions of a polymer so that applications that really depend on the diffusion characteristics of films such as those used for separation processing require careful control of crystallinity. The change in T_m and ΔH_m for the grafted co-polymer at the first and second heating runs with degree of grafting is investigated (Table IV). It can be seen that no remarkable change in T_m by grafting is observed. Also, a slight decrease in (T_{rc}) and a decrease in (ΔH_{rc}) are observed (Table III). This is good evidence that such grafted co-polymer is not highly crosslinked.

TABLE III. EFFECT OF DEGREE OF GRAFTING ON THE THERMAL PARAMETERS OF LDPE-G-P(STY/MAN)

Degree of grafting (%)	ΔH_{m1} (J/g)	ΔH_{m2} (J/g)	ΔH_{rc} (J/g)	T_{m1} (°C)	T_{m2} (°C)	T_{rc} (°C)
0	85.4	88	86.4	108.4	109.1	90.8
37	64.8	53	53.1	107.1	107.7	90.1
81	47.1	42.7	44.3	107.3	106.4	87.4
135	37.8	31.3	34.1	107.3	106.1	86.5
170	29.2	24.6	25.1	107.4	106	85.6
225	21.9	9.2	14.1	107.6	102.3	73.9

2.2. Biocompatible materials interfaced with blood and tissue

Since the major problems for application of biomaterials that contact occur at the blood and for tissue material surface, an attempt has been made to gain insight into the biomedical uses of polypropylene-g-poly (vinyl acetate/maleic anhydride) mode system, that prepared by radiation grafting method, and its treated one with sodium hydroxide and ammonia solutions and tested as biocompatible materials interfaced with blood and tissue. Investigations on the biomedical and histopathological changes which might be caused in the tissue due to muscle implantation of (PP) and PP-g-P (VAc/MAn) graft co-polymer of film having 100% graft were investigated. Histopathological studies of the dorsal muscle of the rabbits surrounded the implanted polymer was investigated. Results showed that no sign of degradation in the tested polymers occurred and much materials seem to be inert. Consequently, they might be used as biocompatible materials.

To investigate the suitable percent grafting that minimize the thickness of capsule which may be formed around the implanted films, PP-g-poly (VAc/sodium maleiate) graft co-polymer film having degree of grafting varied from 0 to 100% are implanted subcutaneously in rabbits. After 4 weeks, capsules with different thickness were formed around the implanted polymer materials.

The thickness of capsules depends on the percent graft of the grafted films under investigation as shown in Table IV. The thick capsules were formed around ungrafted PP and for the grafted films having 5% and 10% grafting. However, the implantation of grafted films having higher percent grafting films resulted in forming thin capsules.

The results indicate that the graft co-polymer of succinate and VAc supported into PP films enables to present biocompatible materials at low percentage of grafting.

TABLE IV. GRADE OF THE ENCAPSULATION OF PP-G-P(VAC/SODIUM SUCCINATE) IMPLANTED SUBCUTANEOUSLY FOR FOUR WEEKS. (+) IS PROPORTIONAL TO THE THICKNESS OF CAPSULE

Degree of Grafting of implanted polymer (%)	Thickness of Fibrous Tissue
0	+++++
5	+++++
10	+++
23	+
40	+
58	+
79	+
98	+

2.2.1. Evaluation of blood compatibility

The main obstacles in the use of non-biological materials in cardiovascular implants are surface induced thromboses. In the present work, series of PP-g-P (VAc/MAn) graft co-polymer films with different percent grafts which treated with various reagents such as diluted HCl, NaOH at 30°C, NH_4OH and NaOH at 100°C were prepared. The whole blood clotting technique was used to evaluate the blood compatibility of such films.

Fig. 5 shows the effect of the percent grafting of treated films that having different functional groups; free –COOH, -COONa, -$CONH_2$, -COONa, -COO-NH_4^+, –COONa, and -OH groups, on the time required for clotting the blood adherent to those films. It is found that for most grafted co-polymer films investigated, the lower the percent grafting, the shorter the time required for clotting the blood is noticed, especially those films containing free carboxylic acid groups. However, for the films containing both –COOH, and –OH groups, and treated with NaOH at 100°C, it was found that the lower the percentage of grafting, the longer the time required for clotting the blood adherent to such film. However, at all high degrees of grafting, it was noticed that the time needed to clot the blood, increases as the degree of grafting increases. But in case of graft co-polymers containing both –COONa and –OH groups, the shorter time for clotting the blood is observed at high degrees of grafting. Also, it can be seen that the time required to clot the blood contacted with films containing –$CONH_4$, -COONa, and -$COONH_4^+$ gave almost the lowest time in comparison with those containing –COOH or –COONa groups.

3. Use of radiation grafted membrane in dialysis of low molecular weight metabolites

The permeability measurements were conducted using a diaphragm-type cell. The permeabilities of membranes were determined for NaCl, urea, uric acid, Vitamin B_{12}, creatinine and glucose. The NaCl was determined by titration with $AgNO_3$. In case of urea, p-dimethyl amino formaldehyde was added to the urea solution and the concentration was analysed colorimetrically at 440 nm. Also, the concentration of uric acid, creatinine, glucose, vitamin B_{12} and phosphate was analysed colorimetrically at 520, 546, 500, 360 and 700 nm, respectively.

To improve the hydrophilicity of the prepared grafted films, the anhydride groups of LDPE-g-Sty/MAn co-polymer were converted into maleic acid or maleic acid sodium salt by treating with HCl or NaOH, respectively. In order to introduce thiosemicarbazide, hydroxamic acid, 2-amino pyridine and aspartic acid into PE-g-Sty/MAn graft co-polymers, the amino group derivatives of such compounds is introduced to the anhydride ring of the graft co-polymers by heating them in dioxane/DMF (1:1 v/v) at 70°C for 2h.

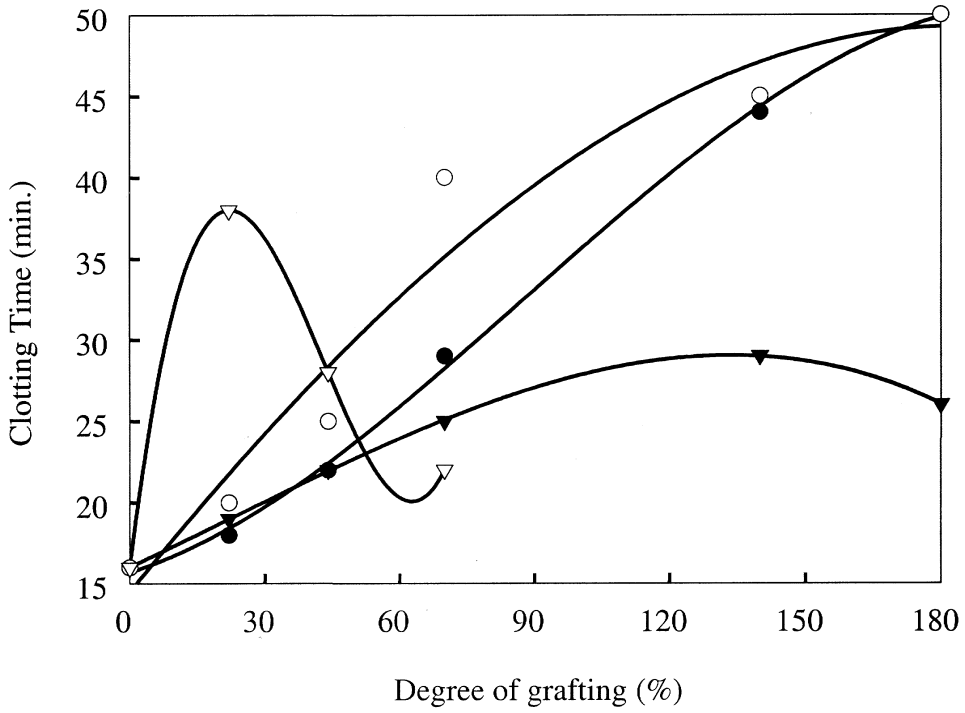


FIG. 5. Effect of degree of grafting of treated grafted co-polymer having different functional groups on the clotting time (●) free COOH, (○) COONa, (▼)COONa, CONH2&COONH4 and (▽)COONa&OH groups.

Fig. 6 shows the water uptake percent for the treated grafted films with HCl and NaOH against degree of grafting. It was observed that the NaOH treated films, that containing sodium carboxylate groups possessed higher hydrophilic properties than that treated with HCl which containing free carboxylic acid group.

The results suggested that the hydrogen bonding formed in the grafted films containing free carboxylic acid groups resulting in crosslinking network structure. As a consequence, the water uptake is reduced. However, the alkali treated grafted films possessed higher water uptake due to the formation of easily ionizable sodium carboxylate groups having good hydrophilic properties. Such hydrophilic grafted material may be of interest for use as supported hydrogel that can be used as biomaterial.

3.3.1. Permeability of solute through grafted hydrogel

For hemodialysis application the effect of degree of grafting and types of cationic and anionic groups introduced into the grafted polymer on the permeability of different solutes through such membranes was studied.

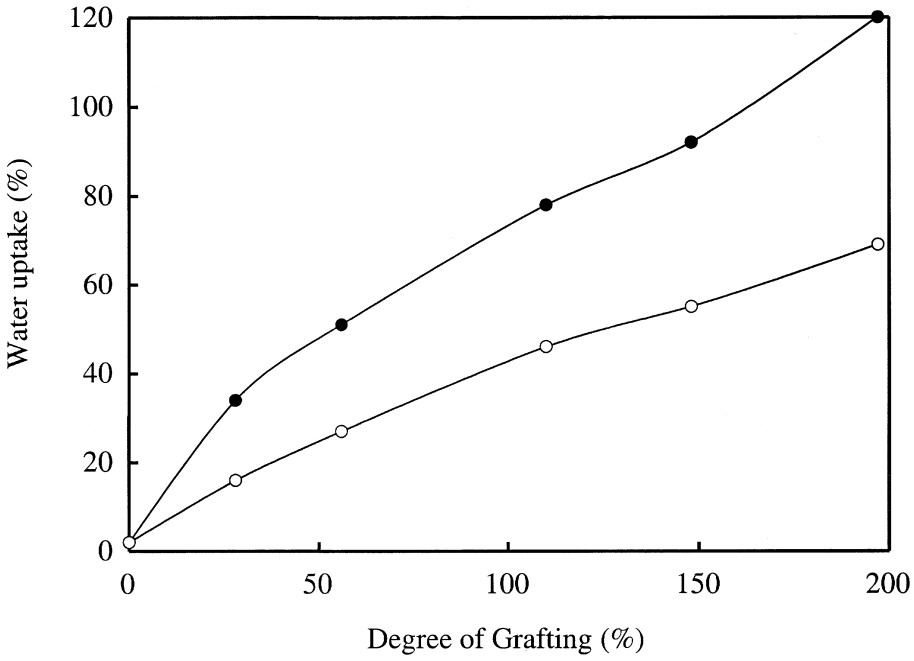


FIG. 6. Water uptake versus degree of grating for HDPE-g-P(Sty/MAn) treated with HCl (○) and NaOH (●).

Figs 7–8 show the effect of degree of grafting on the dialysis permeability of different solutes; urea, glucose and uric acid through the heterografted HDPE films and their treated ones with Hydroxylamine.HCl, 2-amino pyridine and thiosemicarbazide and then NaOH. For all solutes investigated here, the permeability increases with degree of grafting. Also, the permeability of such solutes through the NaOH treated membranes is higher than that through the alkali untreated ones.

The results suggested that the permeation process is a function of the diffusion and solubility of the species under consideration in the membrane. Thus, the enhanced permeability exhibited by the membranes may be interpreted on the basis of solubility and diffusion effect, i.e. the untreated and treated graft co-polymer chains are very swollen in water, as a consequence they enhance the plasticization effect of the water in the membranes as the permeation rate increases.

The permeation of water or water soluble solutes is found to be dependent on the degree of swelling of films and it is assumed that its diffusion only is through the water phases in the water-swollen membranes. It also seems that the permeability of solutes is directly proportional to the equilibrium water content in the grafted membranes.

3.3.2. Diffusion rate of metabolites through grafted hydrogels

The rate of diffusion of solutes through the membranes can be dealing according to the molecular weight of the solute, nature and physico-chemistry of such solute and the type of functional groups in polymers. According to the nature and chemical structure of the functional polymer and metabolises used, the structure of glucose and urea have no pronounced effect on different functional polymers used. However, diffusion rate of urea is faster than that of glucose. This is because of the difference in molecular weight, the maximum diffusion of urea achieved at 4 h, whereas, glucose achieved at 8 h (Figs 9–11).

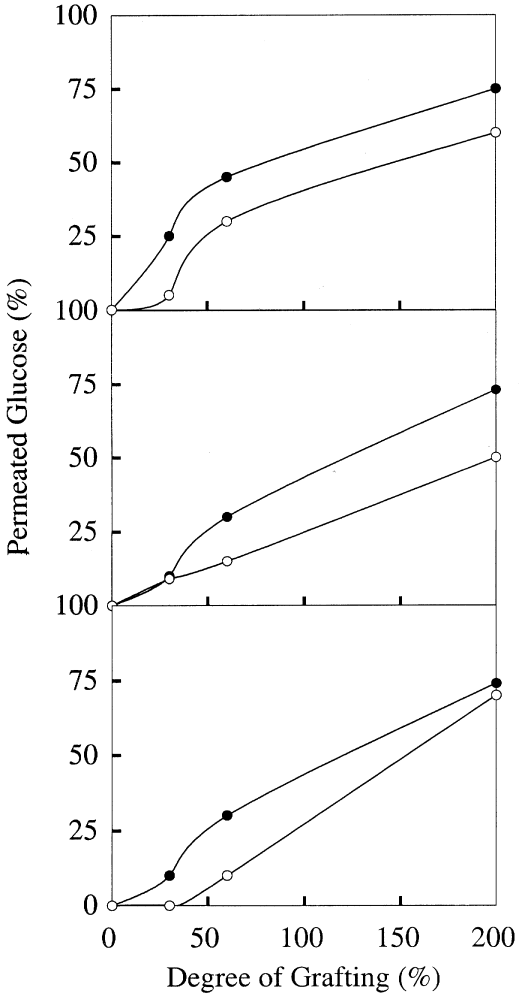


FIG. 7. Effect of degree of grafting on the permeation of glucose of initial concentration 150 mg in100 ml distilled water through grafted films treated with (○).

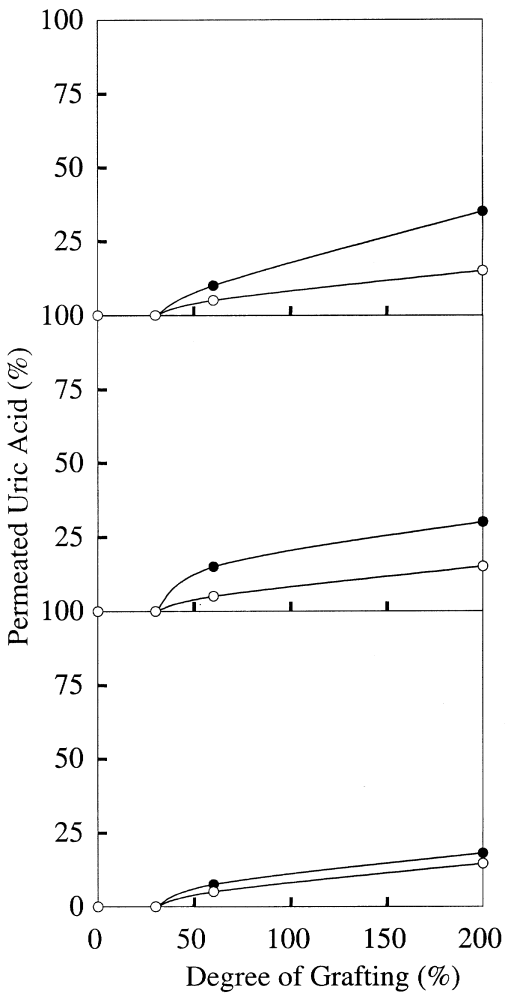


FIG. 8. Effect of degree of grafting on the permeation of uric acid of initial concentration 150 mg in100 ml distilled water through grafted films treated with (○) (A).

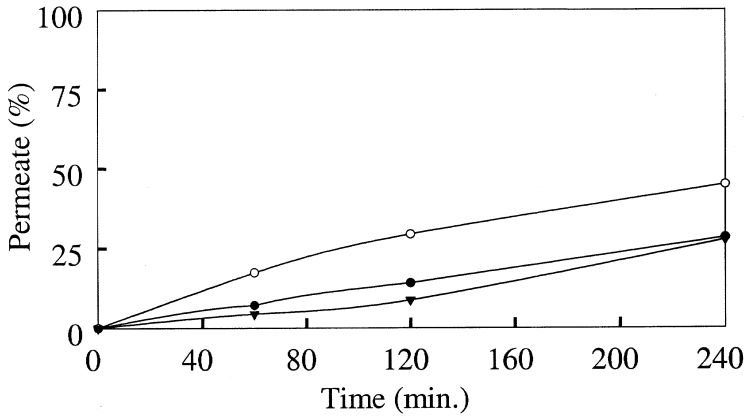


FIG. 9. Amount of permeated glucose (●), urea (○), and uric acid (▾) against time for membranes treated with thiosemicarbazide.

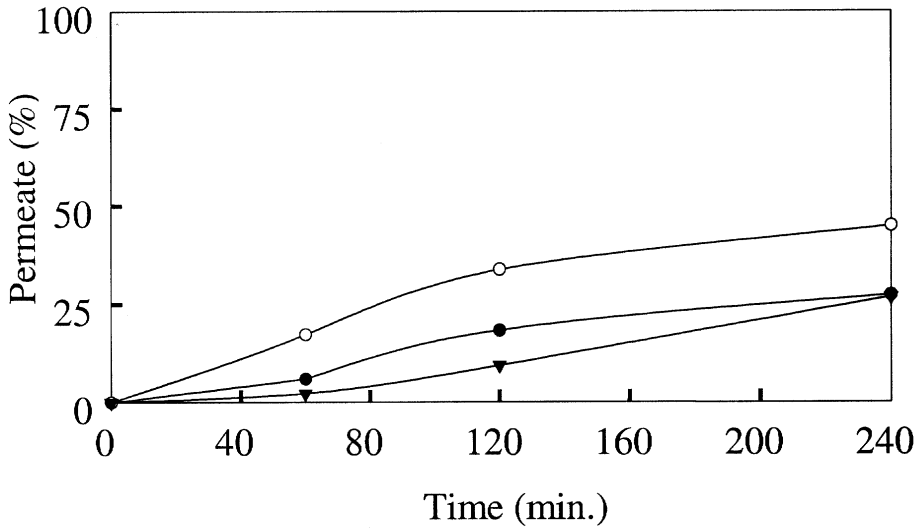


FIG. 10. Amount of permeated glucose (●), urea (○), and uric acid (▾) against time for membranes treated with hydroxylamine HCl.

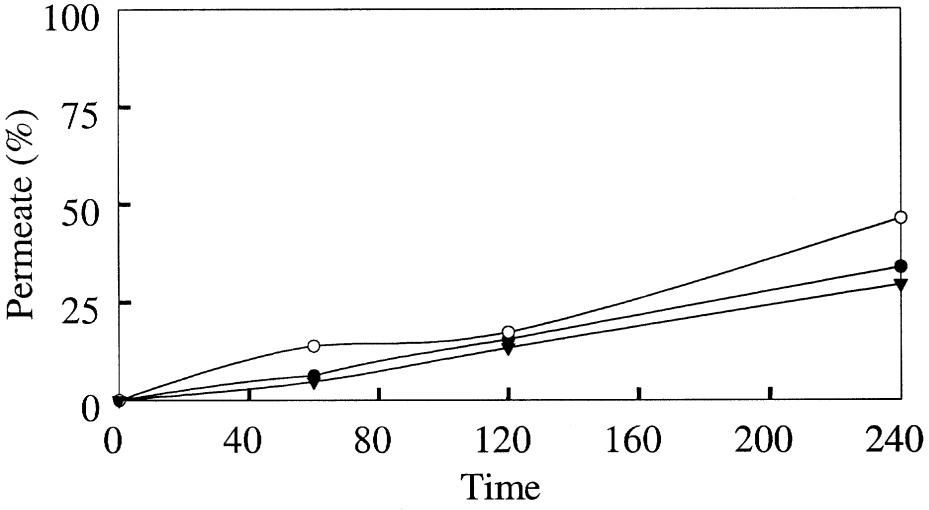


FIG. 11. Amount of permeated glucose (●), urea (○), and uric acid (▾) against time for membranes treated with 2-amino pyridine.

TABLE V. EFFECT OF FUNCTIONAL GROUPS IN GRAFTED MEMBRANES AND CHEMICAL TREATMENTS ON THE DIALYSIS OF THE BASIC METABOLITIES

Treatment reagent	Permeated glucose (%)			Permeated Creatinine (%)			Permeated Urea (%)		
	Acid form	50% alkali treated	100% alkali treated	Acid form	50% alkali treated	100% alkali treated	Acid form	50% alkali treated	100% alkali treated
Untreated	43.3	46.7	50	19.5	48.8	50	41.3	50	51.3
Aspartic acid	36.7	48	50	42.5	47.5	49.5	34.3	50	46.7
Hydroxylamine. HCl	40	45.3	52.7	21	50	40.5	46.7	46.7	48.7
2-Aminopyridine	35.3	45.3	32	43	47.5	50	48	48	50
Thiosemicarbazide	46.7	50	50	40	43.5	50	50	48.7	47.3

3.3.3. Permeability of acidic and basic metabolites

Five types of modified membranes are used to investigate the permeation of basic metabolites such as urea, glucose and creatinine [Table 5].

The membranes of different functional groups seem to be suitable for the separation of different basic metabolises. Furthermore, the alkali-treated membranes showed high permeability towards basic metabolites. However, the membrane containing carboxylic acid group showed lower permeability towards basic solutes. The results obtained can be attributed to the basic character of both membranes and solutes resulting in improving the permeability of such solutes through the membranes investigated here. However, the membranes containing free carboxylic acid groups may interact with the basic metabolises resulting in reduction of metabolises permeation.

Table VI shows the permeability of uric acid through treated membranes that containing various amounts of sodium salt. The permeability of uric acid through membranes containing sodium carboxylate group is greater than that of other films containing free carboxylic acid groups or both sodium carboxylate and carboxylic acid groups. From this result, it can be assumed that the presence of carboxylic acid groups interact with or prevent the diffusion of uric acid through the films, the higher the content of free carboxylic acid groups, the lower the permeation of uric acid through such films.

TABLE VI. EFFECT OF FUNCTIONAL GROUPS IN GRAFTED MEMBRANES AND CHEMICAL TREATMENTS ON DIALYSIS OF ACIDIC METABOLITIES

Treatment reagent	Permeated Uric acid (%)			Permeated Phosphate salts (%)		
	Acid form	50% alkali treated	100% alkali treated	Acid form	50% alkali treated	100% alkali treated
Untreated	21.3	24.7	28.7	19.6	2.8	28.4
Aspartic acid	20	28.3	41.3	7.5	6	4.9
Hydroxylamine. HCl	20.7	26	46.7	3.9	22.9	8
2-Aminopyridine	14	34	38.7	36	17.6	11.8
Thiosemicarbazide	22	41.3	50	4.9	31.4	14

Table VI shows also the permeability of phosphate salt through grafted and treated grafted membranes. Such treated membranes show unsatisfactory behaviour towards phosphate dialysis, except for films treated with 2-aminopyridine.

The results could be discussed in terms of chemical nature and physicochemical interaction of the solutes with the membranes. Phosphate solution prepared from KH_2P0_4, is inorganic in nature and possesses an ionic character and such properties may be aid in interaction between such metabolises and those membranes containing different anion and cation species.

The permeability of potassium chloride through different types of membranes containing different functional groups and various amounts of Na-salt was studied and shown in (Table VII). It can be seen that all films, except that containing free carboxylic acid groups (Untreated films and films treated with aspartic acid), show high permeability towards KCl.

TABLE VII. EFFECT OF FUNCTIONAL GROUPS IN GRAFTED MEMBRANES AND CHEMICAL TREATMENTS ON THE DIALYSIS OF KCL

Treatment reagent	Permeated KCl (%)		
	Acid form	50% alkali treated	100% alkali treated
Untreated	12	50	50
Aspartic acid	10	50	50
Hydroxylamine. HCl	50	50	50
2-Aminopyridine	50	50	50
Thiosemicarbazide	50	50	50

The increment in the permeation of KCl through most treated membranes can be explained by the free volume concept of diffusion for homogeneously water-swollen membrane.

Permeability's of vitamin B_{12} and albumin of high molecular weight substances through PE-g-P(Sty/MAn) membranes and their treated ones with different reagents were studied and are shown in (Table VIII) respectively. It was found that the vitamin B_{12} of 1355 g/mol molecular weight permeates through the treated membranes with NaOH. However, the permeation through other membranes, especially those containing carboxylic acid groups, is lower than that of the other treated membranes. Also, the permeation of albumin of 6900 g/mol molecular weight through all membranes investigated here is negligible, except for films containing pyridine ring (this membranes permeate few amount of albumin).

TABLE VIII. EFFECT OF FUNCTIONAL GROUPS IN GRAFTED MEMBRANES AND CHEMICAL TREATMENTS ON THE DIALYSIS OF THE HIGH MOLECULAR WEIGHT SUBSTANCES

Treatment reagent	Permeated vitamin B_{12} (%)			Permeated bovine albumin (%)		
	Acid form	50% alkali treated	100% alkali treated	Acid form	50% alkali treated	100% alkali treated
Untreated	4	11.5	16.7	0.8	0.9	1
Aspartic acid	3.5	8.5	10	0.8	0.8	0.9
Hydroxylamine. HCl	10	17	20	0.8	0.9	1
2-Aminopyridine	9	14.5	19	8.5	8.9	9.9
Thiosemicarbazide	10	15	20	0.8	0.9	1

4. STIMULI-RESPONSIVE HYDROGELS FOR POSSIBLE USE IN DRUG DELIVERY SYSTEMS

4.1. pH-sensitive hydrogel for drug delivery

A trial has been made to prepare pH-sensitive hydrogel by the use of radiation co-polymerization of binary monomers such as methacrylic acid (MAAc)/Butyl acrylate (BA), methacrylic acid (MAAc)/methyl methacrylate (MMA) and methacrylic acid (MAAc)/methyl acrylate (MA) and the use of ketoprofen as a drug model. The factors affect on the co-

polymerization process such as types of solvent and irradiation dose were studied. The aqueous equilibrium swelling properties of co-polymers based on methacrylic acid (MAAc) and acrylate ester of various chain lengths were investigated as a function of pH at 37ºC. It was found that the % swelling of the co-polymer prepared in acetone is higher than that prepared in other solvents at different pH's used here (Figs 12–14). However, the % swelling of the co-polymer prepared in water or ethanol/water is very low at lower pH (1). Therefore, The most suitable solvent to meet the required properties was ethanol and mixture of ethanol and water. The effect of water/ethanol composition on the swelling behaviour was also studied to find out that the optimum % swelling obtained at (40/60) (water/ethanol) composition (Fig. 15). The extent of the transition from the collapsed hydrophobic state to hydrophilic state depending on co-monomer compositions (Table IX).

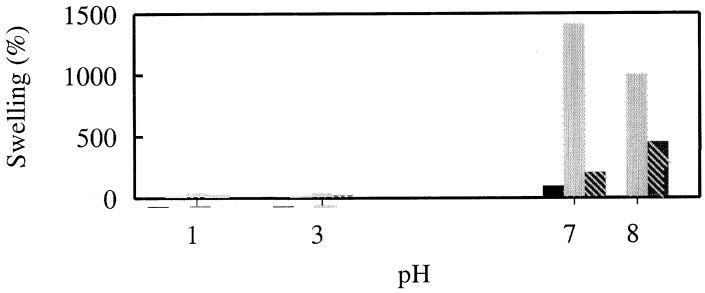


FIG. 12. Effect of pH on the swelling of (BA/MAAc) hydrogel that prepared by co-polymerzatrion of (BA/MAAc) of composition (50/50 mol%) in ( )Ethanol, ( )Acetone and ( ) Ethanol/Water mixture. Swelling time; 3 h.

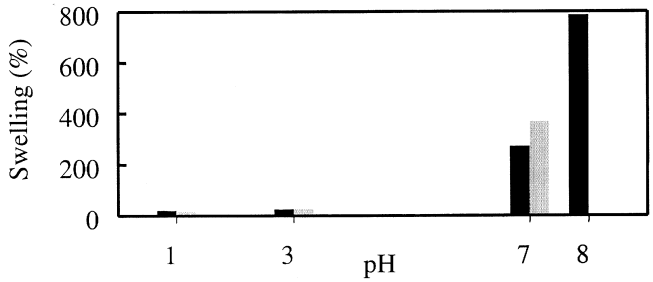


FIG. 13.Effect of pH on the swelling of P(MA/MAAc) hydrogel that prepared by co-polymerzatrion of (MA/MAAc) of composition (50/50 mol%) in ( )Ethanol, and ( ) Ethanol/Water mixture. Swelling time; 3 h.

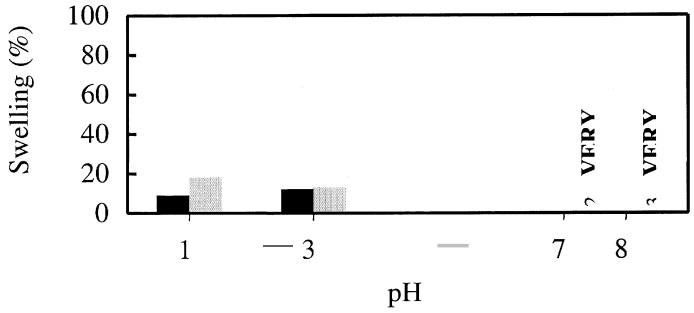


FIG. 14.Effect of pH on the swelling of (BA/MAAc) hydrogel that prepared by co-polymerzatrion of (BA/MAAc) of composition 50/50 mol%) in ( )Ethanol, and ( ) Ethanol/Water(mixture. Swelling time; 3 h.

TABLE IX. EFFECT OF TIME ON THE SWELLING PERCENT FOR HYDROGEL CO-POLYMERS PREPARED FROM DIFFERENT CO-MONOMER (BA/MAAC) COMPOSITIONS IN A SOLVENT MIXTURE OF 40/60 WT% WATER/ETHANOL

Swelling Time (h)	Swelling (%) (BA/MAAc) Composition (wt%)					
	20/80	30/70	40/60	50/50	60/40	70/30
3.5	123	180	285	106	65	29
7	203	280	428	161	121	51

In order to evaluate the release behaviour of ketoprofen -loaded during the radiation process- under different pH conditions, the release of ketoprofen from gel when cycled in buffer solutions between pH1 and pH7.2 was determined (Fig. 16). The effect of dose and drug concentration on the release of ketoprofen was investigated at pH7.2 to find that as the drug concentration increases in co-polymer its release increases. However, as the irradiation dose increases, the release of drug decreases (Figs 17, 18). At pH1, the release of drug has no significant value. The release of ketoprofen from gel — loaded by immersing the co-polymer into concentrated ketoprofen solution for 24h- was investigated. The release of ketoprofen from such gel is much hgiher than that released from gel loaded drug prepared by radiation (Fig. 19).

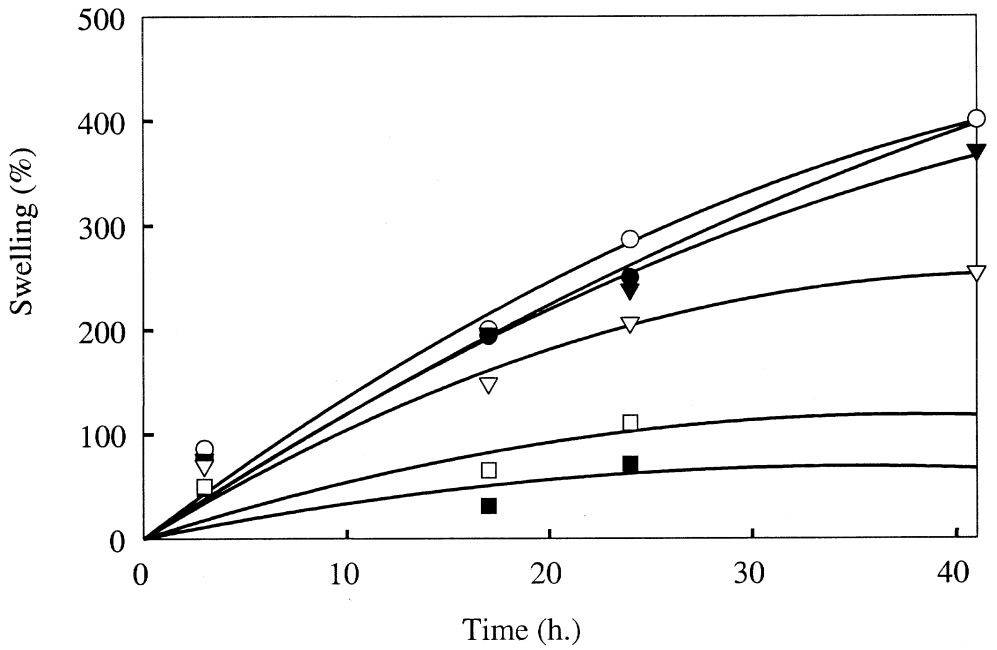


FIG. 15. Effect of time on the swelling percent of co-polymer prepared at different EtOH/water mixture composition (wt%); (■) 30/70, (□) 40/60, (●)50/50, (○)60/40, (▼)70/30, (▽) 80/20.

Analysis of the mechanism of diffusion in swellable polymeric systems has received considerable attention because of its important applications in pharmaceutical engineering. The swelling of MAAc/BA hydrogels in water was investigated and shown in (Fig. 20). The following equation was used to determine the nature of diffusion of water into hydrogel:

$$F = K t^{n}$$

where F denotes the reaction of water diffused into the gel in time t, K is a constant related to the structure of the network, and the exponent (n), is a number to determine the type of diffusion. The value of diffusional exponent is $0.5<n<1.0$. Therefore, water diffusion to the hydrogels is non-Fickian type diffusion. This is generally explained as a consequence of slow relaxation of polymer matrix.

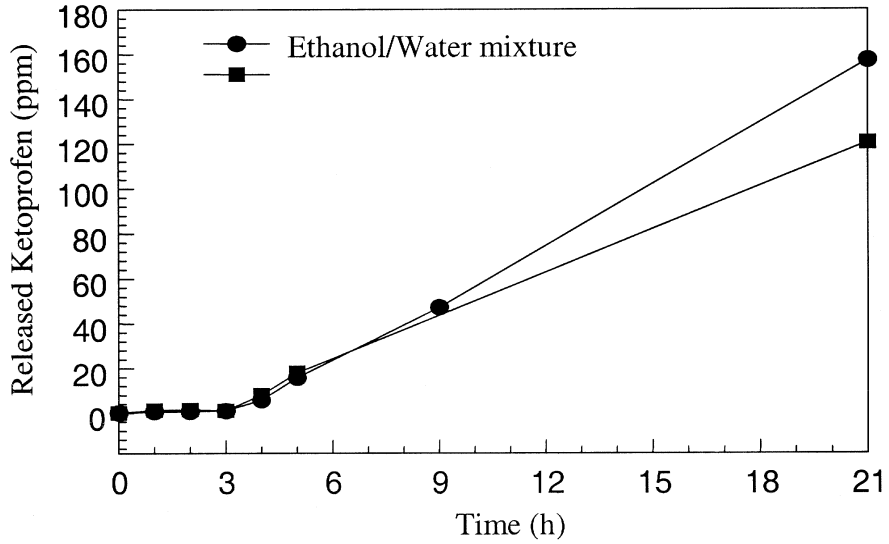


FIG. 16. Effect of time on the release of ketoprofen from (BA/MAAc) (50/50 wt%) co-polymer hydrogel that prepared in different solvents. Irradiation dose; 20 k Gy.

Swelling of (MAAc/BA) co-polymer is a highly pH dependent. At low pH the swelling is less than 10%, however, at high pH the swelling is higher than 300%. This property results in the use of co-polymer system as pH-sensitive hydrogel for drug delivery systems.

4.2. Thermosensitive hydrogels

A study has been made on the preparation of intelligent hydrogels capable of swelling or collapsing in water below or above their low critical solution temperature; respectively, by γ-rays co-polymerization of poly vinyl alcohol and N-isopropyl acrylamide (NIPAAm) and shown in (Fig 21). By altering the co-polymer composition, LCST can be controlled. The kind and the amount of NIPAAm enabled the transition temperature to be easily shifted up and down. Also it was found that M. wt. of PVA and irradiation dose affect the LCST of PVA-NIPAAm co-polymer.

Temperature and pH- sensitive terpolymer for modulated delivery of drugs was also investigated. Terpolymer hydrogels composed of PVA, NIPAAm and different pH-sensitive polymers such as acrylic acid methacrylic acid and N-N dimethyl aminoethyl methacryate were prepared. The equilibrium swelling for the prepared different terpolymer was thoroughly investigated at various pH's. The hydrophilicity of NIPAAm and other pH-sensitive co-monomer greatly influence the critical collapse pH- of the terpolymer (Figs 22-25). The maximum swelling percent for such terpolymer depends on the types of pH sensitive monomer used. It was found that minimum % swelling obtained at pH 1 for all terpolymers used. However, the optimum % swelling differs from terpolymer to another.

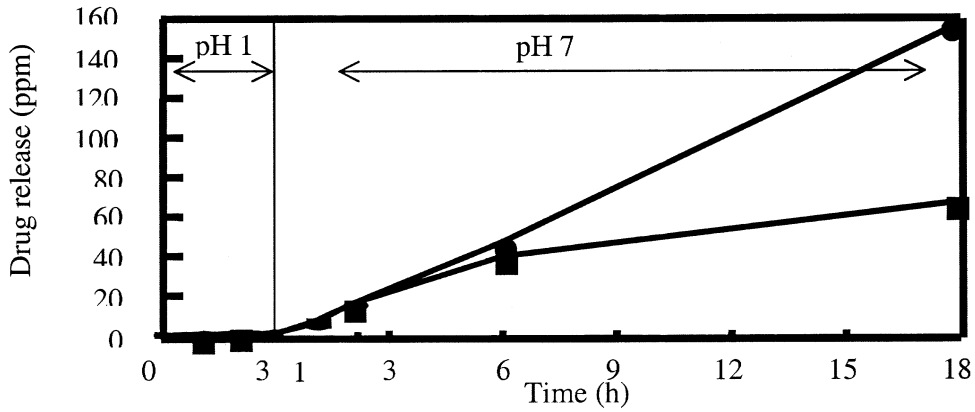


FIG. 17. The release of ketoprofen from BA/MAAc co-polymer hydrogels that prepared at different doses; ( )20 kGy and ( ) 40 kGy. Co-monomer composition; (50/50 mol%).

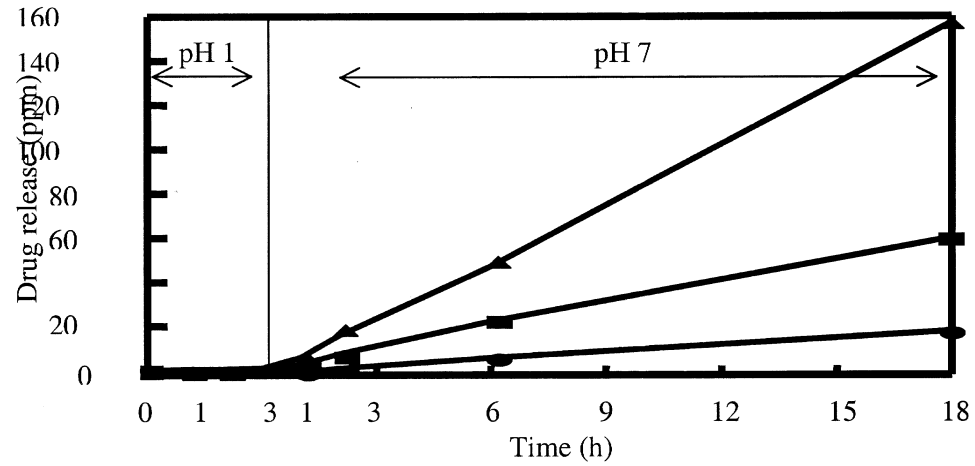


FIG. 18. The release of Ketoprofen from BA/MAAc co-polymer hydrogel contains different ketoprofen concentrations. ( ) 50 ppm, ( ) 125ppm and ( ) 250 ppm. Co-monomer composition; (50/50 mol%), co-monomer concentration; 50%, solvent; ethanol/water mix.; (60/40 mol%), irradiation dose; 30 k Gy.

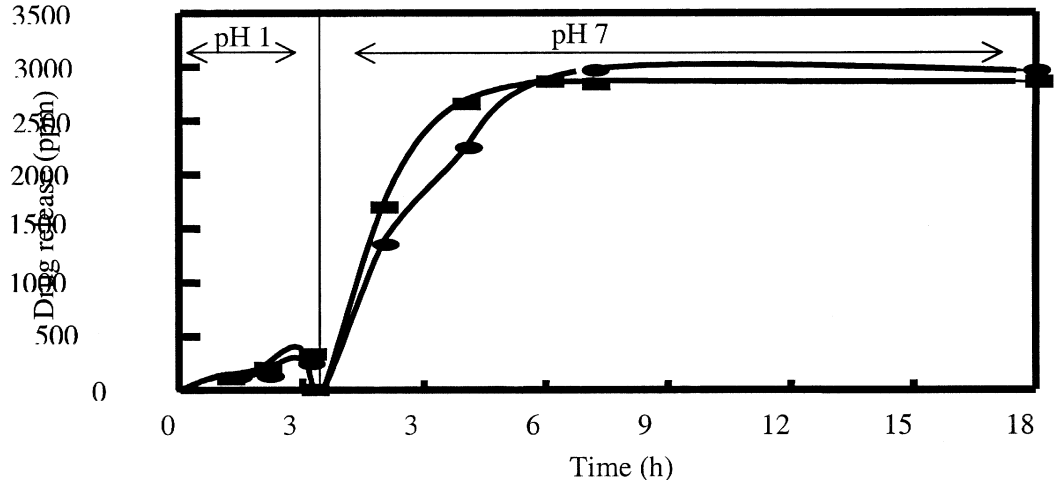
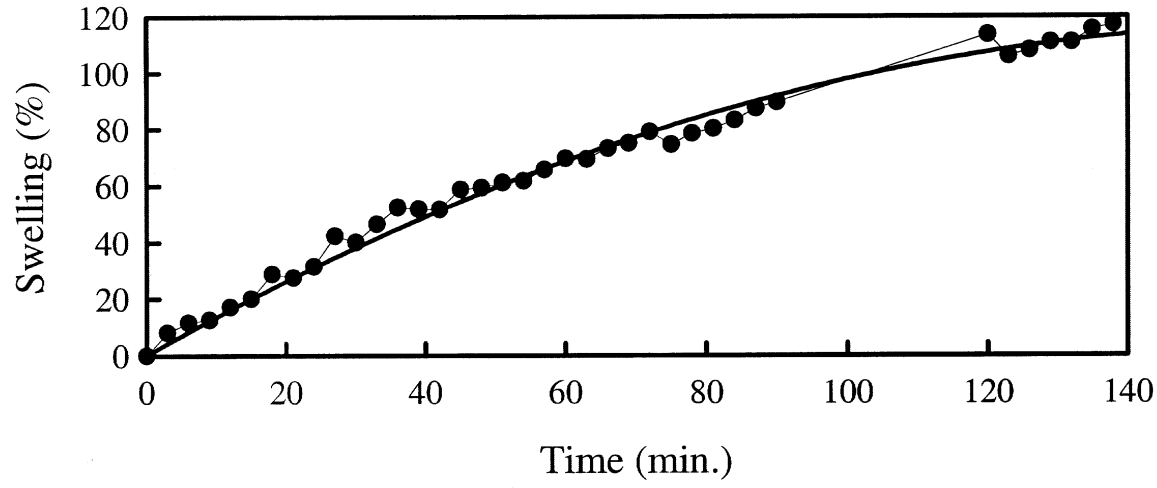
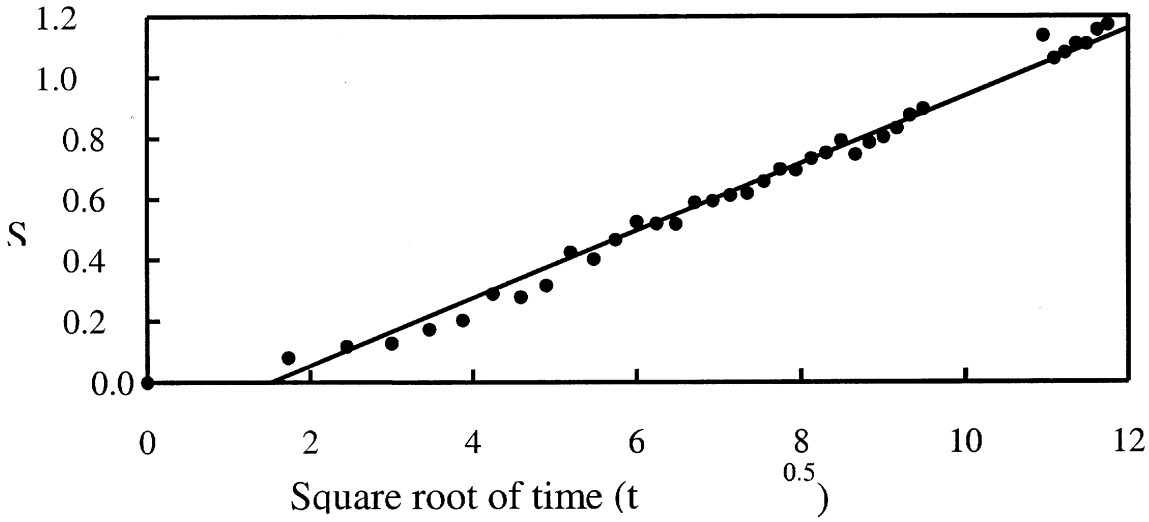


FIG. 19. Release of ketoprofen from BA/MAAc co-polymer hydrogel that prepared at different solvent;( ) ethanol and ( ) (ethanol/water) (50/50). Co-monomer comp.; (50/50mol%), co-monomer concn.; 50%, irradiation dose; 30 kGy.





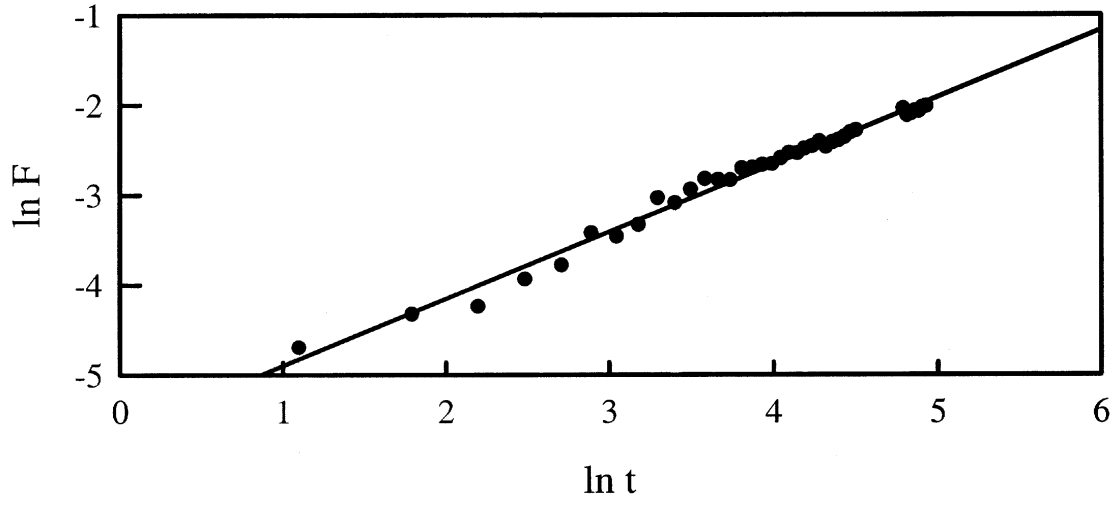


FIG. 20. A)Tthe swelling curve, B) time course of swelling, C) plot of ln F vs. ln t of BA/MAAc hydrogel. Co-monomer concentration: 50 mol%, co-monomer composition: (50/50mol%); total irradiation dose:; 20 kGy.

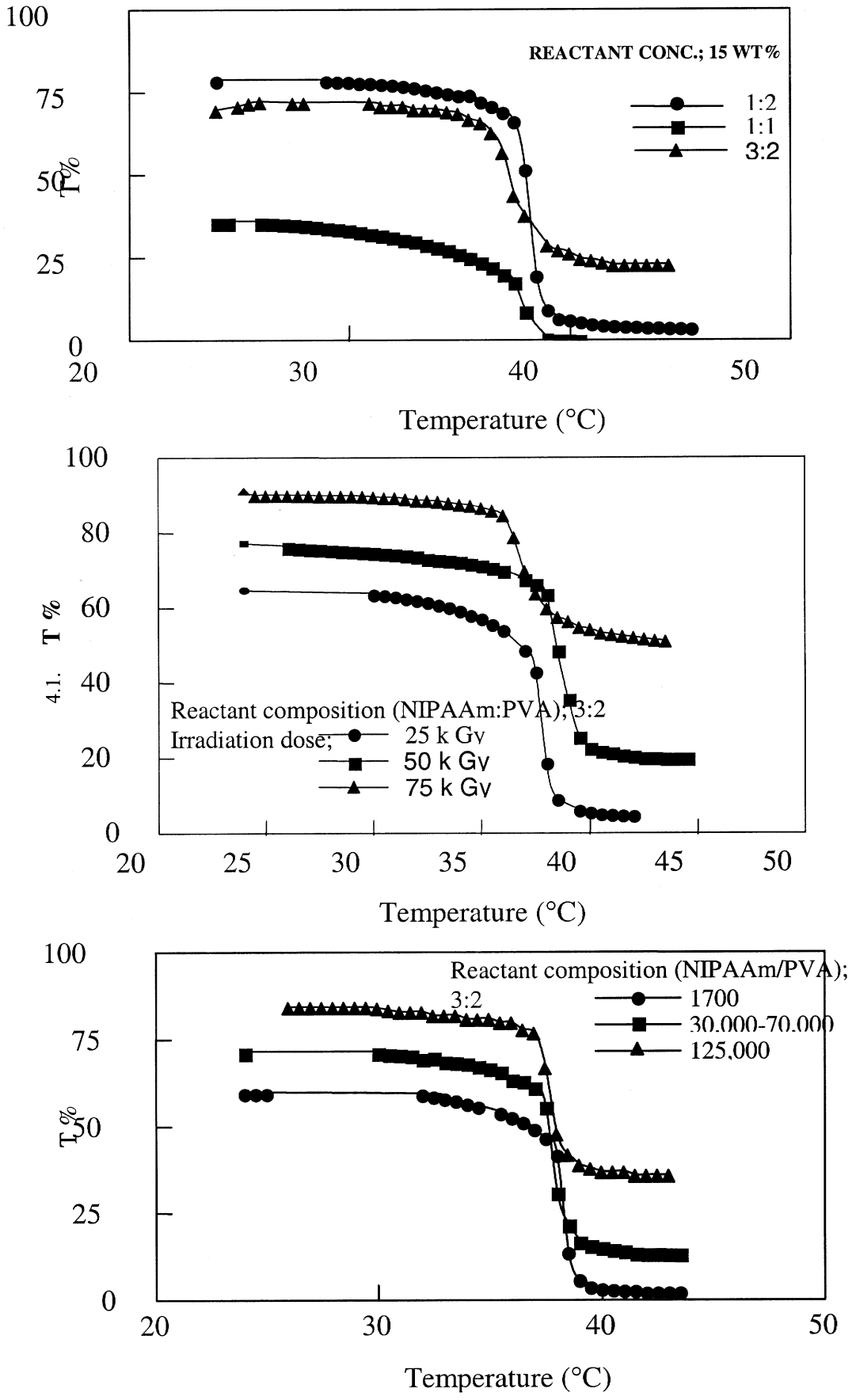


FIG. 21: Effect of temperature on the UV transmittance of the aqueous solution of the PVA-g-NIPAAm, T% was measured at 500 nm.

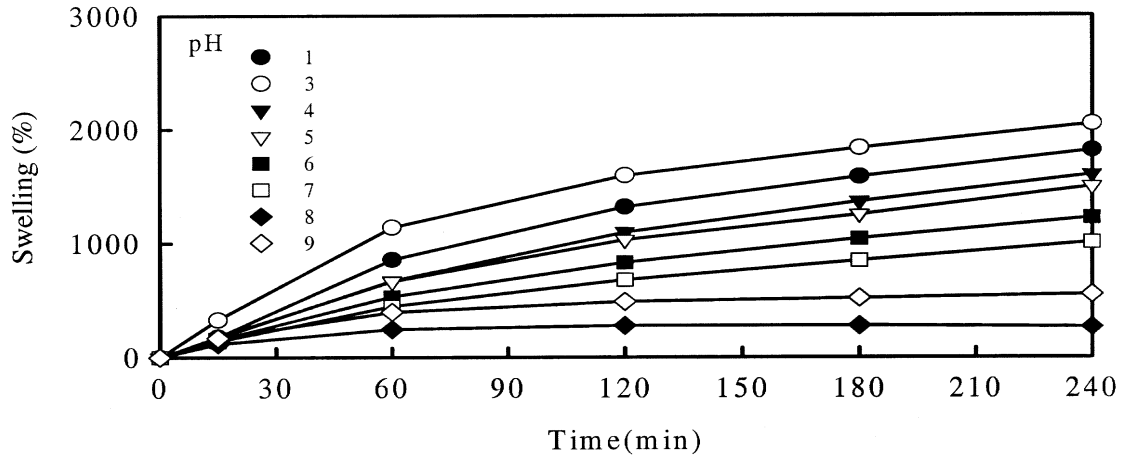


FIG. 22. Effect of time on the swelling percent of PVA-co-NIPAAm at different pH's. Reactant composition; (PVA:NIPAAm)4:2, Reactant concn.; 25 wt%, Irradiation dose; 20 kGy.

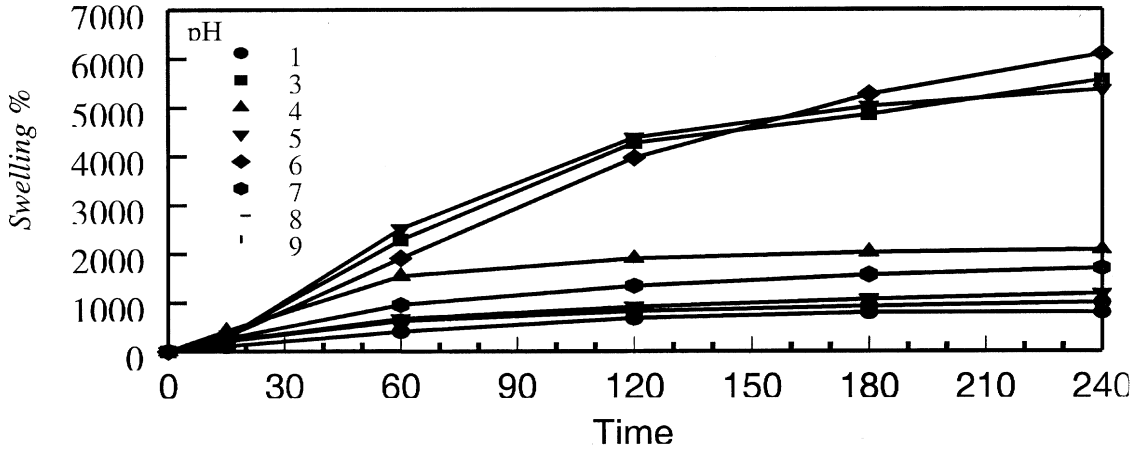


FIG. 23. Effect of time on the swelling percent of PVA-co-NIPAAm-co-DMAEMA at different pH's. Reactant composition; (PVA:NIPAAm:DMAEMA)4:1:1, Reactant concn.; 25 wt%, Irradiation dose; 20 kGy.

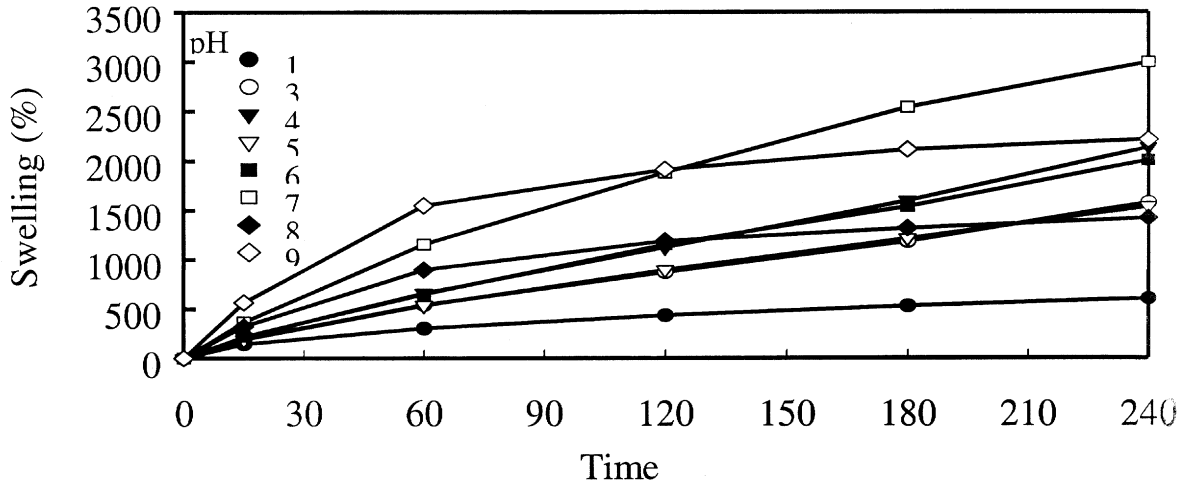


FIG. 24. Effect of time on the swelling percent of PVA-co-NIPAAm-co-AAc at different pHs. Reactant composition; (PVA:NIPAAm:AAc)4:1:1, Reactant concn.; 25 wt%, Irradiation dose; 20 kGy.

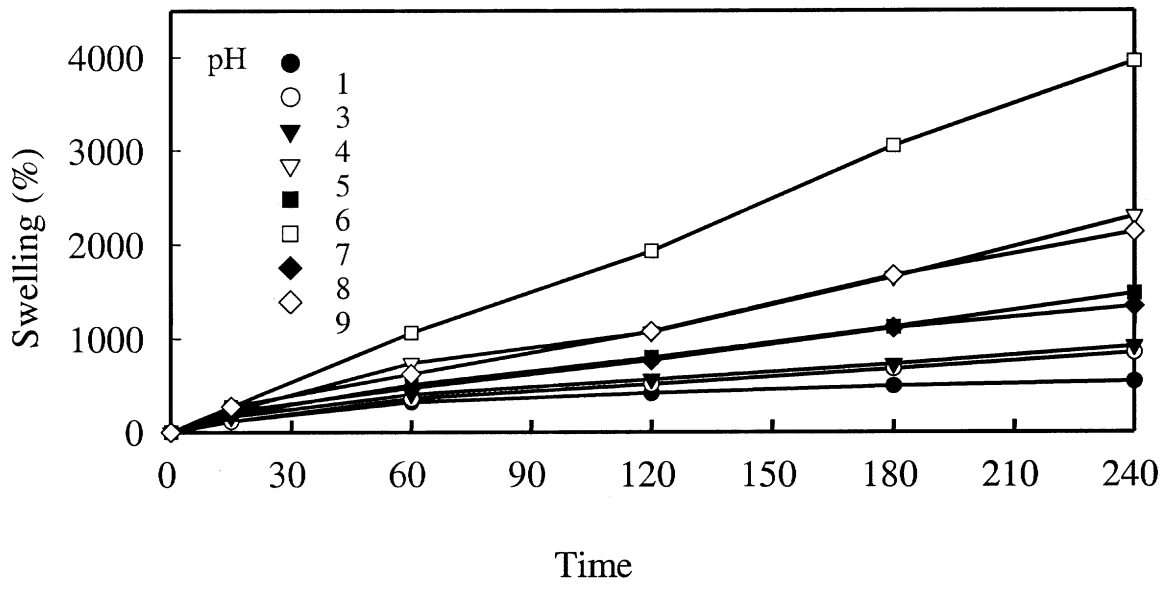


FIG. 25. Effect of time on the swelling percent of PVA-co-NIPAAm-co-MAAc at different pHs. Reactant composition; (PVA:NIPAAm:MAAc)4:1:1, Reactant concn.; 25 wt%, Irradiation dose; 20 kGy.

5. IMMOBILIZATION OF ENZYME BY RADIATION GRAFTED HYDROGELS

5.1. Radiation immobilization of invertase by means of entrapping method

Introduction of enzymes in medical therapies has developed recently, even if slowly. The new technology of protein engineering offers opportunities in obtaining many proteins and drugs as well as modifying their properties such as stability, activity and often specificity. In this respect, a study has been made on the immobilization of invertase by radiation induced polymerization of poly (vinyl alcohol) solution. Acrylamide was also studied using NN-methylene -bisacrylamide (BAAm) as a crosslinking agent.

In order to avoid the deactivation of invertase, the effect of ionizing radiation on invertase enzyme in native and immobilized forms was investigated to determine the dose at which the activity of the enzyme decreased. It was found that the inactivation of invertase by γ□rays was very slow at room temperature and the activity of immobilized enzyme decreases when it exposes to more than 40 kGy.

According to these results, the inactivation of enzyme by radiation could be avoided by adopting the irradiation dose (Fig. 26).

5.2. Effect of crosslinking agent on the Activity of the enzyme

The main effect of the crosslinking agent is therefore to reduce the degree of equilibrium swelling achieved by the network under experimental condition. The effect of crosslinking agent on the activity of enzyme was studied to show that as the content of crosslinking agent increases the relative activity of the enzyme decreases (Fig. 27). The effect of incubation time on the relative activity of immobilized enzyme was investigated to find that it reaches its maximum value after 60 min.

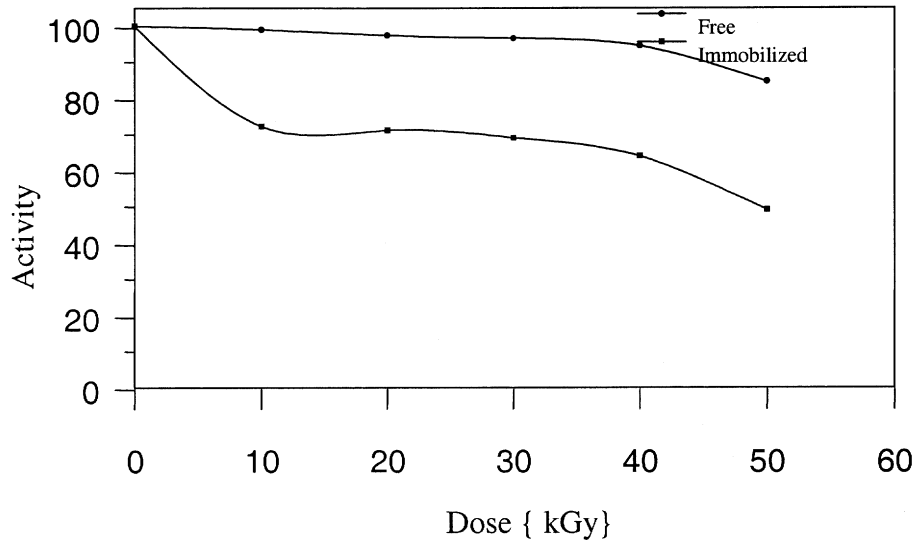


FIG. 26. Effect of iIrradiation dose on the activity of free and immobilized invertase enzyme. The composition of (AAm/PVA/BAAm) terpolymer gel; (57/38/5 wt%). Invertase concn.; 1 mg/ml, dose rate;0.25 Gy/S., incubation time;1 h. at 55 ^{o} C and pH4.5.

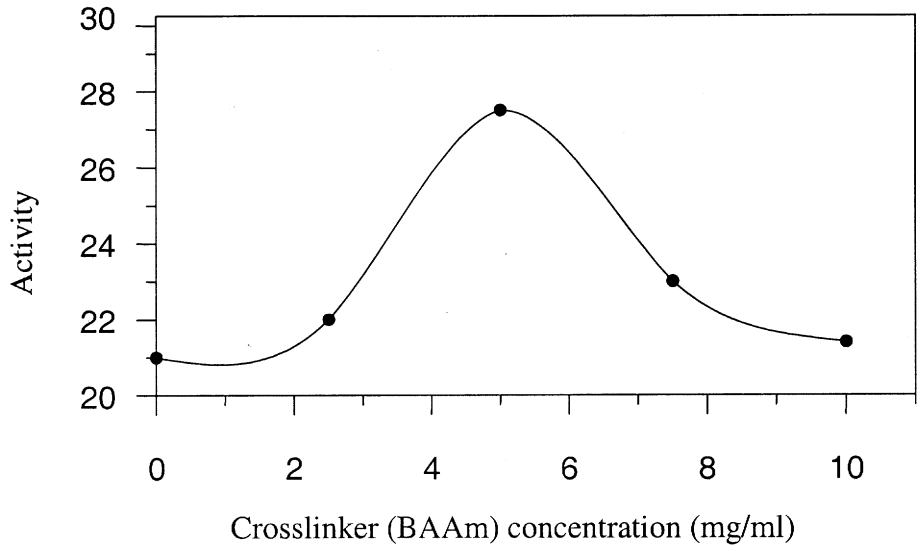


FIG. 27. Effect of crosslinker concentration on the activity of the immobilized invertase enzyme. The composition of (AAm/PVA/BAAm) terpolymer gel; (57/38/5 wt%), invertase concn.; 1 mg/ml, irradiation dose; 10 kGy, dose rate; 0.25 Gy/s.

5.3. Physico-chemical properties of immobilized invertase

The pH effect on the activity of the immobilized invertase was investigated to find that the optimum acting at pH 4.5 which is similar to the free one. This means that the matrix does not affect the optimum pH dependence of enzyme (Figs 28,). The suitable temperature for maximum activity of immobilized enzyme was shifted to 55 ^{o}C (Fig. 29). Michales-Menten constant of immobilized invertase was calculated. The K_m value of the immobilized enzyme is about 2 times higher than that of free invertase. This may be due to diffusion limitation and lower affinity of substrate to the matrix (Fig. 30).

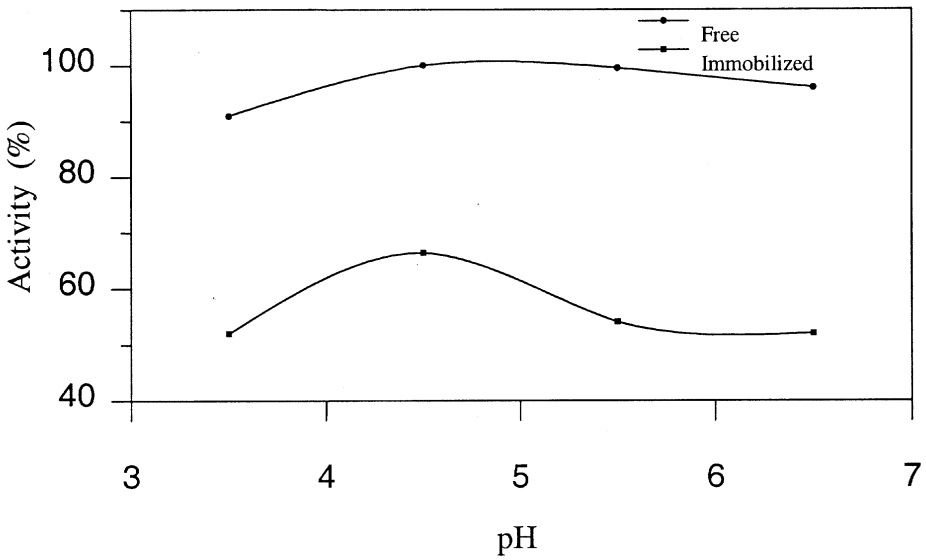


FIG. 28.Effect of pHon activity of free and immobilized invertase enzyme. The composition of (AAm/PVA/BAAm) terpolymer ge; (57/38/5 wt%), invertase concn.; 1 mg/ml,irradiaton dose; 20 kGy and dose rate; 0.25 Gy/s., incubation time; 1 h. at 55 oC.

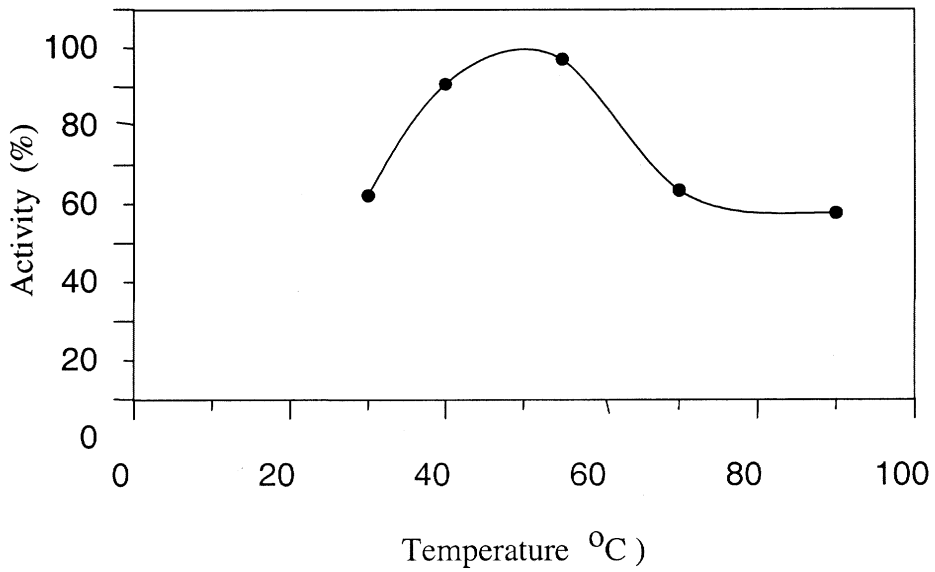


FIG. 29.Effect of temperature on activity of immobilized invertase enzyme. The composition of (AAm/PVA/BAAm) terpolymer gel; (57/38/5 wt%), invertase concn.;1 mg/ml, irradiation dose; 20 kGy and dose rate; 0.25Gy/s., incubation time; 1 h. at pH4.5.

6. ADSORPTION OF PROTEINS BY GRAFTED CO-POLYMERS

Radiation induced graft co-polymerization is well known technique to change the physical and chemical properties of supported polymeric materials. Hydrophobic interaction is the most likely mechanism involved in adsorption of proteins, polystyrene (a hydrophobic polymer) surfaces often have high degree of nonspecific protein adsorption. Studies of protein-polyelectrolyte interactions reported so far, have focused on electrostatic interactions. Therefore, trail has been made to graft different hydrophobic and hydrophilic groups onto PP and the adsorption capacity of such grafted films toward protein is investigated.

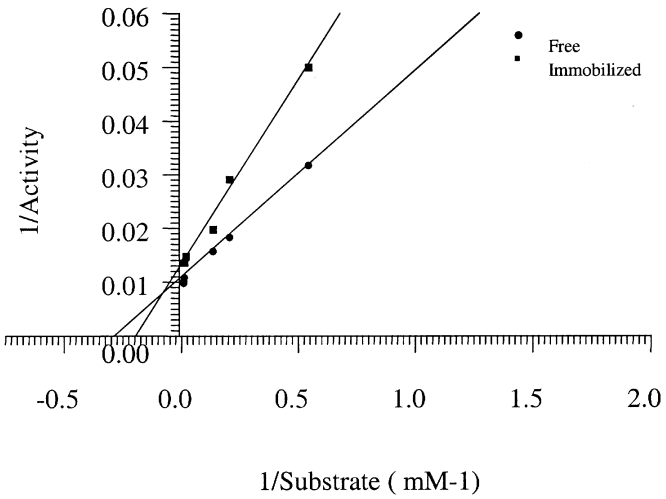


FIG. 30.Linweaver-Burk plot for determining K m for free and immobilized invertase enzyme. The composition of (AAm/PVA/BAAm) terpolymer gel; (57/38/5 wt%), invertase concn.; 1 mg/ml, irradiation dose; 20 kGy and dose rate; 0.25 Gy/s.

6.1. Effect of functional groups

Fig. 31 shows the capacity of the different hydrophilic and hydrophobic functional groups toward adsorption of bovine serum albumen (BSA). It can be seen that the presence of hydrophilic group beside hydrophobic one decreases the adsorption of the protein and the adsorbed protein by PP-g-(Sty/MAn) is lower than that of PP-g-(VAc/MAn). Also the PP-g-(Sty/MAn) treated with aspartic acid adsorbs protein more than the non-aspartic treated one.

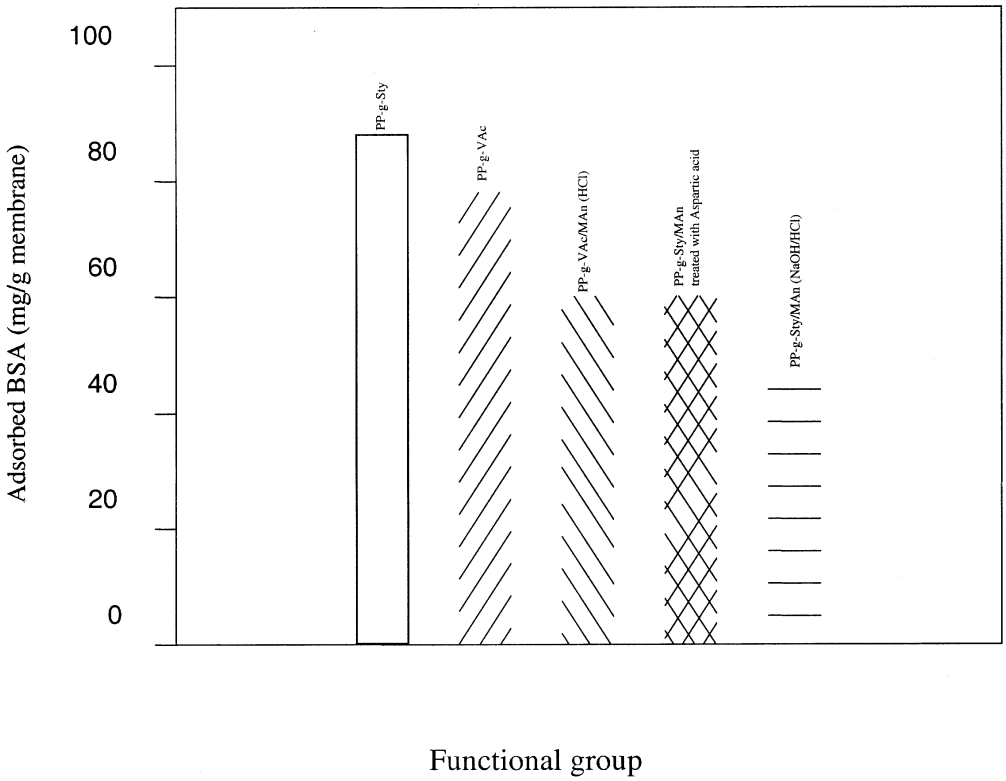


FIG. 31.Protein adsorption by grafted polypropylene membranes having different functional groups. Initial concn. of BSA; 2.5 mg/ml in phosphate buffer, soaking time; 24 h.

6.2. Effect of grafting yield on the adsorption of protein

Fig. 32 shows the BSA adsorption against the degree of grafting, for PP-g-(Sty/MAn) and PP-g-(VAc/MAn). It can be seen that as the percent graft increases the protein adsorption decreases for both systems. The results can be attributed according to that the protein adsorption is a function of chain length — the longer the chain of the polymer the more effective in preventing protein adsorption. Also, as the degree of grafting increases the percent of water content increases resulting in a decrease in protein adsorption.

6.3. Effect of BSA initial concentration and ionic strength

Figs 33a, 33b show that the BSA adsorption capacity initially increases as the initial BSA concentration increases. This behaviour is observed for the grafting and polymers investigated. The influence of external strength on such adsorption process is also investigated to find that as the ionic strength increases, the BSA adsorption decreases. This can be attributed due to the binding of more ions to BSA molecules which may causes a conformational changes in the protein structure and increases its solubility which lead to less adsorption.

6.4. Effect of pH on the adsorption of protein

The effect of pH on the BSA adsorption onto PP-g- (Sty/MAn) and PP-g-(VAc/MAn) and their treated ones was investigated The variation of adsorbed BSA/g dry hydrogel is given in Figs (34a) and (34b). It is generally observed that the maximum adsorption of BSA for PP-g-(Sty/MAn) is around isoelectric point of the protein, which is approximately 4.7. However, the maximum adsorption for PP-g-(VAc/MAn) increases with pH than that for PP-g-(Sty/MAn)system.

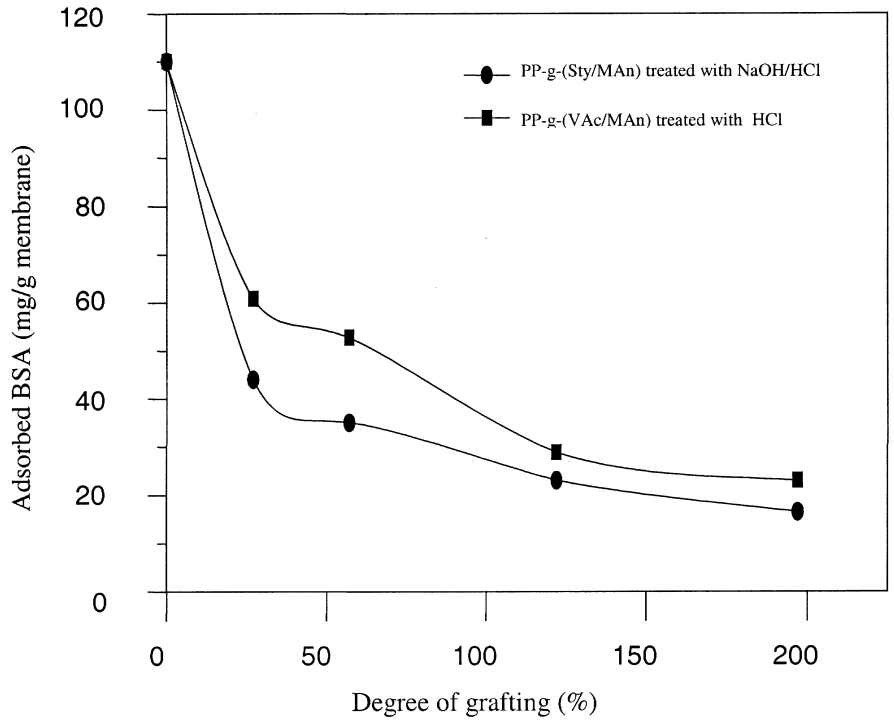


FIG. 32. Effect of grafting percent on protein adsorption. BSA Initial concentration; 2.5 mg/ml in phosphate buffer, pH; 4.7, soaking time;24 h.

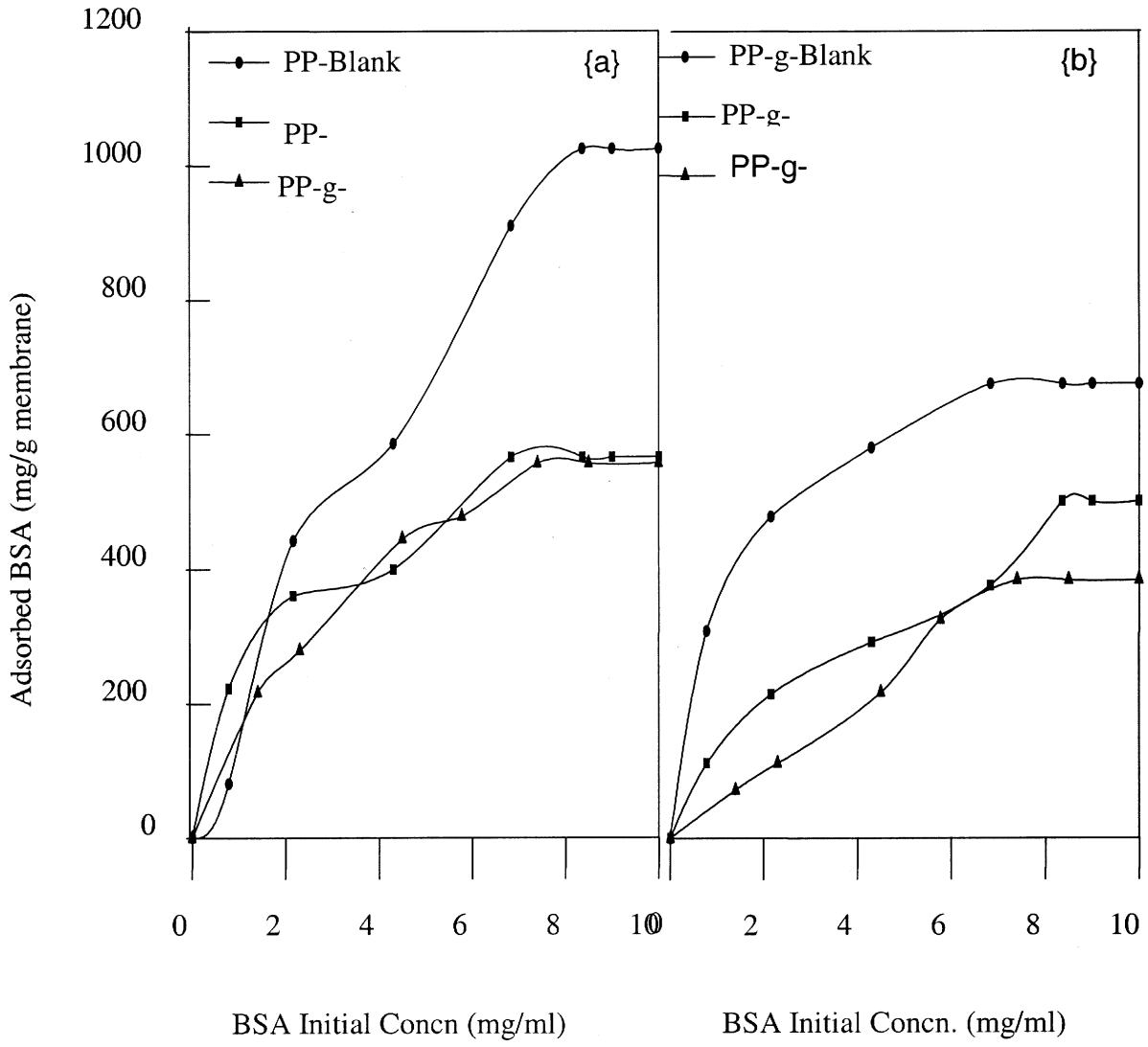


FIG. 33.Effect of Initial concentration of BSA on its dsorption at different ionic strength; (a) 0.01 M NaCl, (b) 0.05 M NaCl, pH = 4.7 for PP-blank and PP-g-Sty/MAn. pH = 8 for PP-g-VAc/MAn. Soaking time; 24 h.

Results can be attributed according to the possible electrostatic interactions between positively charged BSA and negatively charged polymer chains. However, for the PP-g-(Sty/MAn) system the maximum adsorption of protein is reached to the isolelectric point, although the dissociation of MA at this value (pH4.9) is approximately 50%. Several polyelectrolytes as styrene, maleic acid co-polymer are known to undergo a conformational transition in a specific and very narrow pH range. It was reported that the Sty/Maleic complex shows a rapid increase in solution viscosity at pH = 4.5. This polyelectrolyte property is distinct from those of other polycarboxylic acids as poly (vinyl acetate/Maleic acid) and poly (methylvinly ether/Maleic acid)co-polymer[44]. These results suggest that the co-polymer of styrene and Maleic acid undergoes a conformational transition from a tightly coiled chains to an extended one at pH = 4.5. The tightly coiled form is probably adsorb protein more than that of extended one.

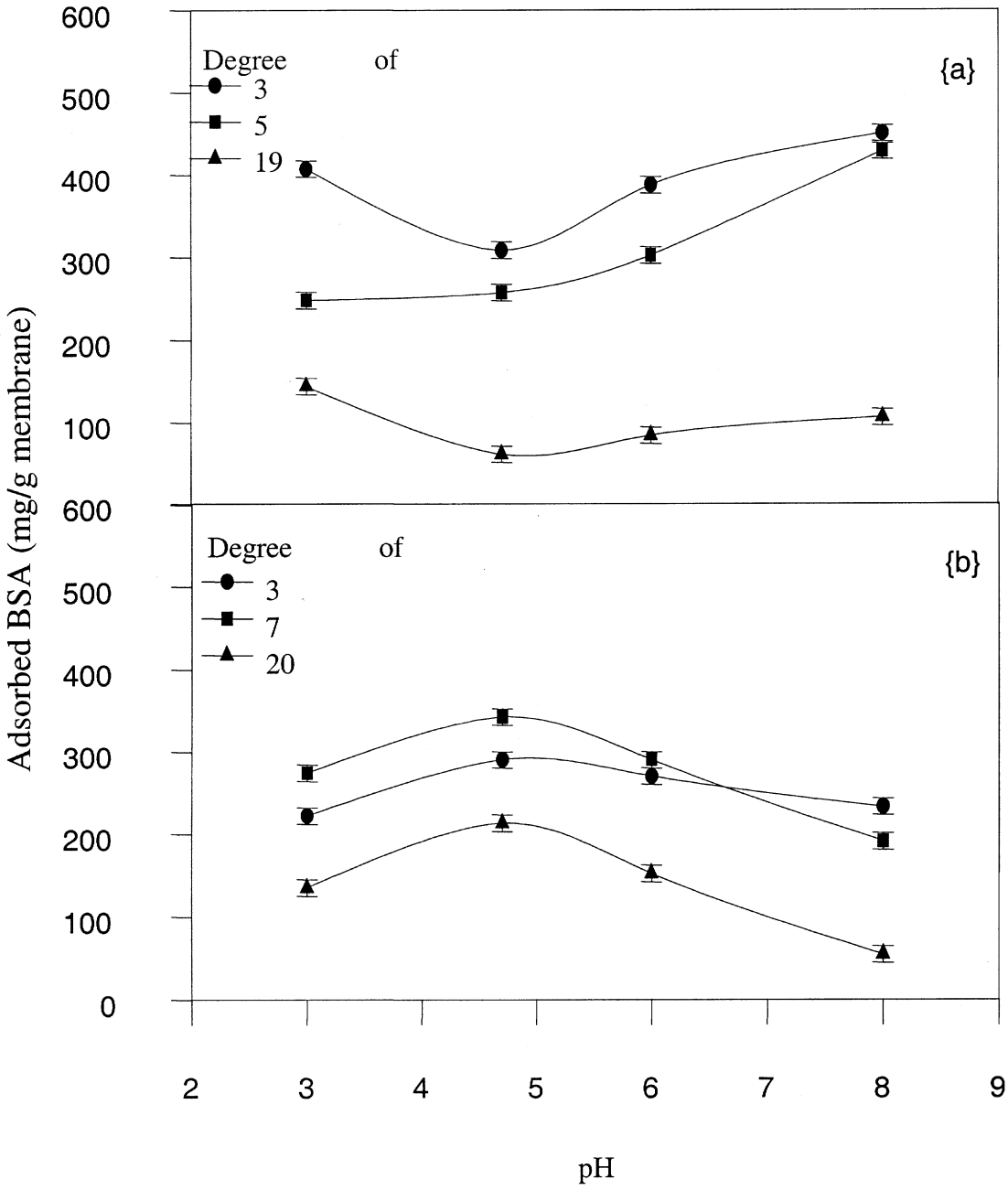


FIG. 34.Effect of pH on protein adsorption at different degrees of grafting by: {a} PP-g-(VAc/MAn)HCl/Heat. {b}PP-g-(Sty/MAn)NaOH/HCl/Heat. BSA Initial concentration; 2.6 mg/ml, soaking time; 24 h.

7. CONCLUSION

Different polymeric materials and monomers were used for the preparation of hydrogels. Characterization and possibility of their practicable uses in biomedical applications were studied. An attempt has been made to gain insight into the biomedical uses of polypropylene-g-poly (vinyl acetate/maleic anhydride). Results showed that such materials seem to be inert Consequently, they might be used as biocompatible materials. For hemodialysis application, the effect of degree of grafting and types of cationic and anionic groups introduced into the grafted polymer on the permeability of different solutes through such membranes was studied.

The grafted polymers are of great interest in the field of separation of toxic materials from blood. Some pH and thermo-responsive polymers were prepared and investigated for drug deleviry. The swelling of (MAAc/BA) co-polymer is a highly pH dependent. At low pH, the swelling is less than 10%, however, at high pH the swelling is higher than 300%. This property makes such co-polymer system to be used as pH-sensitive hydrogel for drug delivery systems. The introduction of the enzyme in medical therapies has been made recently. Immobilization of invertase by radiation induced polymerization of poly (vinyl alcohol) solution and acrylamide was studied as a technique that offers opportunities of obtaining many properties such as stability, activity and often specificity. Different hydrophobic and hydrophilic groups were grafted onto PP to find out that the capacity of hydrophobic functional groups toward adsorption of bovine serum albumen (BSA) is higher than that of hydrophilic ones. The results also showed that as the percent graft increases the protein adsorption decreases for both systems. It can be concluded that some of the prepared hydrogels may be of interest for the practicable uses as biomaterials, especially in the field of drug delivery systems and immobilization of enzymes and protein adsorption.

REFERENCES

[1] N.A. PLATE, A.E. Vasil'ev Fiziologicheski-Aktivnye Polimery (Physiologically Active Polymers) (Moscow: Khimiya, 1986).

[2] S.E. PISKIN, A.S. HOFFMAN (EDS.) Polymeric Biomaterials, Martinus Nishof Publ.New York: (1986).

[3] J. ANDRADE (ED.) *Surface and Interfacial Aspects of Biomedical Polymers,* Plenum Press New York (1985)

[4] V.Y. KABANOV, Russian Chemical Reviews, 67 (9) 783 (1998).

[5] J. B. PARK, biomaterials science and engineering plenum press, NEWYORK (1984)

[6] R.M. KENDED, A Textbook of Biomedical Engineering (1980).

[7] B. JANSEN, biomaterials and biomechanical (1983), (VAN der PERRE, P.G., AUBERT, A.E., Eds.), ELSEVIER Science Publisher B.V., AMESTERDAM (1984).

[8] S.W. SHALABY, A.S. HOFFMAN, B.D. RATNER, T.A. HORBELT, Polymers as Biomaterials, Plenum Press, New York (1984).

[9] C.G. BEDDOWS, M.H. GIL, J.T. GUTHRIE, Biotechnology and Bioengineering, **XXVII**, 579(1985).

[10] J.A. HUBBEL, J controlled release, **39**, 305(1996).

[11] M. JENDRYCHOWSKA-BONAMOUR J. POLYM. Sci., Part A, Polym. Chem., 21, 2589 (1983).

[12] J.P. FISCHER, H. FUHGE, K. BURG, N. HEIMBURGER, ANGEW. Makromot. Chem. 105, 131 (1982).

[13] J. DOBO, Acia Chim. Acad. Sci. Hung., 63, 453 (1970).

[14] H. MAEDA, H. SUZUKI, A. YAMAUCHI, A SAKIMAE, BIOTECH. Bioeng., 17 119 (1975).

[15] I. KAETSU, M. KUMAKURA, M. YOSHIDA, M. HIMEI, M. TANIIRA, K. HAYASHI, Radiat. Phys. Chem. **14**, 595 (1979).

[16] J. ROSIAK, K. BURCZAK, T. CZOLEZYNSKA, W. PEKALA, *Radial. Phys. Chem.,* 22, 917 (1983).

[17] J.M. ROSIAK, P. ULANSKI, L.A. PAJEWSKI, F. YOSHII, K. MAKUUCHI, Radiat.Phys. Chem. **46**, 161 (1995).

[18] D.C WEBER, T. KON, G.L. MARVA, Y., NOSE, Appl. Polym. Sci., 14, 197 (1970).

[19] D.W. MONK, E., WELLISCH, J. Appl. Polym. Sci., 18, 2875 (1974).

[20] L.T., ROZELLE, R.J. PETERSON, R. D. CORNELIUSSEN, Appl. Polym. Sci., **14**, 181 (1970).
[21] T. KANAMORI, K. SAKAI, M. FUKUDA, Y. YAMASHITA, J.Appl. Polym. Sci., **55**, 1901 (1995).
[22] KAETSU, J. MOL. Catal. 23 1 (1984).
[23] C.G. BEDDOWS, M.H. GIL, J.T. GUTHRIE, J. APPI. Polym. Sci., 35, 135 (1988).
[24] J. LIDDY, J.L. GARNETT, S. KENYON, J. Polym. Sci., Polym. Symp., 49, 109 (1975).
[25] S. DEVI, J.T. GUTHIIE, C. J.BEDDOWS, Radial. Phys. Chem., 36, 697 (1 990).
[26] S. DEVI, J.T. GUTHRIC, C. J. BEDDOWS Radial. Phys. Chem. 36 703 (1990).
[27] H. ALLCOCK, R. RUCHER, K. VISSCHER, Biomaterials, 15, 502 (1994).
[28] H. MAEDA, H. SUZUKI, A. YAMAUCHI, A. SAKIMAE, Biotech. Bioeng., 17, 119 (1975).
[29] M. YOSHIDA, M. KUMAKURA, I.KAETSU Polymer, 19, 137 (1978).
[30] M. CARENZA, S. LORA, O. SCHIAVON, F. VERONESE, Radial. Phys. Chem. 42, 897 (1993).
[31] S. TAUNEDA, K. SAITO, T. SUGO, K. MAKUUCHI, Radial. Phys. Chem., 46, 239 (1995).
[32] I. KAETSU, M. KUMAKURA, Radial. Phys. Chem., 30, 263 (1987).
[33] N. NAGAOKA, A. SAFRANJ, M. YOSHIDA, H. OMNICHI, H. KUBOTA, R. KATAKAI, Macromolecules, 26, 7386 (1993).
[34] A. SAFRANY, Nucl. Instrum. Methods Phys. Res., 131 B 376 (1997).
[35] YI MIN, LI JUN, HA HONGFEI, Radial. Phys. Chem., 46, 855 (1995).
[36] S.H. GEHRKE, Advances in polymer Science, Vol. 110, 81.
[37] A. WIRSEN, M. ORIANDER, A. ALBERTSSON, *Biomaterials* 17, 1881 (1996).
[38] K. HAYASHI, N. YAMAMOTO, I. YAMASHITA, *Bull. Gov. Ind. Res. Inst., Osaka,* 35, 117 (1984).
[39] M. RAZZAK, Y. TABATA, K OTSUHATA, *Radiat. Phys. Chem.,* 42, 57 (1993).
[40] A. SAFRANY, A. DEELDER, Rad. Phys. Chem. 55, 681 (1999).
[41] A.M. DEELDER, N. DE JONGE, O.C BOERMAN, Y.E. FILLIE, G.W. HILBERATH, J.P. ROTMANS, M.J. GERRITSW, D.W.O. SCHUT, Am. J. Trop. Med. Hyg, **40**, 268(1989).
[42] J. PIPOTA, V. PLACEKVACEK, K. BURJANOVA, Radiate. Phys. Chem., 42, 949. (1993).
[43] J.H. LEE, BJ JEONG, HB LEE BIOMED. Mater. Res. **34**, 105 (1997).
[44] E. KOKUFUTA, Polymer 21, 177 (1980).

RADIATION CO-POLYMERIZATION AND ITS APPLICATION IN BIOTECHNOLOGY

Ha Hongfei, Zhai Maolin, Li Jun, Yi Min*

Department of Technical Physics, Beijing University, China

Abstract. The main results and achievements that have been done for the full duration of CRP reported as follows:

(1) Radiation preparation of co-polymers, interpenetrating polymer networks and their applications in separation technology.
(2) Preirradiation grafting co-polymerization of NIPAAm and other monomers on cotton cellulose fabric, silicone rubber etc and the discussion on mechanism.
(3) UV-induced grafting and modification of polymers by high LET radiation.

1. INTRODUCTION

The radiation preparation and characteristics of the thermally reversible hydrogels of polyN-isopropylacrylamide(PNIPAAm or polyNIPAAm) have been emphasized in recent years, due to their versatile applications in biomedicine, biotechnology and drug delivery systems(DDS). However, as a hydrogel, it is too soft when it was swollen in aqueous solution. In author's lab for the full duration (1997–1999) of CRP, the co-polymers and interpenetrating polymer networks composed of PNIPAAm and other polymers including natural polymer were prepared by radiation technology and their characteristics such as swelling behaviour, slow release of small molecules and protein were investigated. On the other hand, NIPAAm and some other monomers were grafted on some polymers such as cotton cellulose fabric, silicone rubber and so on by radiation technology to improve the mechanical property and expanded their uses in biotechnology.

In addition, the modification of polymers by UV technology and LET radiation were investigated as well.

These results and achievements have enriched the knowledge of radiation co-polymerization and expanded their applications in biotechnology.

2. RADIATION CO-POLYMERIZATION

2.1. Radiation preparation of co-polymers and use in separation technology

PNIPAAm is a very important kind of temperature sensitive polymers (linear chains) or thermally reversible hydrogels (crosslinking chains) which have a variety of uses in biomedicine and biotechnology. Separation and concentration of Ag^{1+} ions in dilute aqueous solution were realized by using the polymers and hydrogels in author's lab, but it could not be used for heavy metal ions. Some PNIPAAm-based co-polymers solved this problem successfully.

---

* With the assistance and collaboration during the period 1997–1999 of the following students: Jiang Guilin, Wang Ruiyu, Liu Jianqin, Lu Xuequan, Lu Jun, Liu Ning, Guo Kai, Wu Qingquan and Shu Jian.

2.2. Radiation synthesis of binary linear co-polymers and concentration of Er^{3+}, Cr^{4+} and UO_2^{2+} in dilute aqueous solution

2.2.1. Radiation synthesis

Binary linear co-polymers, polyNIPAAm/AAc were synthesized by γ-radiation co-polymerization with total dose 3kGy, dose-rate 38.4Gy/min at room temperature in THF solution of NIPAAm and acrylic acid (AAc) with ratio of 9:1 (mass ratio). The product synthesized here is white powder and possessed of temperature sensitivity

2.2.2. Temperature sensitivity of the linear co-polymers

The co-polymer behaved with temperature sensitivity, whose LCST was affected by pH value of the aqueous solution, due to the existence of $-COO^-$ groups. Fig. 1 showed the relationship between LCST and pH value.

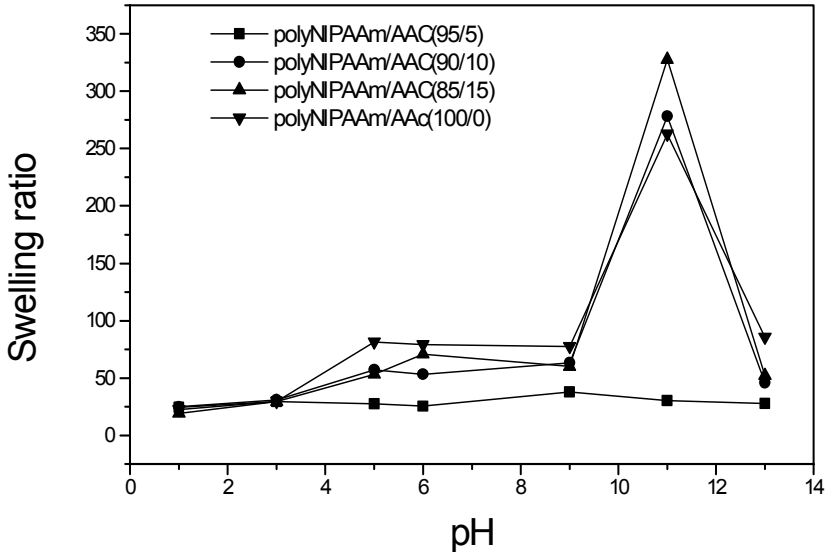


FIG. 1. The swelling ratio of hydrogels as a function of pH.

2.2.3. The concentration of some heavy metal ions

By using the property of solubility and precipitation around the LCST of the co-polymers, the Er^{3+}, Cr^{4+} and UO_2^{2+} in dilute aqueous solution were concentrated and separated successfully. The concentration of co-polymers in solution was 10~15%(mass concentration) and the pH value between 2 and 4

2.3. Radiation synthesis of thermally reversible co-polymer hydrogels polyNIPAAm/x and their applications

2.3.1. Radiation preparation

Poly (NIPAAm/x) co-polymers hydrogels where x is AAc, acrylamide (AAm), N-vinyl pyrrolidone (N-VP) and 4-vinyl pyridine (4-VP) were synthesized by radiation co-polymerization at room temperature. The optimum dose: 3kGy, dose rate: 50Gy/min. The ratio of NIPAAm and other monomers was from 100:0 to 85:15 (mass ratio). All the prepared hydrogels were transparent, elastic and have good mechanical property.

2.3.2. Thermally reversible

The co-polymer hydrogels were thermally reversible but the LCST was different according to the kind of second polymers shown on Table I.

TABLE I. THE LCST OF polyNIPAAm/x

X	LCST/℃
0	35.5
AAc	38.0
AAm	46.0
N-VP	40.2
4-VP	28.0

2.3.3. The concentration of UO_2^{2+}

The co-polymer hydrogels of polyNIPAAm/AAc could be used in concentration of UO_2^{2+} in aqueous solution. The concentrating yields as a function of time and the possible structure of product obtained showed in Fig. 2 and Fig. 3.

Radiation preparation of some interpenetrating polymer networks including natural polymer as the second component.

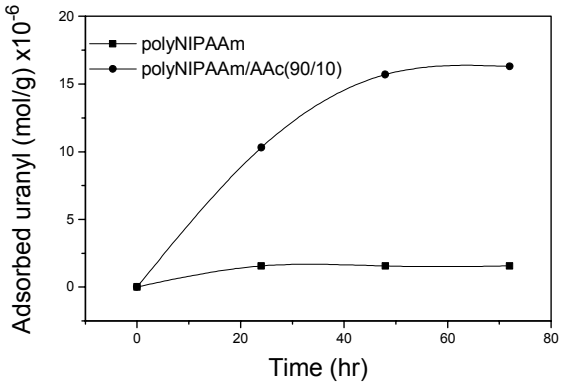


FIG. 2. The adsorbed uranyl per gram.

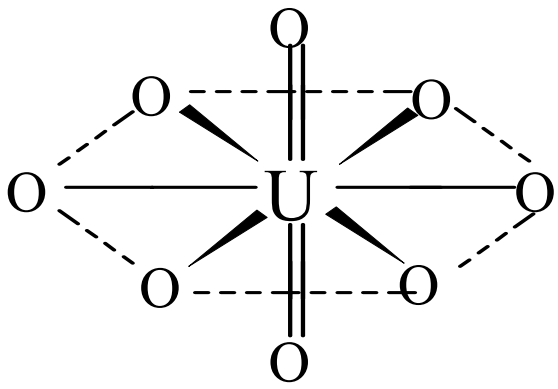


FIG. 3. The structure of uranyl complex ion.

3. RADIATION PREPARATION OF SOME INTERPENETRATING POLYMER NETWORKS INCLUDING NATURAL POLYMER AS THE SECOND COMPONENT

It is well known that the mutil-component polymers, such as the interpenetrating polymer networks (IPNs) which have the microphase-separated structure, exhibit good blood compatibility when they are used in biomedicine, biotechnology and DDS. Here the authors are interested in developing the IPN hydrogels in which the temperature sensitivity of the main component, polyNIPAAm could be effectively kept by changing the content of second components in the IPNs. Two kinds of IPNs were prepared by γ-irradiation technology and some of their properties were investigated.

3.1. Semi-IPNs hydrogels composed of PNIPAAm and hydrophilic polymers

3.1.1. Radiation preparation

In this work, T, C and S were defined by

$$T = (W_m + W_c + W_p)/V_s \times 100\%,\ C = W_c/W_m \times 100\%,\ S = W_p/W_m \times 100\%$$

where W_m, W_c and W_p are weight of monomer (NIPAAm), crosslinking agent(Bis) and polymer, respectively. V_s is the whole volume of the solution (mL). Semi-IPNs hydrogels have been prepared by radiation of aqueous solutions of PNIPAAm and different hydrophilic polymers at room temperature.

3.1.2. The effect of linear hydrophilic polymer components on the swelling behaviour of semi-IPNs

From Fig. 4 it can be seen that the incorporation of polyNaAAc in semi-IPN hydrogels had much higher swelling ratio than pure PNIPAAm. On the other hand, the higher the molecular weight of polyNaAAc used, the greater the swelling ratio of the semi-IPNs.

According to hydrophilicity of the second components in the semi-IPNs, the swelling capacity of polyNIPAAm/polyNaAAc IPNs was higher than that of polyNIPAAm/PVP IPNs and polyNIPAAm/PVP IPNs, respectively (see Fig. 5)

3.1.3. Temperature sensitivity and pH effect of polyNIPAAm/polyNaAAc

The semi-IPNs of polyNIPAAm/polyNaAAc hydrogels still kept obvious temperature sensitivity but the two others did not and the swelling ratio was increasing with pH value in the swelling solutions (see Fig. 6 and Fig. 7).

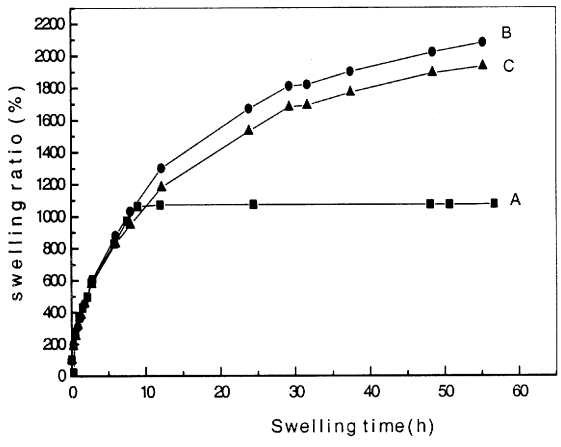


FIG. 4. The effect of polyNaAAc on the swelling behaviour of semi-IPNs at room temperature. A: polyNIPAAm, B: polyNIPAAm/polyNaAAc(Mw = 16000–18000) C: polyNIPAAm/polyNaAAc (Mw = 7000–8000), 3kGy, 103 Gy/min.

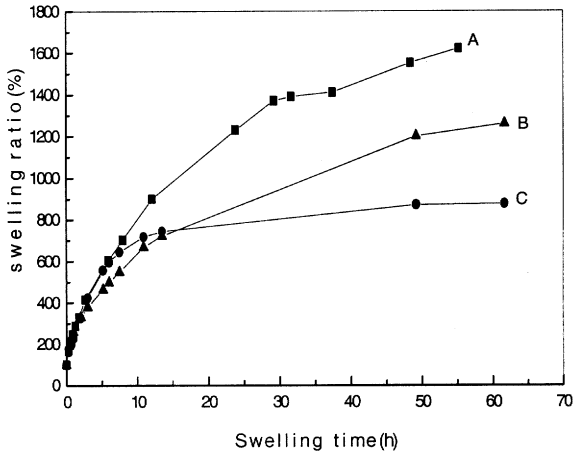


FIG. 5. Comparative swelling behaviour curves of semi-IPNs polyNIPAAm/x hydrogels at room temperature. X = polyNaAAc(A), PVA(B), PVP(C) 5kGly, 103 Gy/min.

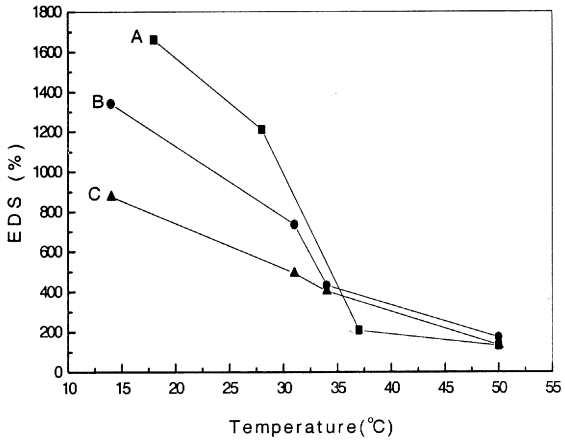


FIG. 6. Comparative EDS-T curves of semi-IPNs polyNIPAAm/x hydrogel, x = polyNaAAc(A), PVA(B), PVP(C), 5kGy, 103 Gy/min.

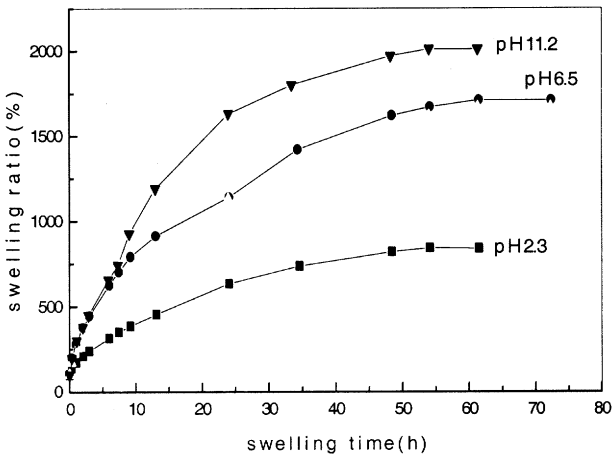


FIG. 7. pH effect on the swelling behaviour of semi-IPNs polyNIPAAm/polyNaAAc at room temperature.

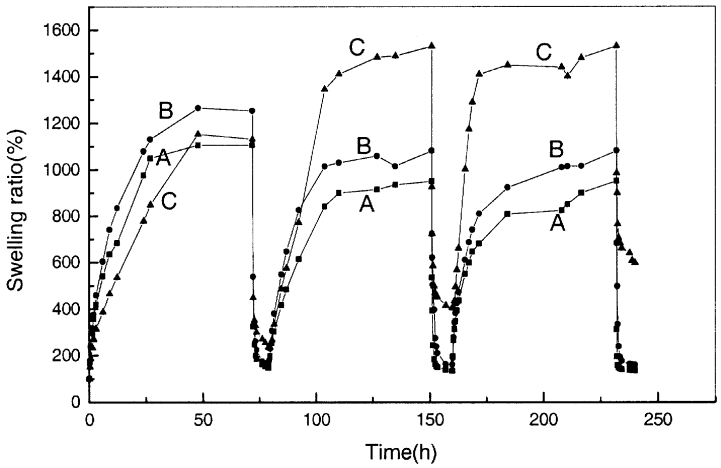


Fig.8 The swelling-shrinking curve of semi-IPNs polyNIPAAm / PAAc hydrogel
A: S = 5%, B: S = 10%, C: S = 15%, 15kGy, 103Gy/min
swelling at room temperature, shrinking at 40°C

3.1.4. The Swelling-shrinking cycles

All the semi-IPNs of polyNIPAAm/PAAc with different content of second polymers still kept obviously thermally reversible property after several swelling-shrinking cycles but the time for swelling to equilibrium was larger than that for shrinking (see Fig. 8)

3.2. The IPNs composed of polyNIPAAm and PMMA

3.2.1. Radiation preparation

The IPNs were prepared by the sequential method. 16.8kGy of dose was used to form matrix polymer polyNIPAAm. The second step was to swell the pieces of polyNIPAAm dry gel in DMSO solution of MMA. The swollen polyNIPAAm were irradiated again with 8.4kGy. Purification and drying treatment.

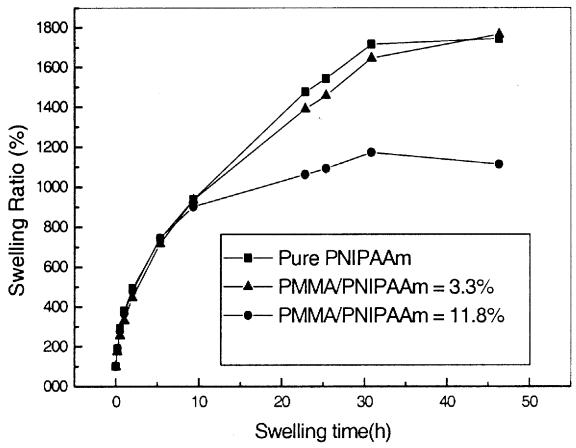


Fig.9 The effect of content of PMMA on the swelling behavior of PNIPAAm/PMMA IPN hydrogel

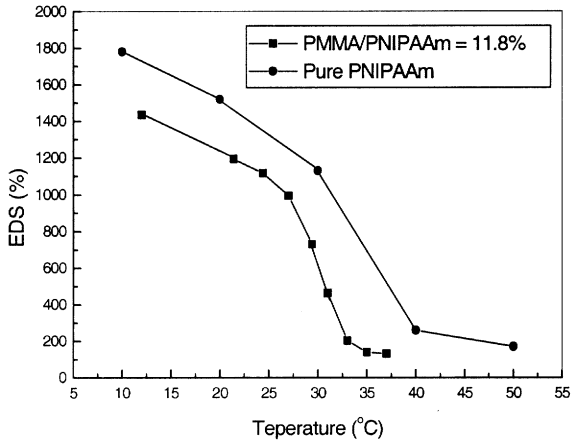


Fig.10 The change of equilibrium degree of swelling(EDS) of the prepared gel with temperature

3.2.2. Swelling behaviour

From Fig.9 it can be seen that with increasing content of PMMA in the IPNs, the swelling ratio of the IPN gels decreased obviously and the time to equilibrium of swelling was shorten as well.

3.2.3. The temperature sensitivity of the IPNs

The IPN hydrogels with PMMA still kept the temperature sensitivity but the LCST was lower than that of pure PNIPAAm due to the hydrophobicity of PMMA (sees Fig. 10).

Swelling-shrinking cycle tests of NIPAAm/MMA IPNs showed that these hydrogels were quite stable after several cycles (see Fig. 11)

3.*2.4. MB release from the IPNs*

The MB release from PNIPAAm hydrogels into water was faster than that from IPN hydrogels that can be explained by structure of IPNs gels with MMA (Fig. 12)

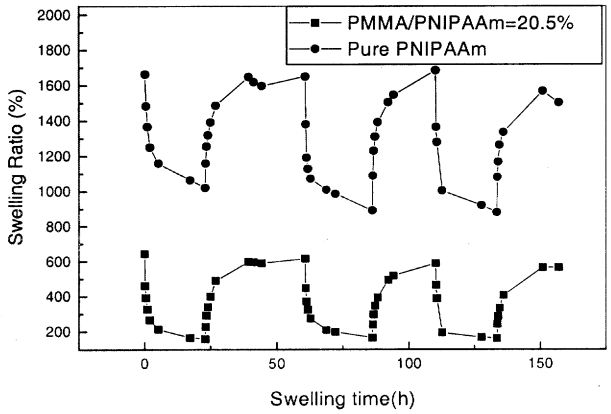


Fig.11 The Shrinking-swelling curve of PNIPAAm and PNIPAAm/PMMA IPN hydrogel. shrinking(37°C), swelling(20°C)

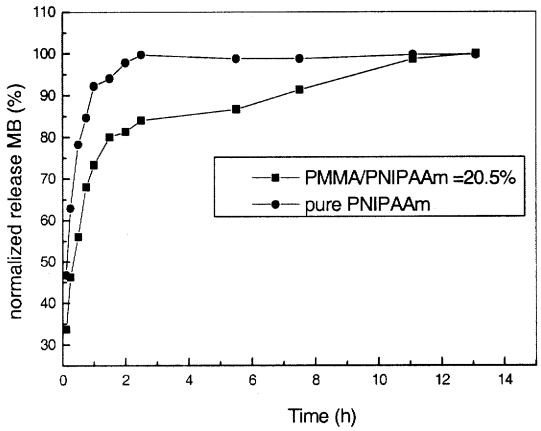


Fig.12 The release of MB at 37°C as a function of time

3.3. Radiation synthesis and characteristic of KC/PVP blend hydrogels

Kappa-Carrageenan (KC) is a kind of natural polymer, polysaccharide, white or light brown pellet/powder. KC can be dissolved in 80°C hot water and formed the transparent and viscous liquid. After cooling to room temperature, KC liquid became KC hydrogel (a kind of thermal reversible gel) which is quite stable and behaves with certain mechanical strength. KC is mainly used in food and drug industry.

In this work a series of blend hydrogels were prepared from KC and PVP by γ-irradiation and some of the improved properties which can be used in biomedical and biotechnology were measured. The discussion of mechanism was given.

3.3.1. Radiation degradation of KC gels

Like the common natural polymer, KC is also a kind of radiation degradation polymer. (see Fig. 13)

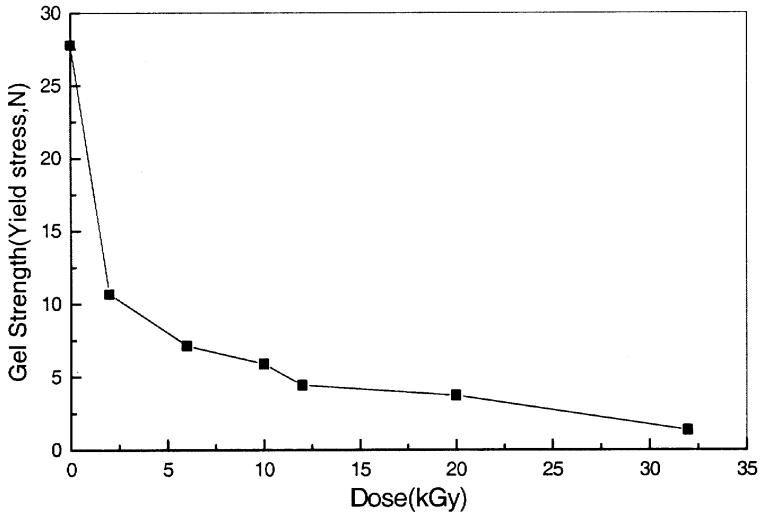


Fig.13 The change of gel strength of 5% KC gel with dose at 2kGy/h

3.3.2. Blend hydrogels composed of KC and PVP

3.3.2.1. Preparation of KC/PVP blend hydrogels

Various contents of KC or KC/PVP aqueous solutions were prepared by dissolving KC or KC/PVP in distilled water at 80°C for 2h. Then the hot mixture was poured into glass tube of 15 mm in diameter. The cooled samples became hydrogels and were irradiated by ^{60}Co γ rays at room temperature.

3.3.2.2. The property tests of the blend hydrogels

The gel strength and swelling ratio of the KC/PVP blend hydrogels appeared unexpected increase simultaneously with the increasing of KC content within a certain dose (see Fig. 14 and Fig. 15).

Besides KC blending with PVP, the radiation preparation of KC/PVP hydrogels by KC mixing with N-VP/14G also be studied. The results indicated that the change of the gel strength and swelling ratio is almost the same as those of hydrogels prepared by KC blending with PVP.

An excellent blend hydrogels can be obtained by adjusting each condition and it would be very useful in biotechnology.

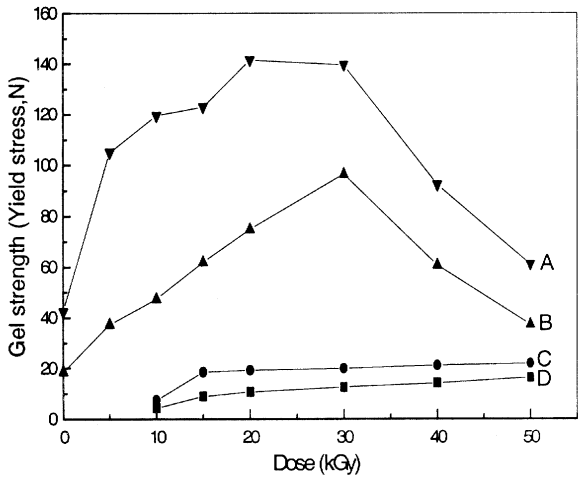


Fig.14 Gel strength of 15% PVP with various concentration KC hydrogel as a function dose. A: 5% KC, B: 3% KC, C: 1% KC, D: without KC

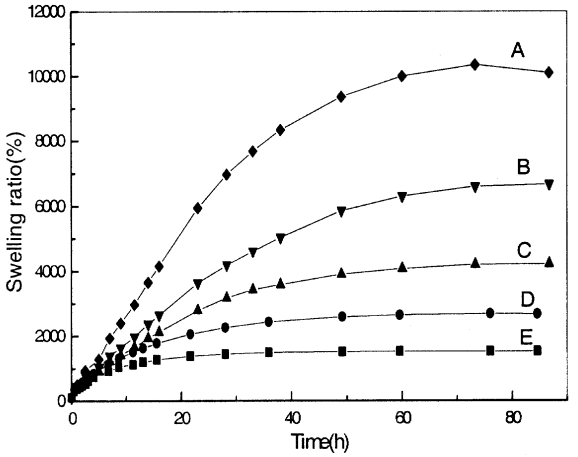


Fig.15 Swelling ratio of KC/PVP hydrogels prepared by KC/PVP with respect to swelling time, A: 5%KC,15%PVP,20kGy, B: 5%KC, 15%PVP, 30kGy, C; 3%Kc,15%PVP,30kGy, D: 1%KC, 15%PVP, 30kGy, E: 15%PVP,30kGy

3.3.2.3. Discussion on mechanism

Before irradiation

In KC/PVP gel the small molecules of N-VP or linear PVP chains were dispersed homogeneously in the network of physical crosslinked KC due to their hydrophilicity

Low doses

During low doses (10~40kGy), the radiation polymerization of N-VP and radiation crolsslinking of PVP (especially in the presence of crosslinkers) would be performed prior to radiation degradation of KC molecules, which means that the molecular chains and physical crosslinking points of KC would be protected by the radiation effect of N-VP and PVP. As polymerization and crosslinking of N-VP and PVP are going on, the IPNs were formed practically.

High doses

When the polymerization of N-VP and crosslinking of PVP have finished, the extra radiation energy would make KC degrade and the structure of KC/PVP IPNs were destroyed which resulted in decrease of gel strength and swelling ratio.

4. PRE-IRRADIATION GRAFTING CO-POLYMERIZATION OF NIPAAM AND OTHER MONOMERS ON COTTON CELLULOSE FABRIC, SILICONE RUBBER, ETC. AND DISCUSSION ON MECHANISM

4.1. Preirradiation grafting of NIPAAm on cotton cellulose fabric and discussion on mechanism

PNIPAAm, a kind of thermally reversible hydrogels, was grafted on some matrix polymers forming the co-polymers which have both good mechanical property and temperature sensitivity on the surface of matrix. As a grafted monomer NIPAAm, some new phenomena were found in preirradiation grafting procedure.

4.1. Cotton cellulose fabric as the matrix

4.1.1. Radiation grafting conditions

Grafting yield as a function of reaction time at different temperature (30~50^{o}C) showed that 3h was enough to finish the grafting reaction in nitrogen. 30kGy of dose could reach reaction equilibrium, the dose rate had no effect on grafting yield and the more concentration of monomer in solution (up to 10%), the higher the grafting yield. 6% monomer in aqueous solution was used in the work.

4.1.2. Effect of reaction temperature on grafting yield

Generally, as reaction temperature increases, the grafting yield should decrease, because the trapped radicals are the main reactive species in preirradiation grafting instead of peroxides formed in samples irradiated with air. Preirradiation grafting of AAm on the cotton cellulose was in accord with this regularity (see Fig. 16 curve 2)

For NIPAAm as the monomer, however, its grafting yields were increasing with reaction temperature (Fig. 16 curve1). It may be induced by temperature sensitivity and special structure of PNIPAAm. In that case peroxides formed in radiation of samples may also attend the reaction together with the trapped radicals as the following reaction.

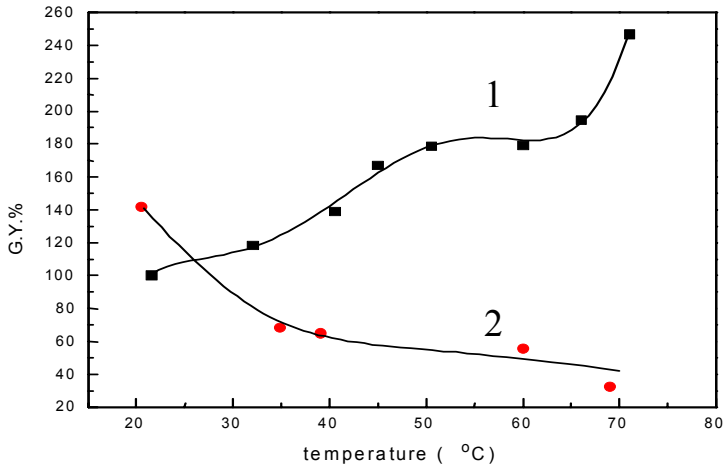
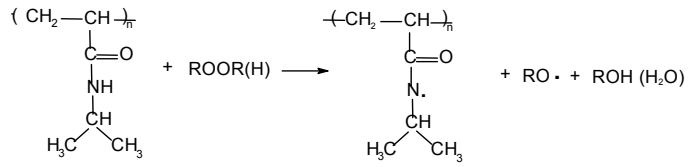


Fig.16: Grafting yield as a function of temperature.
Total dose: 32.3kGy. [Monomer]: 6%.
■ NIPAAm. ● AAm.



The reactive RO· radicals produced in the reaction could induce the extra grafting yields which compensate the decay of trapped radicals.

4.1.3. The location of trapped radicals and development of grafting chains

The trapped radicals are the main reactive species of grafting which was an acceptable conclusion. The problem is where and how the high grafting yield was induced and developed. When the cotton cellulose with about 60% crystallinity was irradiated in air, the free radicals formed in amorphous would react with oxygen immediately and peroxides produced, so the free radicals in amorphous and peroxides formed would not make contribution to grafting at room temperature. The trapped radicals formed in crystalline regions could not make the contribution either because the monomer could not enter into the regions. The trapped radicals in interphase regions between amorphous and crystalline regions, so called the third phases, might be the suitable species to initiate the grafting reaction. Those phases are the unique regions where molecular chains only partly restrained by the crystalline structure nearby, their mobility enable the radicals in those regions to be stabilized. So they are easy to induce the grafting reaction in nitrogen. In addition, the trapped radicals in those regions can be compensated from crystalline regions nearby through immigration of trapped radicals along the molecular chains.

According to this consideration, as grafting chains growing up the decrease of crystallinity would be followed. Measurements of X-refraction of the grafted samples proved this idea (see Fig. 17).

On the view of dynamic procedure of grafting chains developing the grafted chains would intercalate into crystalline regions destroying molecular regularity of surface of crystalline regions step by step. The schematic of this procedure was given in Fig. 18.

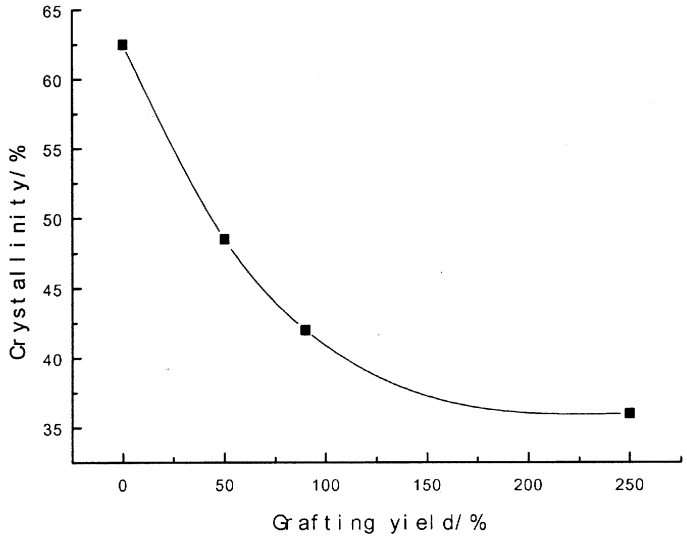


FIG. 17. Crystallinity of the grafted sample as a function of grafting yield of polyNIPAAm.

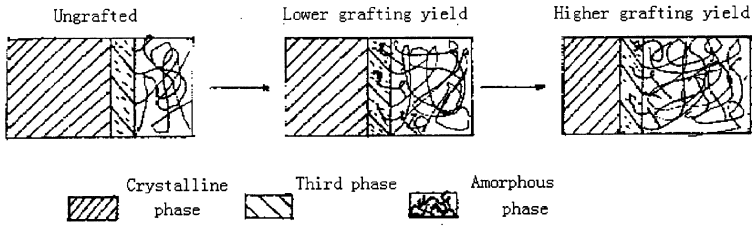


FIG. 18. The scheme of the dynamic procedure of grafted chains developing.

4.2. Silicone rubber as the matrix

4.2.1. Effect of grafting temperature

Pre-irradiation grafting of PNIPAAm on silicone rubber (SR) was completed successfully but the grafting reaction could be done only at higher temperature (see Fig. 19). It means that the grafting reaction was induced by peroxides formed during irradiation of samples in air instead of the trapped radicals like in the case of cotton cellulos

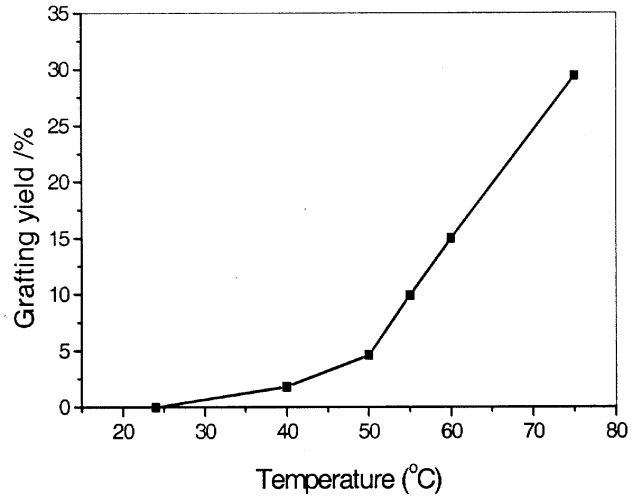


FIG. 19. The effect of temperature on grafting yield Dose: 124kGy; NIPAAm: 10%; grafting time: 5h The thickness of silicone rubber film: 0.5mm.

4.2.2. Production and decomposition of peroxides

Fig. 20 and Fig.21 showed the curves of production and decomposition of peroxides formed during irradiation of samples.

The active energy of peroxide decomposition was calculated which equal to 27.2 kJ/mol.

5. PRE-IRRADIATION GRAFTING OF ACROLEIN ON POLYSTYRENE (PS) AND IMMOBILIZATION OF PROTEIN

Polystyrene spheres are widely used in radioimmunoassay kit but it is limited by weak physical adsorption of protein with surface of PS and hard to control the amount of adsorption of protein. The physical adsorption of protein on the surface of PS was replaced by combination of chemical bond useful to this technology.

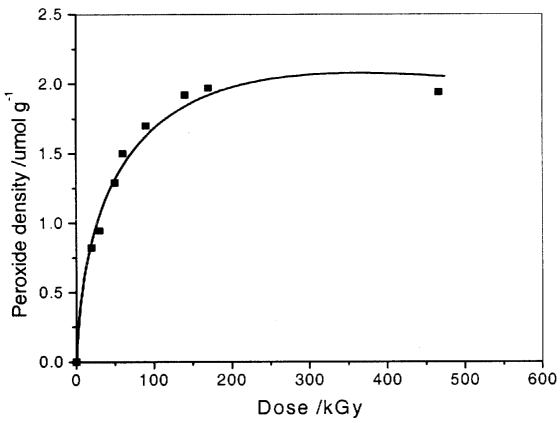


FIG. 20 The effect of dose on peroxide density produced in the SR films. The thickness of silicone rubber film: 0.5mm.

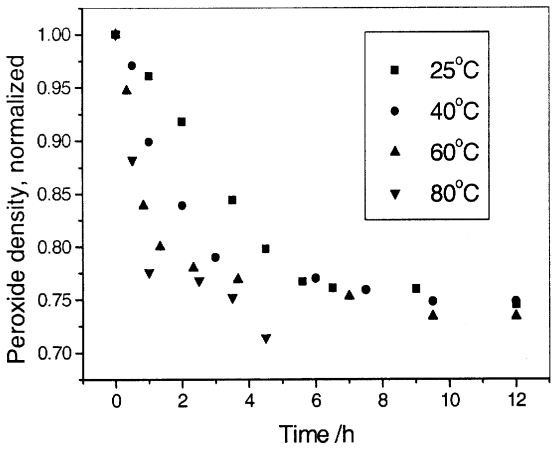


FIG. 21. The effect of storage time in air on peroxides. Dose: 398kGy. The thickness of silicone rubber film: 0.5mm.

5.1. Grafting of acrolein on PS

The aldehyde groups could react with the amino group of protein, so the acrolein was grafted on the surface of PS spheres via γ irradiation. Because the acrolein is easy to induce oxo-addition as side reaction with PS, the grafting efficiency was rather low.

5.2. Immobilization of trypsin and ^{125}I-labeled antibody

The immobilization of trypsin and ^{125}I-labeled antibody on the grafted PS with aldehyde groups showed in Fig. 22 and Fig. 23.

From these results, it can be seen that the radioimmunoassay kit has been improved.

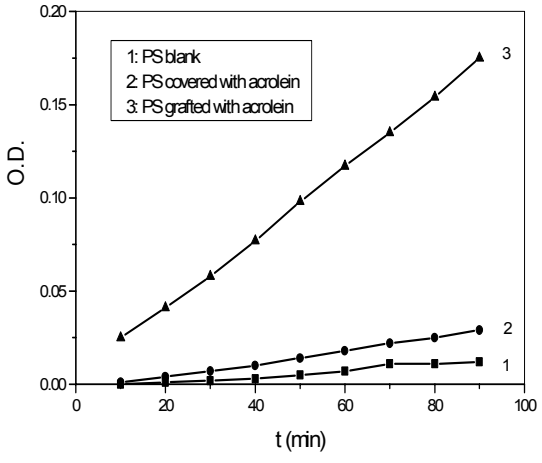


FIG. 22. The activity of different samples treated with trypsin.

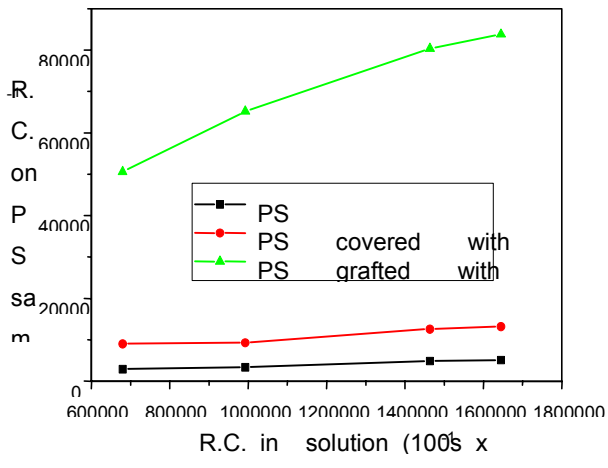


FIG. 23. The effect of protein concentration on the immobilized protein in different samples (R.C. = radiocativity count).

6. RADIATION PREPARATION OF PVA-G-NIPAAM CO-POLYMER AND ITS APPLICATIONS IN CONTROLLED RELEASE

Polyvinylalcohol (PVA) was prepared and used in a variety of chemical, biological separation and biomedical areas. It also can be made as hydrogel through freezing-thawing technology. In this work polyNIPAAm was induced into PVA molecular chains or onto the surface of PVA films in homogeneous or heterogeneous systems, respectively and then the physical crosslinking hydrogels of PVA and PVA-g-polyNIPAAm were made in the same way. The swelling behaviour and slow release of Methylene Blue (MB) from this kind of hydrogels were investigated.

6.1. The effect of total dose and dose rate

The effect of dose and dose rate on grafting yield in both systems were showed in Figs 24 and 25.

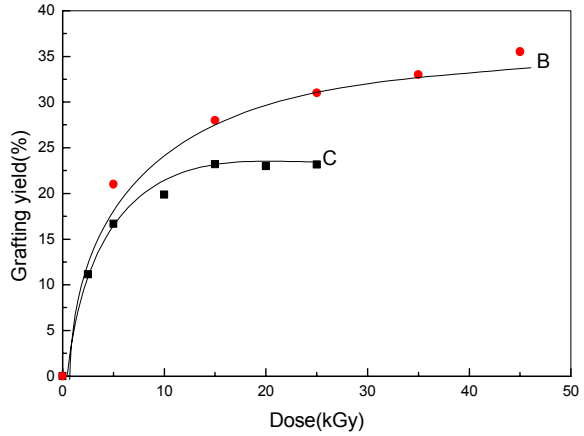


Fig.24 Effect of dose on grafting yield
B: PVA-g-polyNIPAAm copolymer prepared in homogenerous system of DMSO, 102Gy/min, 5%NIPAAm, 5%PVA
C: PVA-g-polyNIPAAm membrane prepared in hetergenerous system of water,103Gy/min, 10%NIPAAm, 0.1mol/L $CuSO_4$

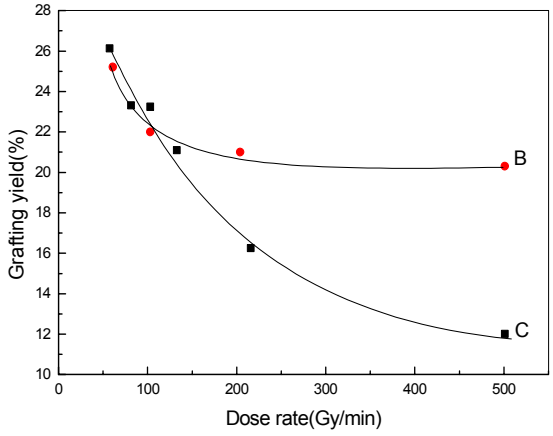


Fig.25 Effect of dose rate on grafting yield
B: PVA-g-polyNIPAAm copolymer prepared in homogenerous system of DMSO, 6.08kGy, 5%NIPAAm, 5%PVA.
C: PVA-g-polyNIPAAm membrane prepared in hetergenerous system of water, 15kGy,10%NIPAAm, 0.1mol/L $CuSO_4$

From the data it can be seen that relationship between dose and grafting yields was similar in both eases but in homogeneous systems the reaction efficiency was higher. However, the decrease of grafting yield with dose rate was more obvious in heterogeneous systems because in this system the grafting reaction was controlled by the diffusion of monomers to polymer films.

6.2. Swelling of PVA and PVA-g-polyNIPAAm in water

From Fig. 26, it can be seen that the EDS of PVA-g-polyNIPAAm gels was much higher at lower temperature than that of PVA abruptly declined around 25 ^{o}C which showed that

PVA-g-polyNIPAAm behaved with temperature sensitivity. The EDS of PVA gels, however, was increased gradually.

6.3. Release from the prepared hydrogels

MB release tests were performed in deionized water 15 □. From the release curve in Fig. 27 it can be seen that the release rate of MB from the grafted gels was faster than that from ungrafted one. It is possible that The PVA-g-polyNIPAAm hydrogels behaved with more loose structure than pure PVA gels which resulted in decrease of physical crosslinking points due to existence of grafted chains of polyNIPAAm.

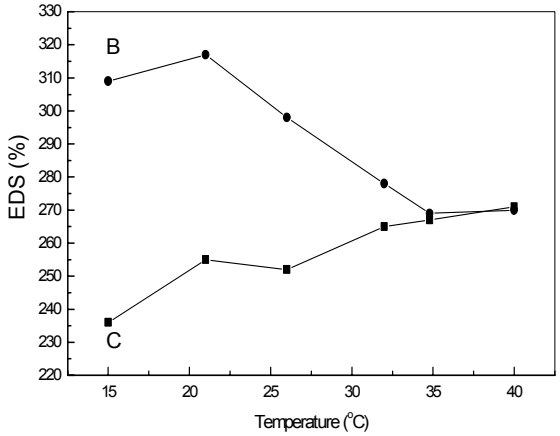


Fig.26 The change of equilibrium degree of swelling of the prepared gel with temperature B: PVA-g-polyNIPAAm gel C: PVA gel

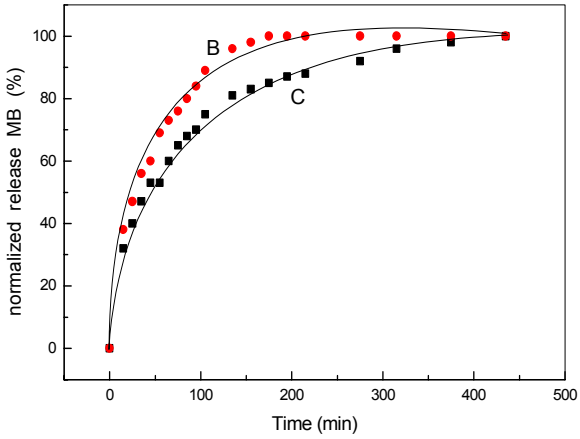


Fig.27 The release of MB at 15°C as a function of time B: PVA-g-polyNIPAAm gel C: PVA gel

7. UV-INDUCED GRAFTING AND MODIFICATION OF POLYMERS BY HIGH LET RADIATION

7.1 UV-induced grafting of SuMA onto PET fiber and its applications of protein immobilization

UV light, a kind of electromagnetical radiation can also be used to induce the grafting reaction. In some cases it is more effective. In this work, the succinimidylmethacrylate(SuMA) was grafted on the surface of polyethyleneterephthalate, PET, by UV-irradiation and then the protein was immobilized with SuMA on PET.

7.1.1. UV-induced grafting

7.1.1.1. Effect of HEMA on grafting yields of SuMA onto PS

The monomer SuMA or SuMA together with HEMA was grafted on the surface of PET fiber successfully under UV irradiation by use of $NaIO_4$. Existence of HEMA in SuMA grafting systems enhanced grafting yields of SuMA (see Fig. 28)

The proteins of trypsin and hepatities surface antibody were immobilized onto SuMA grafted PET fibers in the neutral aqueous solutions (Fig. 29, Fig.30)

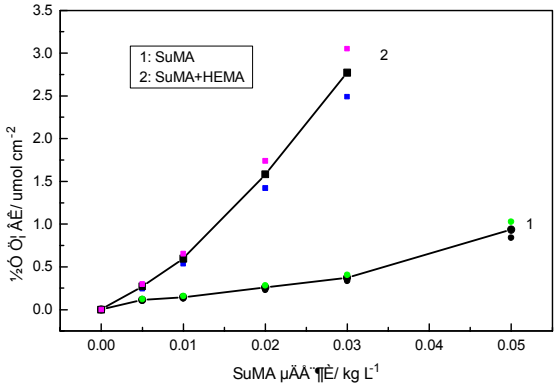


FIG. 28. The effect of SuMA conc. on grafting.

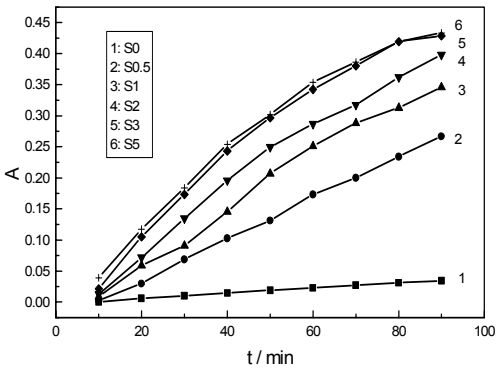


FIG. 29. The activity of the trypsin immobilized on SuMA grafted PET.

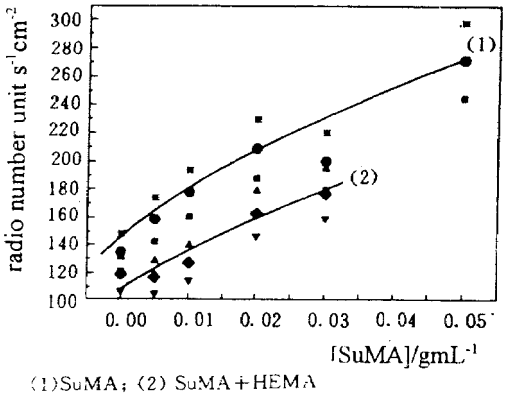


FIG. 30. The radioactivity of the samples with the immobilized protein.

7.1.1.2. Immobilization of trypsin and hepatities surface antibody

From the figures, it can be seen that although the HEMA could enhance the grafting yields of SuMA, the immobilization of proteins in the same system was inhibited remarkably by existence of HEMA.

7.2. Study on oxidation of polymers treated by high LET radiation

Both high linear energy transfer (LET) radiation e.g. ion beam and low LET radiation e.g. electron beam or γ-rays can be used in modification of polymeric materials. High LET radiation has wide energy range and narrow distribution of energy deposition. So it is more useful in surface modification of materials to some extent. Many properties such as optical, electrical and biocompatible properties of materials could be modified by ion implantation. In recent years some applied research has been carried out in this area but the mechanism has not been discussed clearly. In this work the oxidation behaviour of two kinds of polymeric membranes, i.e. silicone rubber (SR) and segmented polyether urethane (SPEU) which are bio -compatible materials was studied by treating with high LET radiation.

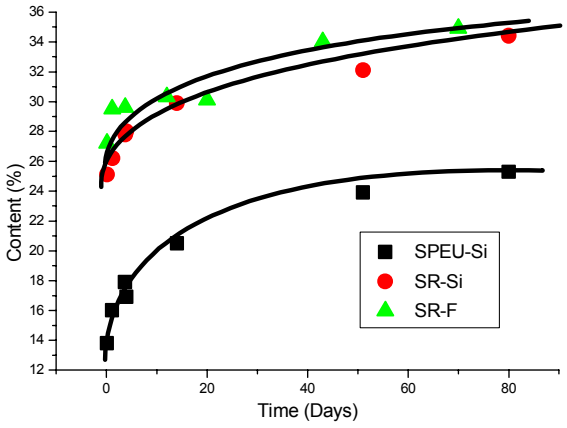
2×1.7 MV Tandem Accelerator, 5SDH-2 NEC (Peking university) was used as the ion source equipment. Si^+ beam with 1MeV energy, 30nA current and ion fluence of 5×10^{14} Si^+/cm^2. For F^+ beam energy was 1.2 MeV. XPS (LAB-5, VG, USA), ESR (ER200D SRC Germany) and UV-Vis spectrophotometer (756 MC China) were used to measure the treated samples.

7.2.1. Oxygen content on the treated surface of membranes

The oxygen and carbon contents on the surfaces of SR and SPEU implanted by Si^+ and F^+ respectively as a function of time stored in air showed that the oxygen content grew up, while the carbon content lowered down accordingly. The treated samples were washed by ethyl alcohol several times and measured again. The results showed that there was no difference between the two XPS data before and after washing. Therefore, the physical absorption of oxygen from air could be eliminated (see Fig. 31)

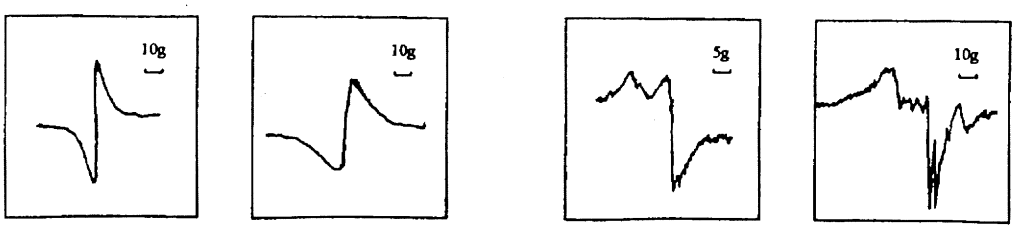
7.2.2. Free radicals in implanted samples

The ESR spectra of SPEU and SR membranes implanted by Si^+ ions showed that the free radicals formed by ion implantation were broad symmetric singlet which were different from that formed by γ-rays (see Fig. 32).



a. Variation of O content with time

FIG. 31. Variation of O content on SR and SPEU surface implanted by Si^+ or F^+.



a. SPEU-Si^+ b. SR-Si^+ c. SPEU-γ d. SR-γ

FIG. 32. ESR Spectra of Si^+-implanted and γ -irradiated SPEU and SR.

Those free radicals are the active species to combine with oxygen forming peroxides. It indicated again that the increase of oxygen content was resulted in chemical reaction instead of physical adsorption.

7.2.3. The adsorbability of BSA on SPEU and SR implanted by F^+ ions

The samples implanted by F^+ ions adsorbed with BSA to be saturated and then put into water under supersonic measuring the adsorbability of BSA on the surface. It was found that the BSA adsorbed implanted SR was more stable, but for SPEU the opposite results were obtained (see Fig. 33)

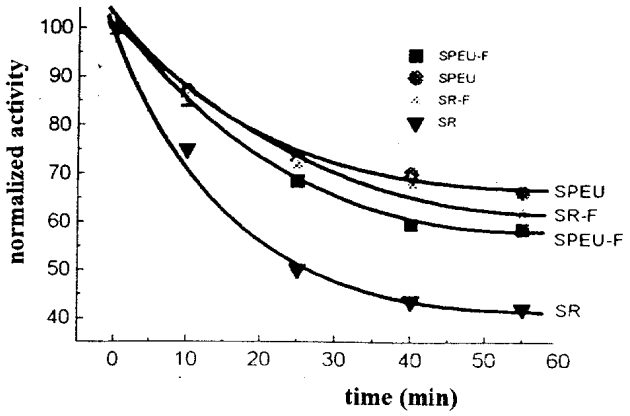


FIG. 33. SR and SPEU adsorbed with BSA and implanted by F^+ ions.

REFERENCES

[1] P. R.CHATTERJI and H. KAUR Interpenetrating Hydrogel Networks: 3.Properties of the Ge1atin-Sodium Carboxymethylcellulose System Polymer l992, 33, 2388-2391

[2] KANG DE YAO, TAO PENG, M.F.A.GOODSEN etal. PH-Sensitivity of Hydrogels Based on ComPlex Forming Chitosan: Polyether Interpenetrating Polymer Network Journal of Applied Polymer Science l993, 48, 343–354

[3] TAKASHI AOKI, MASAHIKO KAWASHIMA, HIROKI KATONO, et al., Temperature-Responsive Interpenetrating Polymer Networks Constructed with Poly (acrylic acid) and Poly (N,N — dimethylacrylamide) Macromolecules l994, 27, 947–952

[4] ANNA GUTOWSKA, YOU HAN BAE, HARVEY JACOBS etal Thermosensitive Interpenetrating Polymer Networks: Synthesis, Characterization and Macromolecular Release l994, 27, 4l67–4175

[5] KANG DE YAO, TAO PENG, HAN BAO FENG, etal Swelling Kinetics and Release Characteristic of Crosslinked Chitosan: Polyether Polymer Network (Semi-IPN) Hydrogels Journal of Po1ymer Science l994, 32, l213-l223

[6] YOUNG MOO LEE, SU HWI KIM AND CHONG SOO CHO. Synthesis and Swelling Characteristics of pH and Thermo-Responsive Interpenetrating Polymer Network Hydrogel Composed of Poly (vinyl alcohol) and Poly (acrylic acid) Journal of Applied Polymer Science 1996, 62, 301–311

[7] XIN CHEN, WENJUN LI, WEI ZHONG etal pH Sensitivity and Ion Sensitivity of Hydrogels Based on Complex-Forming Chitosan/Silk FibrOin Interpenetrating Polymer Network Journal of Applied Polymer Science l997, 65, 2257-2262
[8] HANFU WG, WENJUN LI, WA LU etal Studies on Chitosan and Poly (acrylic acid) Interpolymer Complex. I. Preparation, Structure, pH-Sensitivity and Salt Sensitivity of Complex-Forming Poly (acrylic acid):Chitosan Semi-Interpenetrating Polymer Network Journal of Applied Polymer Science l997, 65, l445-l450
[9] I.CHIKINA, M.DAOUD. Structure of Interpenetrating Polymer Networks Journal of Applied Polymer Science l998, 36,l507-l5l2
[10] BYUNG CHUNL SHIN, MU SHIK JHON, HAI BANG LEE. etal Temperature-Induced Phase Transition of Semi-Interpenetrating Polymer Networks Composed of Poly (N-isopropyl acrylamide) and Hydrophilic Polymers EUr.Polym.J. l998, 34, 171–174
[11] HEUNG SOO SHIN, SO YEON KIM, YOUNG MOO LEE. Permeation of Solutes Through interpenetrating Polymer Network Hydrogels Composed of Poly (viny1 alcohol) and Poly (acrylic acid) Journal of Applied Polymer Science l998, 69, 479-486
[12] A.L.BUYANOV, L.G.REVEL'SKAYA, YU.P.KUZNTZOV. etal Cellulose-Poly (acrylamide or acrylic acid) Interpenetrating Polymer Network Membranes for the Pervaporation of Water-Ethanol Mixtures Journal of Applied Polymer Science l998, 69, 761–769
[13] GIAMMONA G, PHAESI G, CAVALLARO G. Functionalization of a Polyaspartamide with Glycidyl Methacrylate: A Useful Method to Prepare Hydrogels Through Gamma Irradiation Macromolecular Symposia l999, l38, 225-230
[14] ROONEY MTV, SEITZ WR. An Optically Sensitive Membrane for pH Based on Swellable Polymer Microspheres in a Hydrogel Analytical Communications l999, 36, 267-270
[15] TRANQUILAN-ARANILLA C, YOSHII F DELA ROSA AM etal Kappa-carrageenan-Polyethylene Oxide Hydrogel Blends Prepared by Gamma Irradiation Radiation Physics and Chemistry 1999, 55, l27-l33
[16] LEE JW KIM SY KIM SY.etal Synthesis and Characteristics of Interpenetrating Polymer Network Hydrogel Composed of Chitosan and Poly (acrylic acid) Journal of Applied Polymer Science l999, 73, ll3-l20

GAMMA RADIATION TECHNOLOGY OF PRODUCING CROSSLINKED POLYMERS (HYDROGELS) FOR SPECIFIC APPLICATION IN MEDICINE AND BIOTECHNOLOGY

Z.S. Nurkeeva

Al-Farabi Kazak State National University,
Chemistry Faculty, Department of High Molecular Compounds,
Almaty, Kazakhstan

Abstract. New polymeric hydrogels based on vinyl ethers have been synthesized by the γ-initiated polymerization method. Their physical chemistry and physical mechanical properties have been studied. It has been shown that structure and swelling behaviour of the hydrogels can be regulated by the changing of synthesis conditions and nature of monomers. Novel stimuli-sensitive polymers have been synthesized by the varying of macrochains hydrophilic-hydrophobic balance. The some biomedical aspects of application of hydrogels in capacity of drainaging polymeric materials in ophthalmology surgery, implants in plastic surgery as well as drug delivery systems.

1. INTRODUCTION

Due to unique properties, polymeric hydrogels are widely used in various fields of medicine [1, 2].

Vinyl ethers of glycols and aminoalcohols produced on "Alash" Ltd., Kazakstan, are specific and perspective monomers to synthesize a various kinds of hydrogels with unique physico-chemical and physico-mechanical properties. But, they belong to hadrpolymerizing monomers owing to their low activity. For instance in the present of radical initiators the oligomer products are formed; in the presence of acidic catalysts the formation of polyacetales is occurred [3]. It was found by our research team that the gamma-irradiation polymerization method is more effective to obtain both water-soluble and water-swelling (co)polymers, the radical mechanism of reactions was found [4–8]. Molecular-weight and conformation characteristics were studied, main constants were determineted [9, 10].

It was found by ESR-method (spin-trapping method), IR- and Raman-spectroscopy, that increasing of molecular weight and cross-linked structure of the polymersat the γ-initiated polymerization of vinyl ethers of glycols caused by branchy processes with participartion hydroxyl and methylene groups of side substitutes owing to H-atom separation [11–13]. It was shown that new polymer hydrogels are perspective materials for application in medicine [14–19].

Presented work is continuation of the investigation on using γ-irradiation technologies for synthesis of bio-medical cross-liked polymers based on vinyl ethers.

2. GAMMA-IRRADIATION SYNTHESIS OF POLYMERS BASED ON VINYL ETHER

2.1. Polymerization of vinyl ethers of glycols

To optimize the synthesis of polymeric gels based on vinyl ethers, the influence of cross-linking agent (CA) structure and nature as well as conditions of cross-linking process on the equilibrium swelling state, elasticity module and the basic parameters of hydrogels was

studied [20]. The hydrogels based on vinyl ether of ethyleneglycol (VEEG) and vinyl ether of diethyleneglycol (VEDEG) were synthesized in the presence of divinyl ether of diethyleneglycol (DVEDEG), N,N'-methylene–bis-acrylamide (BAA), N'N-bis(2-vinyloxyethyl)urea (BU), N,N'-bis(2-vinyloxyethyl)ethylurethane (BEU) and allyloxyethylcellulose (AOEC), M_n120000 as cross-linking agents. BAA is bifunctional acrylic cross agent, which is widely used at the synthesis of hydrogels. BU and BEU are anologue to DVEDEG and they are vinyl ethers. AOEC is high molecular weight cross agent.

At the using of the vinyl ethers with similar activities of double bonds as a cross-agent, practically same result takes place. In all cases, the increasing of the CA concentration and absorbed irradiation dose D leads to the increasing of gel fraction yield and cross-linking degree. Exception is only BAA, which has high reactivity of its double bounds. As it is seen from fig. 1, the swelling degree α and cross-linking degree (parameter n_c tht is determined from Flory-Rener equation [21]) pass through maximum and minimum respectively and gel-fraction yield increases during the increasing of irradiation dose. It can be supposed that the reason of observed effects is the peculiarities of three-dimensional radical co-polymerization in the system VEEG-BAA with significant difference in monomer and CA activities. BAA as a more reactive agent is consumed at initial conversion degrees. It is accompanied by the formation of high-density network (low α and high n_c). It depresses the effects concerned with increasing of network charge. So then, the significant difference between the monomer and CA activities is accompanied by more inhomogeneous network formation with low and high cross-linking density phases in comparison with systems in which co-monomer activities are similar.

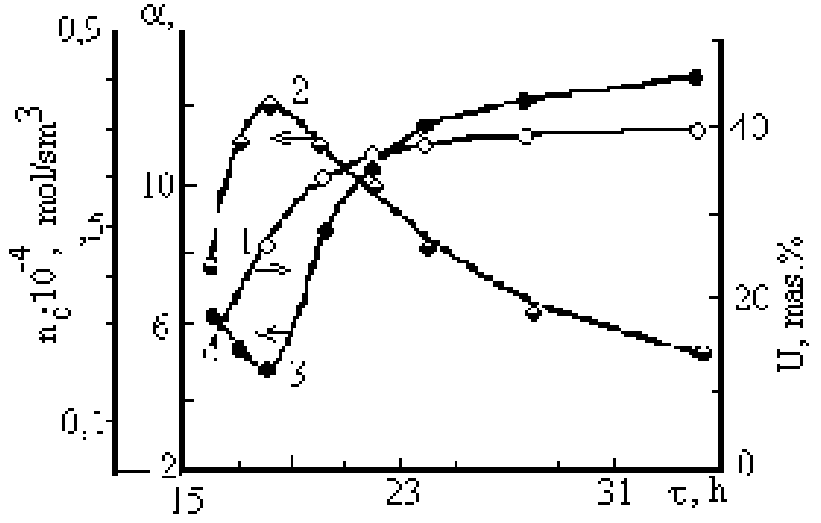


FIG. 1. The influence of irradiation period at dose rate 120 rad/sec on gel-fraction yield (1), swelling degree (2) and cross-linking degree (3) for PVEEG hydrogels with BAA as CA. [BAA]=0,05 mol%; [H_2O]=30 vol.%.

The network formation process through the participation of pendant double bonds on the degree of conversion was detected by Raman spectroscopy. It is proved that the double bonds of more active cross-agent BAA are involved into the polymerization process completely. The swelling degree α of hydrogels decreases with the CA connection increasing except only AOEC. The equilibrium swelling degree value of VEEG polymer (PVEEG) hydrogels cross-linked by AOEC increases due to the polymer nature of cross-linker. AOEC is able to play the

role of rigid spacer between polymer's chains. It makes it possible to obtain the high swelling hydrogels containing up to 100 g of water per 1g of dried polymer. One can proposed that the application of CA having double bounds closed to monomers' double bonds on activity is more affective. Therefore, the DVEDEG, which is closed to vinyl ether of glycols (VEG) on activity and structure, was used as a CA in further experiments.

It was showed earlier that one of the effective way to regulate the co-polymerization process of VEG to carry out the reaction in aqueous solutions [22]. In contrast to organic solutions, we observed the growth of reaction rate and molecular weight of linear polymers, the change of the composition and structure of the polymers in aqueous solutions.

In the present communication, this procedure was also used for the three-dimensional γ-irradiation polymerization of VEEG and VEDEG in the presence of DVEDEG. A considerable influence of water molecules on the kinetics of polymerization, swelling and strength parameters of hydrogels was established. Consequently, the kinetic effect of enhancing during the three-dimensional polymerization leads to the formation of more crosslinked network structure. It is possible to regulate the physico-chemical and physico-mechanical properties of hydrogels by variation of the structure and nature of both monomers and crossagents, changing the concentration of crosslinkers, dose of irradiation and by addition of water molecules into the reaction mixture. The structure of networks can be characterized by the average molecular weight between crosslinks M_c [23]. The values of M_c calculated theoretically (M_e^{theor}), determined from the equilibrium swelling data (M_c^{mech}) and determined from the elasticity module (M_c^{es}) were counted. The experimentally found values of M_c^{mech}, M_c^{es} and as well as theoretically calculated M_e^{theor} are different. The increasing of the irradiation dose D leads to the decreasing of M_c^{mech} and M_c^{es}. M_c^{es} is always higher than M_c^{mech}. At the same time, the values of M_c^{mech} and M_c^{es} for VEEG hydrogels are high than that of VEDEG. It should be noted that $M_c^{mech}/M_e^{theor} < 1$ for both VEEG and VEDEG hydrogels independently on the reaction conditions excepting for the VEEG at low D and concentration of CA. The difference between and M_c^{es} for networks is probably the result of heterogeneity of their structure and can be explained by the presence of regions with low (diluted phase) and high (concentrated phase) volume fraction of macromolecules. The low value of experimentally found M_c in comparison with calculated one is the evidence that the real VEEG and VEDEG networks are more crosslinked. The side substitutes of vinyl monomers (VEEG and VEDEG) also can additionally participate in the formation of three-dimensional structures during the γ-irradiation (co)polymerization. However, the contribution of these reactions was not taken into consideration when M_e^{theor} was calculated. The increasing of the side substitute (for instance in the case of VEDEG) leads to increasing the probability of such reactions.

2.2. Synthesis of new pH-sensitive polymeric hydrogels

Novel pH-sensitive anionic polymers were synthesized by γ-initiated radical co-polymerization of AA vinyl ethers vinyl alkyl ethers (VBE and VIBE) of hydrophobic nature [24-27]. The relative activity of vinyl ethers and AA has been determined via the binary co-polymerization investigation of at low conversion degree in aqueous solutions. It has been found that vinyl ethers are considerably less active in comparison with AA:

$$r_1(AA)=5.4\ r_2(VBE)=0.01$$
$$r_1(AA)=5.6\ r_2(VIBE)=0.01$$

The co-polymerization rate depends on active acrylic co-monomer concentration in the feed. With conversion and enriching of reaction mixture by low active acrylic monomer the co-polymerization rate is decreased, AA units content in polymer composition is decreased and composition inhomogeneity is appeared.

Similar regularities were observed for three-dimensional co-polymerization of vinyl alkyl ethers (VAE) with AA in the presence of DVEDEG as c cross-agent. An increasing of acrylic co-monomer concentration in the feed increases the rate and yield of sol and gel fractions. As it is well known, for polyelectrolyte gels the high equilibrium swelling degrees (α) are reached due to the ionic contribution of ionic groups into whole swelling pressure of polymeric network. But, the swelling degree of cross-linked co-polymers VAE-AA does not increases and falls down with the increasing of AA concentration in the feed until definite value (fig. 2). It can be supposed that the reason of observed effects is the peculiarities of three-dimensional radical co-polymerization in the system VAE-AA with significant difference in monomer activities. AA as a more reactive co-monomer, is consumed at initial conversion degrees. It is accompanied by the formation of high-density network (low α and high n_c). It depresses the effects concerned with increasing of network charge. These results are in accordance with data on VEEG three-dimensional polymerization with BAA as a cross agent. Moreover, co-polymers are enriched with hydrophobic component. The significant difference between the monomers activities is accompanied by more inhomogeneous network formation with low and high cross-linking density phases in comparison with systems in which co-monomer activities are similar.

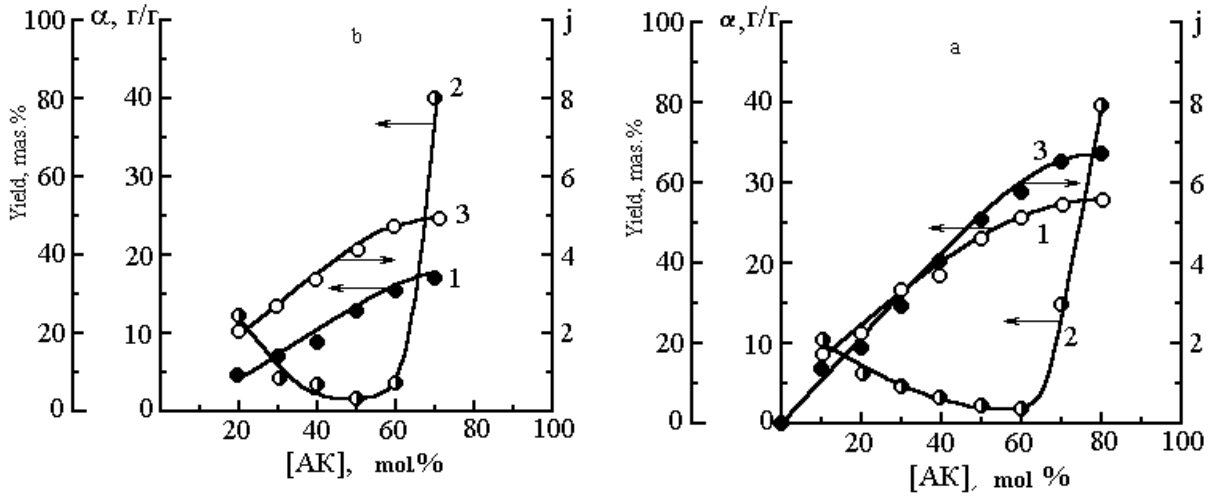


FIG. 2. The influence of the feed composition on gel-fraction degree (1), swelling degree α (2) and cross-linking index j (3) of co-polymers VBE-AA (a) and VIBE-AA (b). [CA]=4 mol.%; D=102, 1 kGy; alcohol 50 vol.%.

The comparative analysis of the data on the synthesis of co-polymers of vinyl alkyl ethers with acrylic acid showed, that the values of gel-fraction yield and cross-linking density are higher and the equilibrium swelling degrees are lower for VBE-AA system than for VIBE-AA system. Thus, the equilibrium swelling of hydrogels in water can be regulated not only by an increase of hydrophilic fragments concentration in the network but, by changing of the structure of vinyl alkyl ether. Namely, the transition from VBE to VIBE in the line of cases is accompanied by an increase of α in approximately two times at the same synthesis conditions.

2.3. Synthesis of thermo-sensitive co-polymers

Most of thermo-sensitive polymers are synthesized at the homopolymerization of water-soluble monomers, containing in their structure simultaneously both hydrophilic and hydrophobic fragments. Co-polymerization method discovers wide possibilities to obtain thermo-sensitive polymers. It is significant that for synthesis such co-polymers, the monomers, which forms their homopolymers without thermo-sensitivity, can be used. As a hydrophilic monomer we chose VEEG, and VBE and VIBE as a hydrophobic monomers.

The synthesis of VEEG-VBE and VEEG-VIBE water-soluble and water-swelling polymers has been carried out by n γ-initiated co-polymerization. The co-polymerization constants indicate the higher activity of VEEG in radical co-polymerization compared with VBE and VIBE:

$$r_1(\text{VEEG})=1.2\ r_2(\text{VBE})=0.2$$
$$r_1(\text{VEEG})=1.3\ r_2(\text{VIBE})=0.02$$

Kinetics investigation of VEEG-VBE co-polymerization demonstrated the lower activity of VIBE compared with VBE. It is in accordance with results obtained on co-polymerization VAE with AA and it caused by different influence of normal and isomeric structure substitutes on VAE double bonds activity.

It should be noted that even small difference in the reaction ability of co-monomers influences on the process of polymeric network formation. It demonstrates by the data on three-dimensional co-polymerization of VEEG with VBE [27, 28] and VIBE (fig3.). Both for linear co-polymerization and three-dimensional one the co-polymers yield are decreased with increasing of concentration of low active component VAE in the feed. The dependence of swelling degree of hydrogels in water is passed maximum. The anomalous rising of α values with hydrophobic units in network structure caused by the decreasing of cross-linking density in these conditions. The α values are reduced after maximum, because of increasing of hydrophobic properties at negligible changing of cross-linking density. At the same time, in isopropyl alcohol the monotonous rising of equilibrium swelling values versus growth of VAE in initial monomer mixture has been observed.

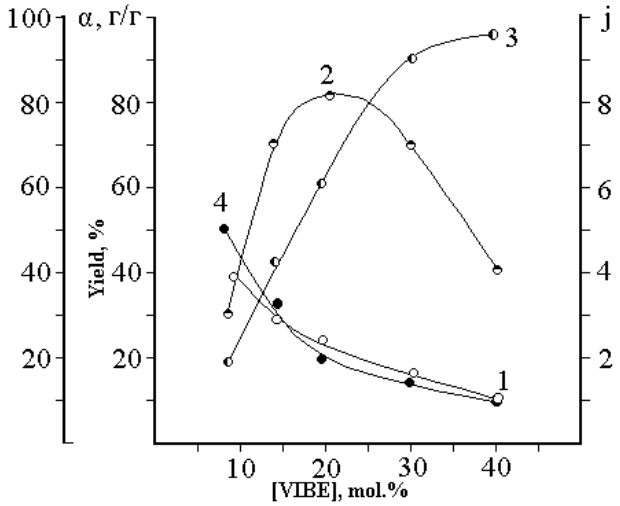


FIG. 3. The influence of feed composition on gel-fraction yield (1), swelling degree α in water (2) and in isopropanol (3) and cross-linking parameter j (4) of VEEG-VIBE co-polymers сополимеров ВЭЭГ-ВиБЭ. [CA]=4 mol%; D=61.2 kGy.

2.4. Synthesis of new hybrid hydrogels

As it is known the injection of ionic units in thermo-sensitive networks composition as increased the collapse amplitude as let to create the polymers that are sensitive both temperature and pH changing. Such pH-dependent thermo-sensitive hydrogels have been synthesized by three-dimension radical co-polymerization of VEEG, VBE and ionic monomer AA at presence of DVEDEG.

The structure formation of these hydrogels is characterized by phenomena concerned with considerable difference in reaction ability of co-monomers. Thus, these hydrogels are characterized by extremal dependence of α on AA content in initial monomer mixture (fig. 4) [29]. It is caused with different activity of co-monomers and the formation of inhomogenous structure networks. These dependences are in accordance with data obtained on AA-VAE systems.

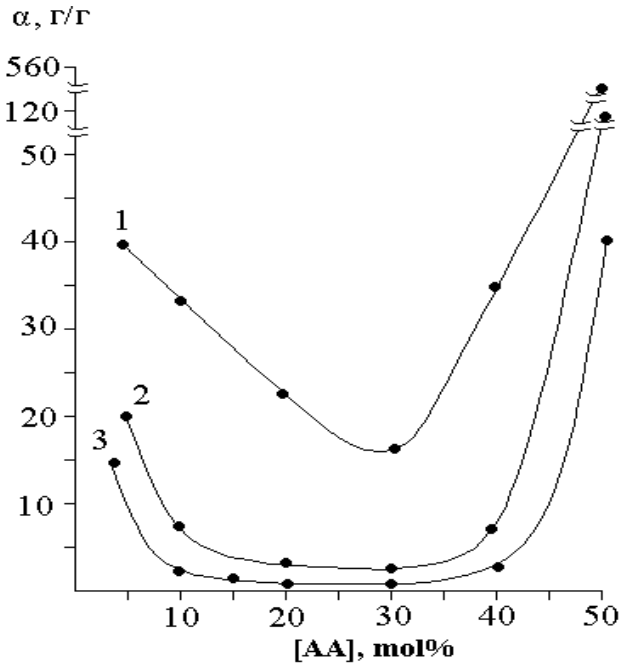


FIG. 4. The dependence of swelling degree of VEEG-VBE-AA hydrogels on the concentration of AA in the feed. [CA]=4 mol%; 30 vol% alcohol; D=146.9 kGy; [VEEG]:[VBE], mol%: 1–80:20; 2–70:30; 3–60:40.

3. PHYSICAL CHEMISTRY BEHAVIOUR OF STIMULI-SENSITIVE POLYMERS BASED ON VINYL ETHERS

3.1. Swelling behaviour of pH-sensitive co-polymers VAE-AA

The behaviour of co-polymer VAE-AA contained both ionic pH-sensitive carboxylic groups and hydrophobic VAE units has been studied in wide interval of pH medium.

Polymeric networks based on co-polymers VAE-AA characterized by pH-induced collapse (the equilibrium swelling degree of hydrogels abruptly decreases at narrow range of pH) (fig. 5).

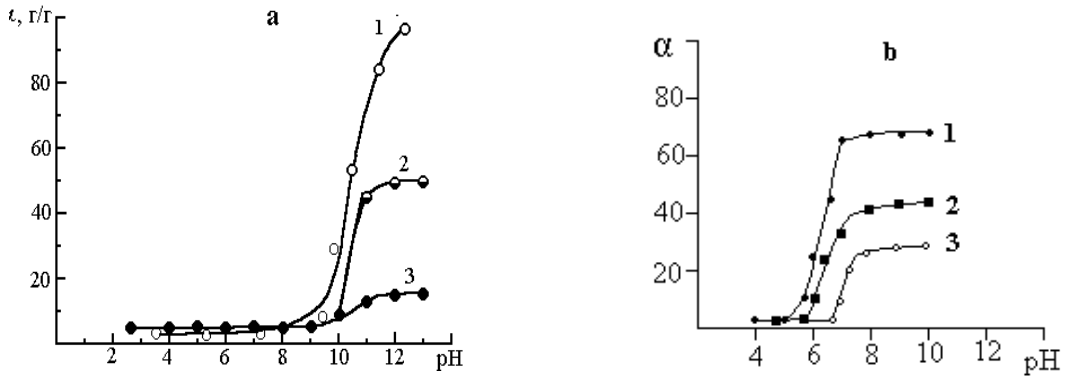


FIG. 5. The dependence of swelling degree α on pH of surrounding solution for VBE-AA (a) and VIBE-AA (b) hydrogels. (a) μ=0,01; [ВБЭ]:[АК], мол.%: 1–10,9:89, 1; 2–17,5:82,5; 3–28,2:71, 8; (b) μ=0,05; [ВиБЭ]:[АК], мол.%: 1–9,8:90,2; 2–15, 1:84,9; 3–27,7:72,3.

The value of pH transition for VBE-AA hydrogels is located in alkaline area and practically independent on their composition. An increase of VBE content in cross-linked co-polymer is accompanied only by a decrease of transition amplitude. In contrast to VBE-AA, the pH of transition of VIBE-AA hydrogels is located in acidic area and an increase of VIBE content in co-polymers shifts the transition pH to the higher values. The observed different behaviour of networks is caused with more intensive hydrophobic interactions between normal structure butyl radicals than isomeric one. The additional ionization of carboxylic groups is required to overcome the hydrophobic interactions in VBE-AA, therefore the transition of these gels occured in alkaline pH region. The changing of pH from neutral to more acidic region depresses the ionization of carboxylic groups and decreases the electrostatic repulsion of macrochains for novel synthesized anionic networks. Besides an increase of ionic strength of the solutions shifts the pH transition of hydrogels of co-polymers VIBE-AA to the higher values region and decreases the collapse amplitude.

3.2. The swelling behaviour of thermo-sensitive co-polymers

Aqueous solutions of VEEG-VBE and VEEG-VIBE behave lower critical solution temperature (LCST). The value of LCST depends on co-polymer composition. An increasing of VBE content in co-polymer decreases the LCST value due to the strengthening of hydrophobic interactions. The values of critical temperature are lower for co-polymers enriched by hydrophobic components. Besides, for VEEG-VBE co-polymer characterized by lower values of LCST in comparison with VEEG-VIBE co-polymers. The reason of that is that alkyl substitutes with normal structure are more inclined to hydrophobic interactions, than isomeric substitutes. Moreover, the values of LCST can be decreased by low molecular weight salt addition because of salt-off effect.

Thermo-induced swelling behaviour of VEEG-VAE co-polymer network has been investigated via determination of swelling ratio V/V_0 where V is swollen gel volume at goven temperature, V_0 is synthesized hydrogel volume. The influence of the temperature has been investigated for polymer network with various compositions, but the similar cross-linking parameters (j). The temperature of transition from swollen state to collapsed one is reduced at increasing of containing of hydrophobic components in polymeric network and some abrupt of collapse i.e. temperature interval of transition is became more narrow (fig. 6). For temperature induced collapse of VEEG-VIBE co-polymers hydrogels with higher α values than that for VEEG-VBE networks the higher amplitudes of transition are shown.

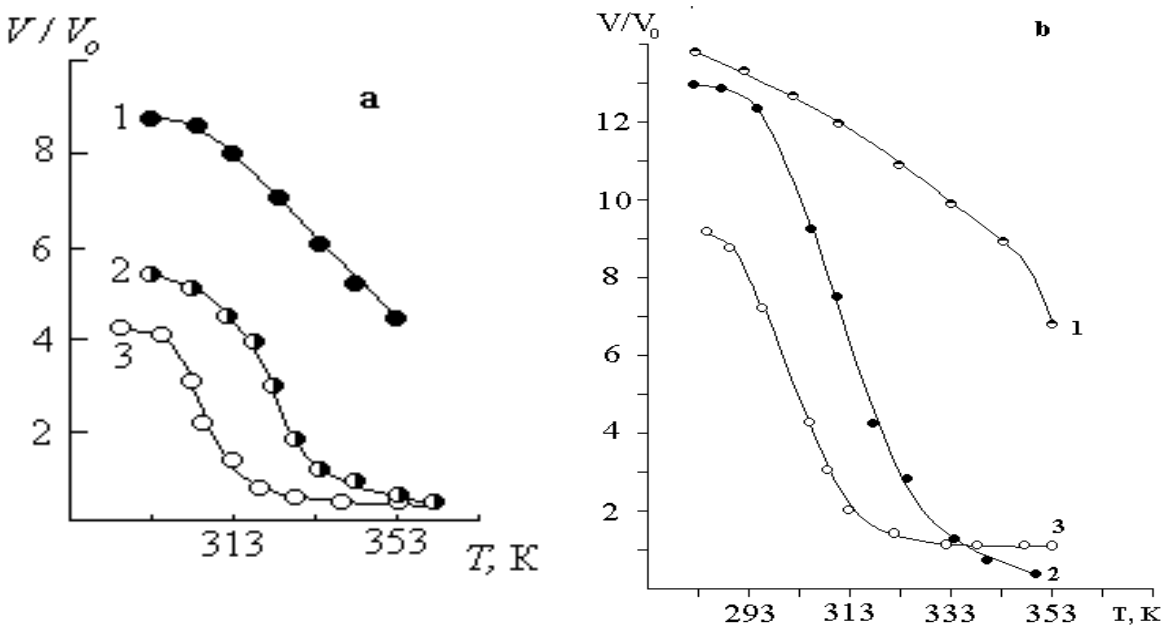


FIG. 6. The swelling behaviour of VEEG-VBE (a) and VEEG-VIBE (b) co-polymers depending on temperature. [VEEG]:[VBE], mol%: 1–93.2:6.8; 2–84.1:15.9; 3–77.9:22.1; 1–α=72,3; j=2,6; 2–α=40,1; j=2,6; 3–α=28,7; j=2,6; [VEEG]:[VIBE], mol%:1–93,4:6,6; 2–83,5:16,5; 3–77,1:22,9; 1–α=77,2; j=2,6; 2–α=66,3; j=2,4; 3–α=56.8; j=2,4.

The cross-linking density of VEEG-VBE and VEEG-VIBE co-polymers hydrogels of same composition almost does not influence the temperature interval of transition, but it reduces the amplitude significantly.

With low weight salt addition the VEEG-VBE co-polymer hydrogel transition from swollen to collapsed state was observed at lower temperatures, and contraction amplitude became sharper. This effect is less noticeable for VEEG-VIBE co-polymer: collapse amplitude and transition temperature are reduced, but abrupt change is not observed. The character of temperature-induced collapse depends on the low weight salt cation and anion nature. It has been found that when transition temperature is decreased, the amplitude and discontinuity degree are amplified at $Li^+<Na^+<K^+$ cations line, and $I^-<Br^-<Cl^-$ anions line. It is necessary to note that anion nature change is accompanied by stronger effects compared with cation nature. It is in accordance with to the data/14/.

Therefore it is possible to affect on parameters of temperature induced collapse by variation of hydrophilic-hydrophobic balance of VEEG-VAB neutral network macrochains, their cross-linking density, ionic strength and low weight salt nature in the environmental medium.

3.3. Stimuli-sensitive behaviour of hybrid hydrogels

The practical application of stimuli-responsive systems frequently requires the possibility to control the parameters of hydrogels by simultaneous varying of both pH and temperature. Especially it is important for bio-medical application of polymers because many pathologies in organism are accompanied by simultaneous changes of pH and temperature.

Novel pH-dependent thermo-sensitive hydrogels, i.e. the polymeric networks with simultaneous sensitivity in respect to pH and temperature, were synthesized by co-polymerization of VEEG, VBE and AA with DVEDEG as a cross agent.

At the study of the swelling behaviour of three components of cross-linked co-polymers, the significant differences from binary systems VEEG-VBE and VBE-AA were found (fig. 7). At the increasing of the temperature, in dependence on cross-linked co-polymers compositions three types of curves were observed. The monotonous swelling (curve 1), the swelling and following collapse (curve 3) and more complicated dependence, including swelling, collapse and the following repeated swelling (curve 2) were revealed.

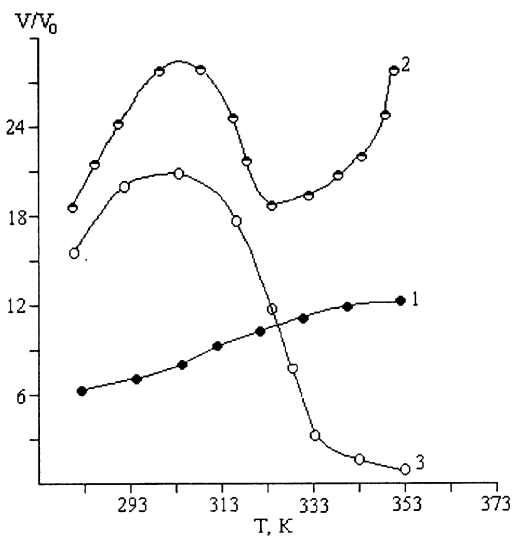


FIG. 7. The dependence of VEEG-VBE-AA hydrogel swelling ratio on the temperature. [AA]=17,3 mol%; [CA]=5,0 mol%; [VEEG]:[VBE], mol%; 1–63,2:14,5; 2–57,6:20,1; 3–52,7:25,0.

The dependencies described by curves 1 and curve 3, are usual for thermo-sensitive hydrogels containing some ionogenous groups. The extremal dependences of swelling ratio of the VEEG-VBE-AA hydrogels with the increasing of the temperature qualitatively can be explained as a result of competitive influence of two factors. First is the increasing of hydrophobic interaction with the temperature that leads to the network contraction. Second is increasing of thermal motion of countr-ions that promotes to swelling of network. As explanation of this phenomenon, the diffusion approach based on ratio of Debye length λ of mobile ions and the characteristic size of inhomogeneity region d_i has been applied [30].

Analysis of physical chemical behaviour of multi-components polymer networks obtained in this work, let us suggest that for thermo-sensitive polyelectrolyte hydrogel containing various size hydrophobic regions, the realization of oscillatory swelling changes are possible. With this goal, the co-polymerization of VEEG, VBE and AA with DVEDEG as a cross agent were performed in water-ethanol medium in the condition of closing to micro aliquation of reaction mixture. The compositions of hydrogels obtained in ethanol and water-ethanol mixtures are practically identical. However, the networks formed in mixed solvent are characterized by the more expressed structure inhomogeneity. The consequence of that is oscillating change of hydrogel volume versus the temperature (Fig. 8).

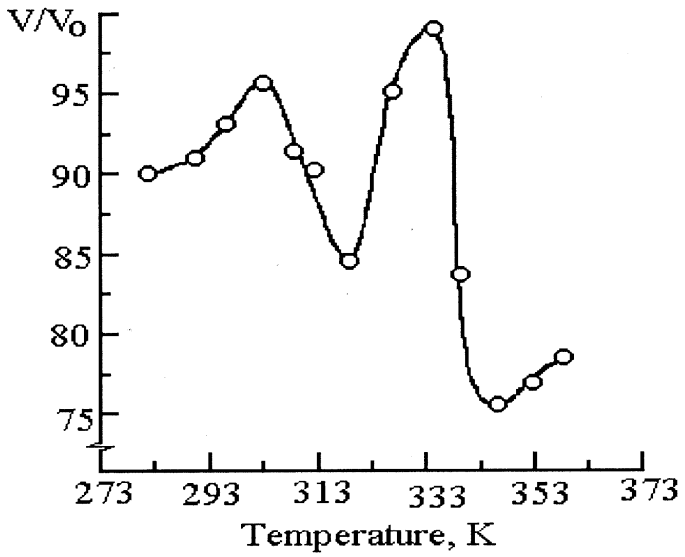


FIG. 8. Oscillating changing of swelling ratio of VEEG-VBE-AA hydrogel at the temperature increasing. [VEEG]:[VBE]:[AA] = 57.6:20.1:17.3 mol%; [CA] = 5 mol%.

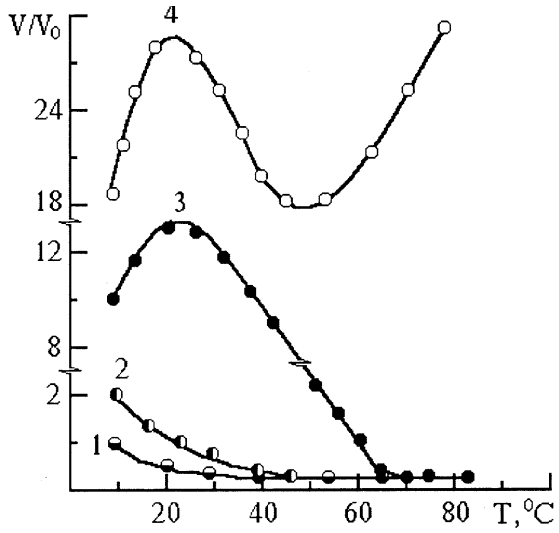


FIG. 9. The pH influence on the temperature dependence of swelling ratio of VEEG-VBE-AA hydrogel from the temperature. [VEEG]:[VBE]:[AA]:[CA]= 57,6:20,1:17,3:5,0 mol%; pH= 1–2,00; 2–3,09; 3–4,29; 4–5,50.

For the multi-components networks, the ratio of ionic and hydrophobic constituents and the swelling behaviour can be regulated by changing the pH and ionic strength of the medium. With increasing acidity, hydrogels undergo collapse (Fig. 9). Apparently, it is caused by reduction of ionic component contribution to common swelling pressure.

Thus, the new water-swelling co-polymers based on vinyl ethers possess the properties, which are typical for stimuli-sensitive polymers. Their swelling behaviour at the changing of the pH and temperature can be regulated by variation of chemical composition of networks, hydrophilic-hydrophobic balance of macrochains and ionic strength of medium. The possibility to use these hydrogels as a material for controlled drug release systems was shown.

4. BIOMEDICAL INVESTIGATIONS OF POLY (VINYL ETHERS OF ETHYLENEGLYCOL) HYDROGEL

Earlier it was shown that neutral polymeric hydrogels can be used as a contact medium for ultrasonic diagnostics, for creation of liver cyst model, plastic surgery of soft tissues. In present work these investigations are continued.

4.1. New stimuli-sensitive hydrogels as drug-delivery systems

The possibility to use new hydrophilic thermo- and pH-sensitive hydrogels as controlled drug delivery systems. The main idea is based on ability of such hydrogels to sharply change the swelling degree and permeability in response on environment conditions. Brilliant green (BG) and Malachite Green (MG) were used as a model drug substances. The sorption and desorption processes of BG and MG from hydrogels were studied in dependence on amount of drug in gel, environmental conditions (temperature, pH), hydrophobic-hydrophilic balance of macrochaincs, and initial state of polymeric matrixes.

It has been found that the diffusion rate and amount of released drug from dry AA-VIBE gels sharply increases at elevation of pH from 6 to 7 by the reason of increasing of their swelling rate at these conditions. The increasing of AA content in co-polymers leads to an increase in amount of BG and MG released from matrixes.

Sorption of BG and MG by hydrogels is accompanied by complex formation due to the ionic interactions with additional stabilization by hydrophobic interactions and it induced collapse of networks.

The desorption rate of BG from dry thermo-sensitive hydrogels of VEEG-VIBE co-polymers is decreased in the interval 293–318 K (fig.10). For swollen samples the desorption rate is increased in these conditions. The first case is concerned with slower swelling of gels that disturbs the diffusion of drug. The second case is concerned with forsing-out effect of water molecules and dissolved drug caused of gel collapse [31].

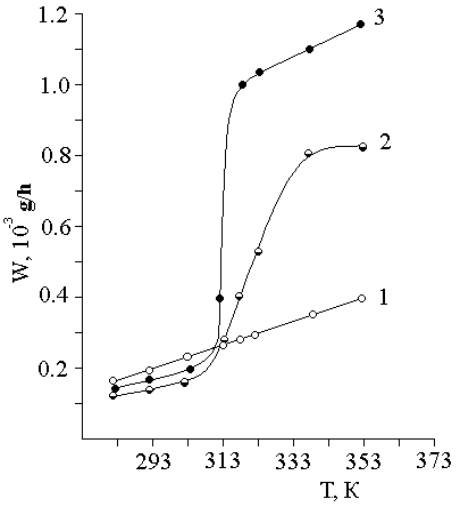


FIG. 10. The temperature influence on the desorption rate of brilliant green dye from polymeric matrixes of PVEEG and VEEG-VBE co-polymers. Load of model drug 10 mg per 1 g dry gel; 1– PVEEG, α=23.5; [VEEG]:[VBE], mol%: 2–83.8:16.2, α=24.0; 3–77.7:22.3, α=24.5.

Thus, the adjusting of temperature and pH medium can regulate the kinetics of desorption of drug substances from polymeric matrixes.

4.2. Hydrogel PVEEG composition in injection form

Nowadays the hydrophilic polymers are widely adopted in the plastic surgery for the increasing the volume and correction of anatomical organs and soft tissues. Unlike traditional implants, the polymer hydrogels have a jell consistence and they can be injected into soft tissues throughout a syringe and needle in the needed quantity to reach the good prolonged functional and cosmetic effects. The advantages of this method are atraumaticness, absence of postoperation scars and possibility of realization the manipulation under local anesthesia at the ambulatory conditions.

Obtained pre-clinical results showed that PVEEG hydrogel, entered deep into soft tissues through injector, does not change its physico-chemical properties, staying inert and biocompatible. It contains permanently in tissues, organs and systems of the human organism resulted in a stable cosmetic and functional effects. The PVEEG polymer material in injection form can be used in capacity of implant for contour plastics of soft tissues, for increasing the volume of various parts of body ands for obtaining the additional skin surface. The hydrophilic cross-linked polymer PVEEG can be recommended to be used for atrophy of muscles of lower extremities after child poliomyelitis as well as for correction of cosmetological deformities — size and shape of breast, hips, buttocks and other things.

4.3. Hydrogel drainages for application in ophtalmosurgery

New water-swelling absorbing polymer of vinyl ether of ethyleneglycol (PVEEG) in both pure and saturated with silver ions forms has been investigated. Acute and chronic toxicity, pyrogen action of the PVEEG *in vitro* and *in vivo* were investigated experimentally [32]. Easy, effective for multiple dynamic research and economical method of the "implantation test" was offered. The results of researched new absorbing material based on PVEEG were accepted as the basis for its clinical use for the treatment of extraorbital suppurative inflammations.

Surgical treatment was performed on 155 patients. The comparative estimation of rubber turunds, the PVEEG drainages and the PVEEG drainages saturated with silver ions was carried out. The offered method of the treatment of extraorbital suppurative inflamations provides active, long drainaging and fast lowering of the inflammation. The period of treatment reduce to 1.5-2 times in comparison with the control traditional method.

5. CONCLUSIONS

It has been shown that the gamma-irradiation (co)polymerization is one of the effective method for obtaining of various types of hydrogels on the basis of vinyl ethers of glycols and aminoalcohol. Physico-chemical and physico-mechanical properties of hydrogels can be regulated by variation of synthesis conditions. New types of hydrogels derived from hydrophilic and hydrophobic monomers are thermo- and pH-sensitive and they can be used as controlled drug releasing system. The phase and volume transitions of hydrogels can be regulated by varying of the hydrophobic/hydrophilic balance of network, by introduction of ionic groups into the neutral network and by addition of low-molecular-weight salts.

Composition of polymer hydrogel based on vinyl ether of ethyleneglycol has successfully passed the pre-clinical tests for application in plastic surgery in injection form. It was shown that the polymer provides stable functional and cosmetic effects and it is very promising material for injection method in plastic surgery of soft tissues.

The pre-clinical and clinical tests in capacity of drainage in ophthalmology surgery were implemented. Shown, that the using of drainage for surgery treatment of extraorbital purulent inflammations allows to reduce the healing period in 1,5 times in comparison with traditional materials. At the using of polymer saturated by silver ions as drainages, the period of treatment is reduced in 2 times because of more active catchment, aspiration and bactericidal functions.

REFERENCES

[1] Hydrogels in Medicine and Pharmacy.Ed. By N.A.PEPPAS, BOCA RATON CRC PRESS, 1987, V.I, 180p.

[2] ROSIAK J.M., YOSHII F. HYDROGELS and their medical applications//Nucl. Instr. and Meth/in Phis. Res. B 151 (1999), 56–64.

[3] M.F.SHOSTAKOVSKII//Prostye vinilovye Efiry (in Russoan. Akad. Nauk SSSR, Moscow 1952. P. 102.

[4] SHAIKHUTDINOV E.M., NURKEEVA Z.S., TLEUBAEVA G.S., Saikieva C.Kh. Radiation polymerization of monovinyl ethers of glycols under the action of γ-irradiation (In Russian)//Reports of Acad. Sci. Of Kazak Sov. Rep. Ser. Chem. 1976. № 1. P. 63–66.

[5] NURKEEVA Z.S., SHAIKHUTDINOV E.M., SEITOV S.Z. Radical polymerization of monovinyl ethers of glycols//Vysokomolek. Soed. 1980. T.22B. № 8. P. 636–638.

[6] NURKEEVA Z.S., SHAIKHUTDINOV E.M., SEITOV A.Z. Determination of elementary constants of radical polymerization of monovinyl ethers of ethylene- and diethyleneglycol (in Russian)//Reports of Acad. Sci. Of Kazak Sov. Rep. Ser. Chem. 1982. № 2. P. 47–52.

[7] NURKEEVA Z.S., SHAIKHUTDINOV E.M., SEITOV A.Z., SAIKIEVA S.KH. About radiation polymerization of vinyl ethers of glycols and aminoalcohols//Vysokomolek. Soed. 1987. V. 29.A. № 5. P. 932–937.

[8] T.ASKENOVA, D.K.DAUKEEV, B.M.ISAKOV, YU.A.ZAIKIN, N.R.MAZHRENOVE, Z.S.NURKEEVA. Investigations on radiation processing in Kazakstan//Radiat. Phys.Chem. 1995. Vol. 46. No4–6, p. 1401–1404.

[9] NURKEEVA Z.S., LEGKUNETS R.E., ERGOZHIN E.E., DAURENBEKOVA G.B. Hydrodynamic properties of water-soluble polymer monovinyl ether of monoethanolamine//Vysokomolec. Soed. –1986.-V.B. 28.-#9.-P. 649–651.

[10] NURKEEVA Z.S., KASAIKIN V.A., IVLEVA E.M., DAURENBEKOVA G.B., LEGKUNETS R.E., SIGITOV V.B., ERGOZHIN E.E. light-scattering and viscisity of the solutions of water-soluble polymers based on vinyl ethers//Vysokomolek.Soed..–1990.-V.B.31.-N6.-p. 415–418.

[11] NURKEEVA Z.S., MUN G.A., GOLUBEV V.B., ERGOZHIN E.E. Application of spin-trapping method for investigation of chain-transfer reaction on polymer (in Russian)//Reports of Acad. Sci. of Kazak. Sov. Rep. Ser. Chem. 1990. № 3. P. 47–51.

[12] NURKEEVA Z.S., GOLUBEV V.B., MUN G.A., SHATYKOVA D.B., SIGITOV V.B., ERGOZHIN E.E. Spectroscopic investigation of branched and crosslinked structure formation of vinyl ethers of glycols//Vysokomolek. Soed. 1990. V.31.B. № 11. P.874–878.
[13] NURKEEVA Z.S., MUN G.A., GOLUBEV V.B. On the activity of monomers and polymers on the basis of vinyl ethers of glycols in radical reactions//Macromol. Chem. 1991. V.193. P.1117–1122.
[14] IKRAMOV R.Z., VISHNEVSKIY V.A., NURKEEVA Z.S., VORONKOVA L.S., SAVVINA T.V., ABULKHANOV A.R., TROSTENIUK N.V. Methodic of formation and morphological characteristic of artificial liver abscess (in Russian)////Bulletin of Exp. Biology and medicine. 1991. № 11. P. 553–556.
[15] NURKEEVA Z.S., MAKEEVA G.A., MEERMANOVA ZH.B., MUN G.A., ERMUKHAMBETOVA B.B., Nam I.K. Polymeric hydrogel dreinages in the treatment of infected cavity wounds//2nd Int. Conf. “Modern approaches to the development of effective dressings, suture materials and polymer implants”. Moscow. Russia. 1995. P. 62–63.
[16] MUN G.A., MAKEEVA G.A., NURKEEVA Z.S., MEERMANOVA ZH.B., ERMUKHAMBETOVA B.B., NAM I.K. Experimental preconditions of a new water-swelling polymer usage for endoprosthetics of soft tissue//2nd Int. Conf. “Modern approaches to the development of effective dressings, suture materials and polymer implants”. Moscow. Russia. 1995. P. 282-284.
[17] NURKEEVA Z.S., ALBAZAROV B.SH., AMINOVA G.P., ADILGEREEVA A.P., ADAMYAN A.A., MUN G.A., SIGITOV V.B. Application of new hydrogel in experiment as a material for closing of bronchial fistula//Actual Questions of Pulmonology. Alma-Ata, 1989. P. 43–45.
[18] ADAMYAN A.A., TROSTENYUK N.B., NURKEEVA Z.S., MUN G.A., SKUBA D.A., CHUPIN A.V. Endoprotesis of soft tissues/Authors certificate 1806694, 1990. USSR.
[19] NURKEEVA Z.S., MUN G.A., SIGITOV V.B., SHALTYKOVA D.B., ERMUKHAMBETOVA B.B., OSPANOVA F.E., DILBARKHANOV R.D. Hydrogels of vinyl ether of ethyleneglycol as basis for drug delivery system//Materials of 1str Int. Conf. “Modern Approaches to the Development of Effective Dressings and Polymer Implants”. 1992. Moscow. P. 80.
[20] YERMUKHAMBETOVA B.B., NURKEEVA Z.S., MUN G.A.. Influence of monomers activity on the structure formation and properties of polymeric networks//5th Int.Symp. of Scientists of Turkic Languages Countries on Polymers and Polymer Composites, Proc. –Almaty, Kazakhstan, 1999. –P.31–36.
[21] P.J.FLORY//Polymer, 20, 1317 (1979).
[22] NURKEEVA Z.S., ERGOZHIN E.E., SIGITOV V.B., MUN G.A., USHANOV V.ZH. IR, Raman and EPR-spectroscopic investigation of reaction ability of vinyl ethers of glycols and aminoalcohols//Reports of Acad. Sci. of Kazak. Sov. Rep. Ser. Chem. 1990. № 5. P. 54–59.
[23] FASINA A.B., STEPTO R.T.F. Formation and Properties of Triolbased Polyester Networks. Macromol.Chem., 182, 2479 (1981).
[24] SHAIKHUTDINOV E.M., NURKEEVA Z.S.,YERMUHAMBETOVA B.B., MUN G.A. Some peculiarities of formation of hydrogels based on monovinyl ether of ethyleneglycol and sodium acrylate//Доклады НАН РК. –1994. –5. С. 50–54.
[25] G.A.MUN. Synthesis and physico-chemical behaviour of hydrophilic polymers of vinyl ethers//5th Int.Symp. of Scientists of Turkic Languages Countries on Polymers and Polymer Composites: Proc. –Almaty, Kazakhstan, 1999. –P. 3–8.

[26] MUN G.A., NURKEEVA Z.S., NAM I.K., KARZHAUBAEVA R.G. Stimuli-sensitive polymer hydrogels of vinyl ethers//5th Int.Symp. of Scientists of Turkic Languages Countries on Polymers and Polymer Composites, Proc. –Almaty, Kazakhstan, 1999. –P. 277-283.

[27] NURKEEVA Z.S., MUN G.A., NAM I.K. Thermo- and pH-activated hydrogels on the basis of vinyl ethers of glycols (in Russian)//Abstract of Int. Conf. "Fundamental problems of science about polymers". Moscow. 1997. P. 2–62.

[28] KUDAIBERGENOV S.E., NURKEEVA Z.S., MUN G.A., YERMUHAMBETOVA B.B., NAM I.K. Temperature-responsive swelling and deswelling of the co-polymers from vinyl ether of ethylene glycol and butyl vinyl ether//Macromol.Rapid Commun. – 1995. -16, P. 855–860.

[29] MUN G.A., NURKEEVA Z.S., ERMUKHAMBETOVA B.B., NAM I.K., KAN V.A., KUDAIBERGENOV S.E. Thermo- and pH-sensitive Amphiphilic Gels of Co-polymers of Vinyl Ether of Ethylene Glycol//Polym.Adv.Technol.-1999. –10,3. P. 151–156.

[30] MUN G.A., SULEIMENOV I.E., NURKEEVA Z.S., KUDAIBERGENOV S.E., NAM I.K., KAN V.A. Effect of the Structural Inhomogeneity of Polyelectrolyte Gels on Their Thermal Sensitivity//Polymer Science. –1998. -Ser.A,40, 3. P. 253-259.

[31] URKIMBAEVA P.I., MUN G.A., NAM I.K., YERMUKHAMBETOVA B.B., KURBANOVA G.K. Drug delivery systems based on pH and thermo-sensitive polymers of vinyl ethers//5th Int.Symp. of Scientists of Turkic Languages Countries on Polymers and Polymer Composites, Proc. –Almaty, Kazakhstan, 1999. –P. 354–355.

[32] MAKEEVA G.A., MEERMANOVA ZH.B., NURKEEVA Z.S. MUN G.A., ERMUKHAMBETOVE B.B. Method of treatment of soft tissue abscesses and phlegmon. Patent of Republic of Kazakstan. KZ (B) N5672 A61B 17/00, A61F9/00 on 15.01. 98.

IRRADIATION OF POLY (VINYL ALCOHOL) AND POLY (VINYL PYRROLIDONE) BLENDED HYDROGEL FOR WOUND DRESSING

M.T. Razzak*, D. Darwis **, Zainuddin, Sukirno*****

* Center for Utilization of Nuclear Science and Technology
** Center for Research and Development of Isotopes and Radiation Technology
*** Ministry of Health of The Republic of Indonesia

Jakarta, Indonesia

Abstract. Poly (vinyl alcohol) and poly (vinyl pyrrolidone) (PVA-PVP) blended hydrogel for wound dressing has been prepared by using gamma rays irradiation technique. The gel fraction, mechanical properties, the water content and water absorption performance of the hydrogels were measured. It was found that the gel fraction increases with increasing irradiation dose but never reaches 100% of gel. The PVA/PVP blended hydrogel has a water content in the range between 60% and 80% and water absorption between 40% and 250%. The water vapor transmission rate value (WVTR) of the PVA/PVP blended hydrogel varies between 50 and 200 $g/m^2/h$. The hydrogel could be considered as good barrier against microbes. According to *in vitro* assessment it was found that the PVA/PVP blended hydrogel was very useful material that can meet the efficacy requirement and its healing rate was comparable with sterilized gauze and sofratulle.

1. INTRODUCTION

Polyvinyl pyrrolidone (PVP) has been used successfully as a basic material for the manufacturing of hydrogel wound dressing (Rosiak at. al., 1991). There are some commercialized hydrogel wound dressing under the trade name of Vigilon, Ivalon, Aqua gel an Kik gel which are all sterilized by using irradiation technique. Various other types of hydrogel dressing have also been reported in the literature (Peppas, 1987; Corkhill at. Al., 1989; Ohsaki et.al., 1991; Kroschwitz, 1992). Polyvinyl pyrrolidone hydrogel wound dressing was normaly prepared in the presence of agar as a second component to enhance the mechanical properties of hydrogel. The present of agar, however, may cause easier penetration of microorganisms into the hydrogel particularly in a tropical environment where humidity is high.

Research work on the preparation of hydrogel wound dressing, which are particularly appropriate to tropical environment or local requirement are continued (Hilmy N. et. al., 1993; Jie Chen et. al., 1993). For example, Hilmy N. et.al., 1993 have added polyethylene glycol to the PVP hydrogel composition. They reported that the presence of polyethylene glycol could improve the hydrogel barrier against bacteria.

Instead of using agar as a second component, the present work reports the preparation of Polyvinyl pyrrolidone and Polyvinyl alcohol blended hydrogel for wound dressing by using gamma rays irradiation. The gel fraction, mechanical properties, microbe penetration test, and *in vitro* assessment were studied to obtain an applicable hydrogel wound dressing for tropical environment.

2. MATERIALS AND METHOD

2.1. Materials

Polyvinyl pyrrolidone (PVP) with an average molecular weight of 30 KD was purchased from Fluka AG, Germany. Polyvinyl alcohol (PVA) with degree of polymerization of 1700–2400 and degree of saponification of 99 mol% was supplied by Kuraray Poval Co. Ltd., Japan. Both polymers were used without further purification. Other chemicals such as hematoxylene-eosin (HE), Zoletil 50, Barium sulfide solution and Alcohol were use as received. Solfratulle (Roussel, England) and Gamma sterilized Gauze were used for comparison in healing performance. Double-distilled water was used as solvent.

2.2. Preparation of PVA-PVP blended hydrogel

PVA (20 wt%) and PVP (8 wt%) were dissolved in double-distilled water and heated by using an autoclave at temperature 120 ^{0}C and pressure 2 atm for 50 minutes and 15 minutes, respectively. The two solutions were mixed with a composition of 40 parts of PVA and 60 parts of PVP at temperature of 80 to 90 ^{0}C. The mixed solution was poured into a plastic mould or plastic bag, sealed and squeezed between two glass plates and stored overnight at room temperature. It was then irradiated by gamma — rays from a cobalt-60 sources with a selected dose and dose rate at room temperature. The obtained hydrogel was in c.a. 3 mm thickness.

2.3. Determination of gel fraction

The samples were extracted by water in Sokhlet apparatus for 24 hr. Then dried to a constant weight in vacuum. The gel fraction was than calculated gravimetrically by using the following formula:

$$G = \frac{Wg}{Wo} \times 100\%$$

where
G is gel fraction (%)
Wg and Wo are the weight of sample after extractions and the weight of sample before extractions, respectively.

2.4. Determination of mechanical properties

Tensile strength and elongation at break were determined by using the hydrogel specimen which are cut into dumbbell shape according to ASTM standard and tested with an instron universal testing instrument (Strograph-1, Toyoseiki, Model 1122) with a constant extention rate of 50 mm/min, at room temperature (30 ^{0}C).

2.5. Determination of equilibrium water content (EWC) and water absorption

The samples were immersed in water with the proportional of mass of gels to the mass of water about 1: 500 at room temperature. Swelling continued to reach of constant weight of gel. Before weighing the sample, any surface water was removed with filter paper. The swelled gel was then slowly dried to the constant weight.

The equilibrium water content (EWC) and the water absorption were (Aw) calculated as follows:

$$EWC\ (\%) = \frac{Ws - Wd}{Ws} \times 100\%$$

where
Ws and Wd are the weight of swollen state and dried state respectively
AW (%) = (Ws – Wo) × 100% Wo
where
Aw is water absorption, and
Wo is the weight of initial gel sample (before immersed in water)

2.6. Degree of adhesiveness

The degree of adhesiveness of the obtained hydrogel was measured based on adhesive to aluminum plate by using Rhesca Tackiness Tester (Rhesca Co. Ltd. Japan) with a gross weight of 100 gf, a constant extention rate of 1 mm/min and the pressure time for 30 second at room temperature.

2.7. Measurement of water vapor transmission rate

The water vapor transmission rate (WVTR) was measured according to monograph of the European Pharmacopiae. It consists of measuring the weight loss of a bottle which containing 25 ml of water. The bottle has a mouth with a diameter of 35 mm. The hydrogel sample with a diameter of 40 mm was than put at the bottle mouth as a cap, and placed in an oven at 35 ^{0}C for 24 hrs. The water vapor transmission rate (WVTR) was calculated by using the following formula;

$$WVTR = \frac{(Wi - Wt)}{A \times 24} \times 10^6\ g/m^2hr$$

where
WVTR is expressed in g/m^2 hr
A is the area of bottle mouth (mm^2)
Wi and Wt are the weight of bottle before and after placed in oven, respectively.

2.8. Microbe penetration test

The gel with a thickness of 2–3 mm was cut into a size of 2 × 2 cm^2, put on the TSA (Tryptose Soy Agar) that had been incubated previously for 18 hr at 30 ^{0}C. On the upper surface of the sample was dropped a suspension of bacteria (B pumilus, Sarcina lutea, and E. coli) with concentration of 10^9/ml and flated by sprayer, then the sample was incubated at 30 ^{0}C. The observation for bacteria's passing through the hydrogel was done day by day for 14 days.

2.9. *In vitro* assessment

In vitro assessment was done by using 28 rabbits (Japanese white rabbits with an average weight of 2500–3000 grams). The procedure of assessment was discussed previously (Zainuddin, et al., 1999). Briefly, after acclimatization for one week, the rabbits were cleaned with barium sulfide solution, anesthetized under Zoletil 50 and the epidermis incision was

prepared. The sample of PVA/PVP blended hydrogel was placed in two sites of wound and was compared to sofratulle and sterile gauze which are placed at other wound positions. It was then analysed for inflammatory effect, comfortability and the absorptions performance of the wound exudate. This histological observations was also done by using Nikon Microscope ophtiphot Camera.

3. RESULTS AND DISCUSSION

3.1. Gel fraction

Irradiation of PVA-PVP blended aqueous solution leads to the formation of insoluble polymer network (gel). A typical dependence of gel fraction on the irradiation dose is given in Fig. 1. It can be seen that the gel fraction increases with increasing dose and it seems never to reach 100% of gel. This certainly indicates that in the PVA-PVP system chain scission also accompanies the crosslinking. The course of chain scission is probably due to oxidative degradation as a result of the presence of residual oxygen. The variation of the gel fraction at difference composition of the polymer blend is not reported.

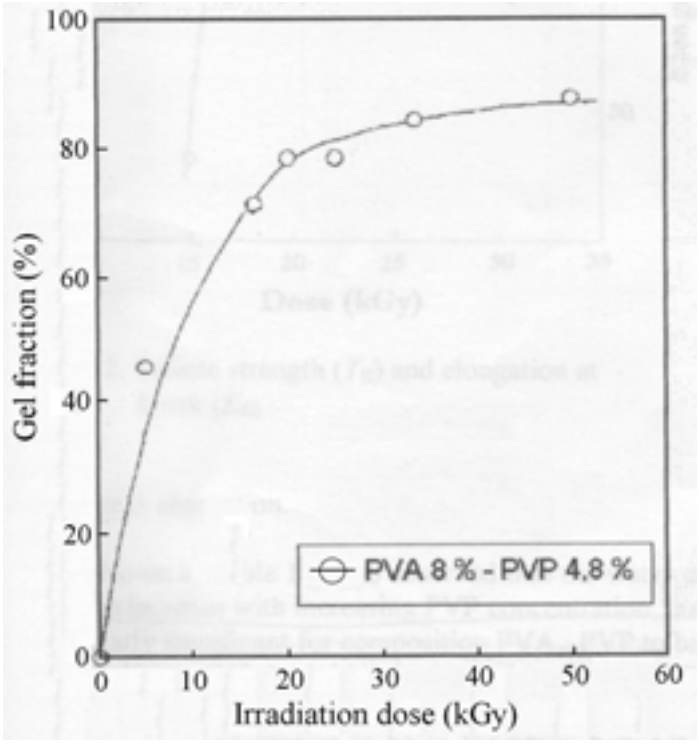


FIG. 1. Gel fraction vs irradiation-dose curve of PVA-PVP hydrogel.

3.2. Mechanical properties

The tensile strength and elongation at break of PVA-PVP blended hydrogel are measured. The results are shown in Fig. 2. Both tensile strength and elongation at break increase with increasing of dose and then decrease. The increase of the tensile strength was believed due to cross-linking. But, the increase of elongation at break may be explained as the effect of grafted chain that occurred simultaneously with crosslinking (Matsuda, et al., 1961). As can be seen in Fig. 2, the tensile strength of 15×10^{-4} Kg/cm^2 and elongation at break 175% were achieved at irradiation condition of 20 KGy. These values are enough to fulfill the mechanical properties required for wound dressing.

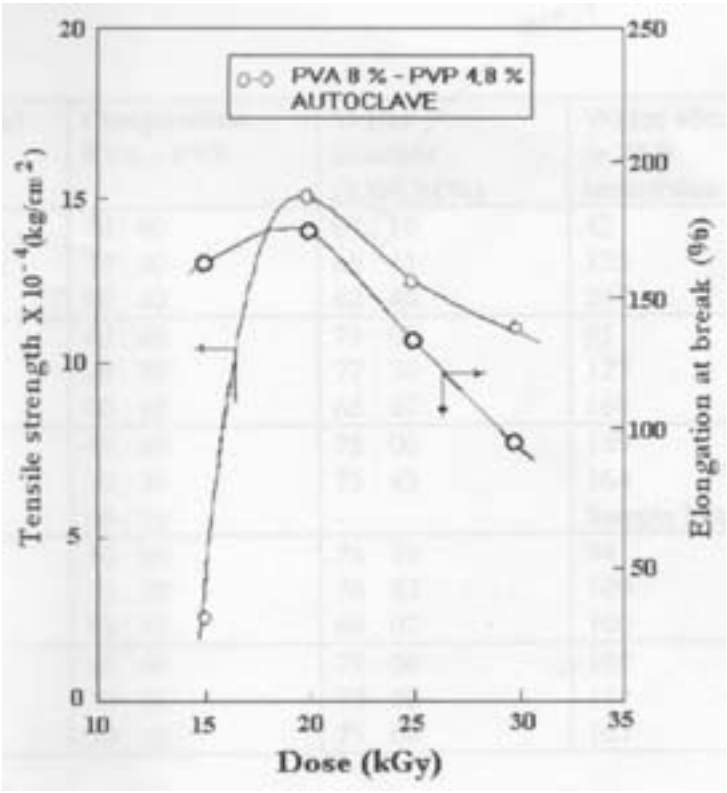


FIG. 2. Tensile strenght (T_B) and elongation at break (E_B) off.

3.3. Water content and water absorption

As shown on Table I it was observed that the water content of PVA-PVP blended hydrogel tends to increase with increasing PVP concentration, but in turn reduces the water absorption. This fact is certainly understandable because if the initial water content of the same sample increases then the ability of the sample to absorp more water will become lower. The PVA-PVP blended hydrogels show the water content to be in the range between 60% and 80% and water absorption (24 h immersed in water) between 40% and 250% (Table I).

Even though the obtain PVA-PVP blended hydrogel has an enough amount of water content, but in fact it can absorb more water. The absorption of water, by the hydrogel blend is much also depend on irradiation dose. The relationship between water absorption and the time of immersion for different dose are shown in Fig 3. It can be seen in Fig 3, the higher the irradiation dose, the lower the water absorption. This is because the cross-linking will be higher at a higher dose. The absorption of water will sharply higher at the immersion time of less than 5 hours, whereas at more than 5 hour immersion time, the rate of absorption will be only slowly increased.

The adhesiveness of PVA-PVP blended hydrogel depended on the concentration of PVP in the hydrogel. Higher PVP concentration, however, affects the decreases of water content but is significantly increases of adhesiveness as shown in Table II. The phenomenon can be explained that the higher PVP concentration will increase the cross-linking therefore decreases water content, but at the same time the functional group contributed from PVP will also increases which causes the improvement in adhesiveness.

TABLE I. WATER CONTENT, WATER ABSORPTION AND WATER VAPOR TRANSMISSION RATE OF DIFFERENT COMPOSITIONS OF PVA-PVP BLENDED HYDOGEL

PVP conc. (%)	Composition PVA:PVP	Water content (EWC) (%)	Water abs. (%) in 24 h immersion time	WVTR (g/m2/h)
4	40:60	67.19	42	89.64
	70:30	65.45	125	100.9
	90:10	62.46	247	110.20
6	40:60	75.01	95	173.97
	70:30	77.30	127	126.49
	90:10	65.07	168	139.10
8	40:60	78.00	139	139.28
	70:30	75.43	164	164.96
	90:10	-	Sample lost	-
10	40:60	78.30	94	156.76
	70:30	76.82	109	118.45
	90:10	68.07	160	138.90
12	40:60	79.06	105	150.60
	70:30	77.50	117	131.39
	90:10	75.00	167	129.40

TABLE II. RELATIONSHIP BETWEEN WATER CONTENT AND ADHESIVENESS

PVP Concentration % w/w	Water content (EWC) (%)	Adhesiveness (gt)
2.4	89.5	6
3.6	88.2	7
4.8	87.1	9
6.0	85.6	10
7.2	74.7	12

3.4. Water vapor transmission rate (WVTR)

According to PEPPAS at al., 1987, the most problem in take care of the burned victim was the fact that the victim may lost of their body liquid due to evaporation and exudation. These will affect the decrease of body temperature and accelerating the metabolism. Therefore the hydrogel wound dressing must avoids or at least reduces the body liquid lost i.e. by controlling absorption and transmission as well as to be able to kept the high humidity in wound area, in order to accelerate the formation of granule and epitelesation process. Based on Table I, it can be seen that the WVTR values of PVA-PVP blended hydrogel are around

80 to 200 gr/m^2/h. These values seem to be an ideal range for wound dressing. The higher value of WVTR causes the faster the drying of wound. Although there are not an exact ideal value of WVTR for wound dressing, but in fact the value must not so high because it will make a dry condition in wound area. On the other hand, if the WVTR value is so low, then it will make the accumulation of exudates which may cause the deceleration of healing process and opening the risk of bacterial growth. For comparison, Table III shows WVTR for some commercial wound dressing values (Bruin, P., et al., 1990). According to Bruin, P. et al., 1990, an occlusive wound covering, such as Op Site (see Table 3) with a WVTR of 33 gr/m^2/hr, has a weakness point, i.e. causes an accumulation of exudates under the covering and in turn causes infection.

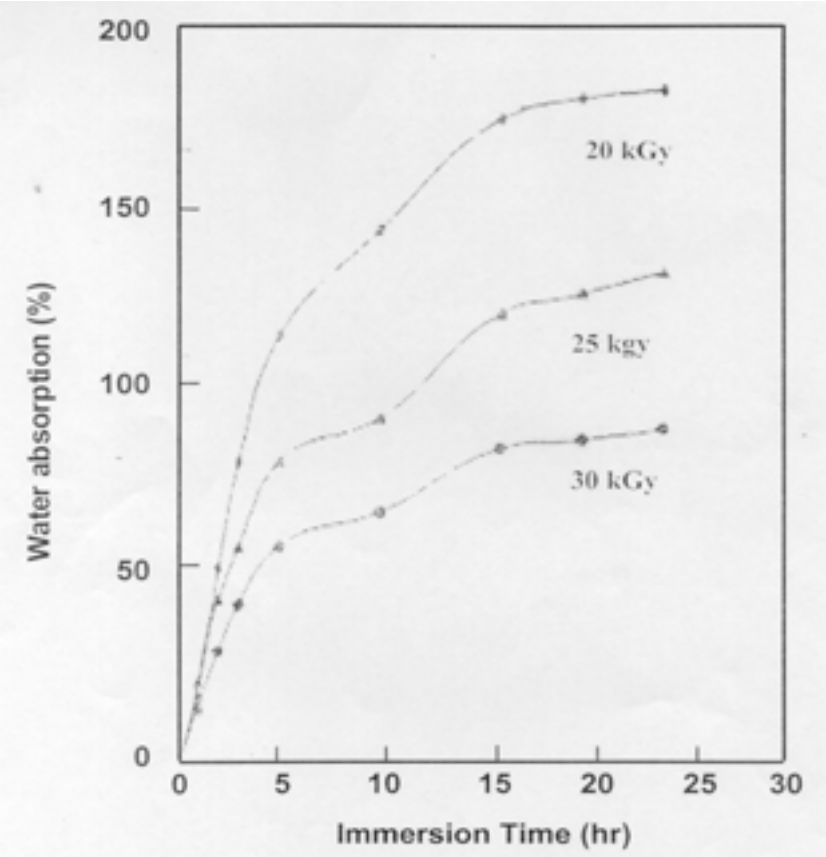


FIG. 3. Relationship between water absorption (%) and immersion time (hr) at different irradiation dose.

TABLE III. THE VALUE OF WVTR OF SOME COMMERCIALIZED WOUND DRESSING

Wound dressing types	WVTR (g/m^2/hr)
Biabrone	154
Metalline	53
Op Site	33
Omiderm	208
Human skin (we)	15
Pig skin (we)	9

3.5. Microbe penetration test

Based on the microbe penetration test, there was no bacteria passing through the hydrogel during day-by-day observation for 14 days. Without bacteria found on the TSA medium, the PVA-PVP blended hydrogel could be considered as a good barrier against microbes. This characteristic is very important for hydrogel dressing, especially in protecting the wound from further infection so that it may accelerate the healing of wound.

3.6. *In vitro* assessment

The macroscopic observations of the wound healing effect in term of comfortability and the excudate absorption performance of the hydrogel sample as compared to Sofratulle and Sterilized Gauze were done. It was found that the PVA-PVP blended hydrogel and Sofratulle have a better comfortability than that of the sterilized gauze when dressing are removed from the wound. The hydrogel adhered slightly to the wound and caused only a little hemorphagic. The capability of the PVA-PVP blended hydrogel in absorbing wound exudates was observed as high as the Sterilized Gauze, while Sofratulle nearly did not absorbed any exudates. Furthermore the PVA-PVP blended hydrogel was better in preventing the wound from contamination compared with the sterilized gauze and sofratulle. This is possible because it has a good comfortability that enables to cover the wound perfectly. It can be seen in Fig. 4, that the healing process which are reflected by the reduction of the wound surface area seems to be proceeded quite fast up to 10 days, then it will be slowly until the wound was fully recovered at the day of 18. There were no significant differences on the time of complete recovering of the wound. However, the surface of the recovered wound treated with PVA-PVP blended hydrogel or sofratulle was observed to be smoother than that of treated with sterilized gauze.

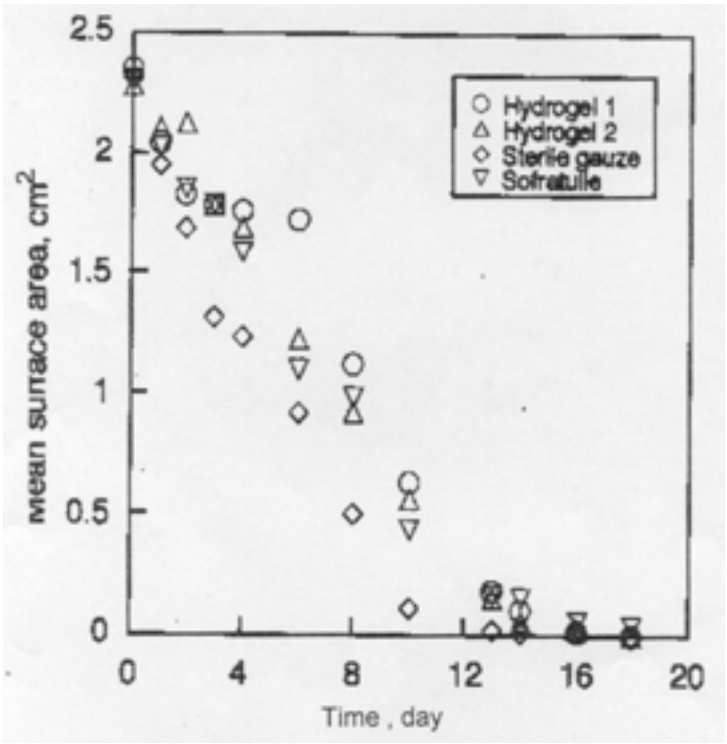


FIG. 4. Relationship between the mean surface area of the wound.

Histological study was done by microscopical observation of the wound healing process. Microscopical observation of the formation of the new tissue at day 3, 7, 14 and 18 revealed that at Day 3, the wound shows no hair follicle and no sebaceous gland under the wound surface (see Fig. 5). The wound surface was covered by exudates layer which was consisted of the mixture of the fibrin, tissue debris and polymorpho nuclear cell (PMN). The tissue bond underwent oidema (macrophage) and blood vessel look hyperemis. Besides that, the initial granulation started to be formed. There were no bacteria of fungi colonies found. At day 7, all wounds either tested with PVA/PVP blended hydrogel, sofratulle or sterilized gauze showed a significant inflammatory reaction.

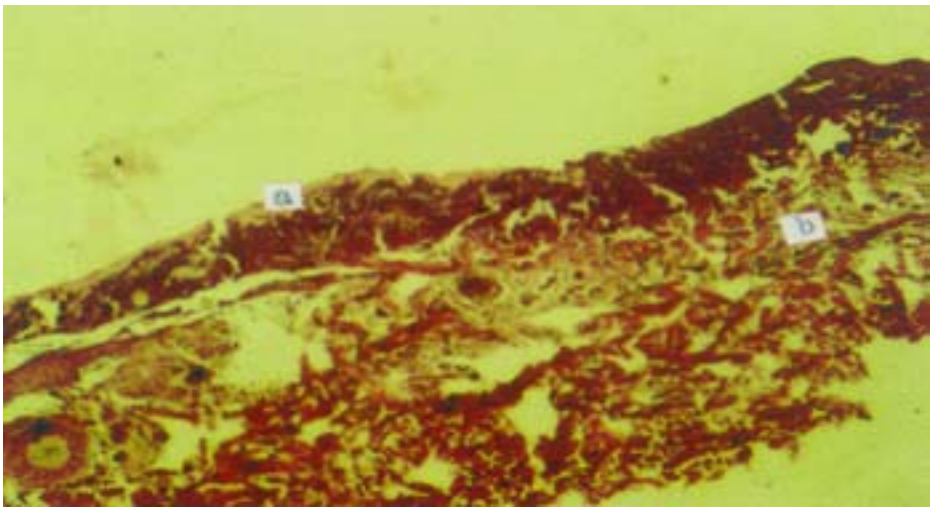


FIG. 5. Histology of wound after 3 days of treatment with PVA-PVP blended hydrogel: (a) the wound covered by fibrin, debris and PMN excudate, (b) hair follicles undergo degeneration and integration. Magnification: 40 X.

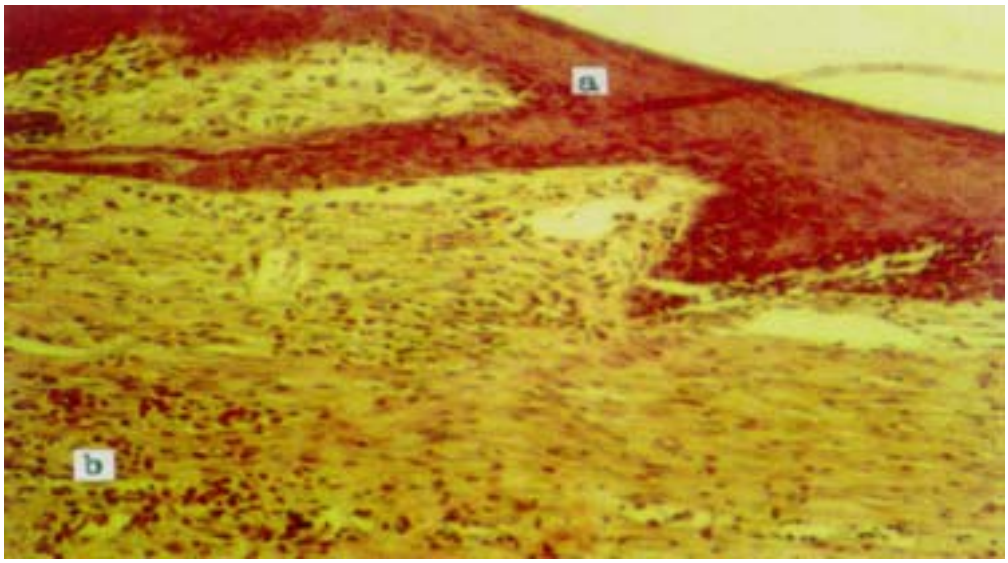


FIG. 6. Histology of wound after 14 days of treatment with PVA-PVP blended hydrogel. Epidermis layer undergoes proliferation: (a) and information of dermis tissue bond which is consisted of PMN, eosinofil and macrophage (b) Magnification: 40 X..

At Day 14, although the wound healing was almost completely achieved and granulation tissue has been formed, the wound surfaces were still covered by PMA exudates (see Fig. 6). The epidermis did not yet contain hair follicle and sebaceous gland, and the edge of the wound became thicker due to proliferation of the epitel cells. These macroscopic and microscopic observations under *in vitro* assessment revels that the PVA-PVP blended hydrogel can meet the efficacy requirement and its healing rate was comparable to that Sterile Gauze and Sofratulle. The hydrogel was also pleasant fell, comfortable, and does not disturb the formation of cells and new tissue on the skin.

4. CONCLUSION

The PVA-PVP blended hydrogel shows some properties thatcan meet the requirements of an ideal wound dressing. For example, it effectively absorbs the fluid, is pleasant to touch and painless to remove, exhibits high elasticity but good mechanical strength and good transparency, and can act as a barrier against microbes. This hydrogel wound dressing is highly potential for use in tropical environment.

ACKNOWLEDGEMENT

The author wishes to thank Prof. Dr. J.M. Rosiak and Dra. Nazly Hilmy, PhD., for their valuable suggestions. The authors also would like to appreciate Dr. Iwan Budiarso for his valuable discussion for *in vitro* assessment. Assistants to experiments by Ms. Dewi SP, Ms. Yuharni, Ida and Rachmi are also acknowledged. This research work is partially supported by IAEA under a Research Contract No. 8978/R2/DPA.

REFERENCES

[1] BRUIN P., JONKMAN M.F., MEIJER H.J., PERMINGS A.J., 1990, A new porous Polyether urethane wound covering, J. Biomed. Mater, Res. 24, 217-226.

[2] CORKHILL, PH, HAMILTON C.J AND TIGHE B.J. (1989), Synthetic Hydrogel. VI Hydrogel Composition as Wound Dressing and Implant Materials, Biomaterials 10, 3–10.

[3] HILMY N, DARWIS D, AND HARDININGSIH L, 1993, Poly (N-vinylpyrrolidone) Hydrogel: 2. Hydrogel Composition as Wound Dressing for Tropical Environment, Radiation. Phys. Chem. 42, 4–6, 911–914, 993.

[4] JIE CHEN, YUEQI YANG, PINGBO QIAN, ZUETEH MA, WEIBIN WU, PEIZHI SUNG, XINGGUO WANG AND JINGHUI LI, 1993, Drug Carrying Hydrogel Base Wound Dressing, Radiation Phys. Cem. 42, 4–6, 915–918, 993.

[5] KROSCHWITZ J.I., 1992, Polymers Biomaterials and Medical Applications, Encyclopedia Reprint Series, Wiley, New York.

[6] MATSUDA T., LIN C.C., SAYAKAWA K., 1961, Radiation Induced Effect on Water Soluble Polymers in their Aqueous Polution I. Crosslinking between Two Different Polymers, Kobushi Kogiku 18, 492.

[7] OHSAKI K., KONSHI J., IKEGAMI K., KOIDE M., 1991, A new reconstructive Method for Skin Defect by Using The Artificial Dermis, Jpn., Journal Artificial Organs 20(2), 497–502.

[8] PEPPAS N. A(Ed), 1987, Hydrogel in Medicine and Pharmacy II and III, CRC Press, Boca Raton FL.

[9] QUINN K.J, COURTNEY J.M., EVANS J.H. AND GAYLOR J.D.S. (1985) Principle of burn Dressing, Biomaterials 6, 369.
[10] RAZZAK M.T., ZAINUDDIN, ERIZAL, S.P. DEWI, H. LELY, E. TATY AND SUKIRNO, The Characterization of Dressing Component Materials and Radiation formation of PVA– PVP Hydrogel, Radiation. Phys. Chem. 55 (1999) 153–165.
[11] ROSIAK, J.M., 1991, Hydrogel Dressing, Radiation Effects on Polymers (Eds.: Clough. R.L, Shalaby S.W), ACS Book series, Washington DC, 475.
[12] ZAINUDDIN, DARWIS D., S.P. DEWI., H. LELY AND RAZZAK, M.T., (1995) Analysis of PVA-PVP Hydrogel by Gamma Irradiation and its Characteristics, Proceeding of 1st Indonesian Polymer Society Symposium, 12.
[13] ZAINUDDIN, S.P. DEWI, SUKIRNO AND RAZZAK, M.T., Efficacy of Radiation Formed PVA/PVP Hydrogel Dressing, Atom Indonesia (submitted 1999).

NUCLEAR METHODS FOR SURFACE MODIFICATIONS OF POLYMERS BY ION INDUCED COGRAFTING

R.O. Mazzei, E. Smolko, D. Tadey, L. Gizzi

Ezeiza Atomic Center,
National Atomic Energy Commission,
Buenos Aires, Argentina

Abstract. Polymer surface modifications are obtained by the application of radiation treatments and other physic-chemical methods: fission fragment irradiation (ff) or ion implantation and etching and/or grafting procedures. A method of surface modification consisting in the physical adsorption of protein after radiation was assayed. The biocompatibility of the surface was observed by cell seeding and cell adhesion experiments. Track membranes were prepared by ff radiation treatment, etching and grafting techniques of commercial foil polymers. PC, PET, PVDF, CTA, PMMA and PS were assayed as bulk polymers and NIPAAm, AAc, VP, Styrene, MMA and HEMA as grafting monomers. Fission fragment radiation from Cf-252, α-particles from Am-241, 25 MeV protons particles from CAE cyclotron accelerator and γ-rays from Co-60 sources were employed in the experiments.

1. INTRODUCTION

Approaches to improve the cell adhesion are obtained by different methods.

In PMMA, Lhoest et al [1], after bombarding the polymer, reconditioned the surface before cell inoculations with surfactant and proteins. Suzuki, et al. [2] improved cell adhesion in PS by ion implanting and Daubresse et al [3] assayed cell adhesion on nuclear track membranes prepared from PVDF films. In our work, we are following these methods but

New materials are prepared using radiation techniques using swift heavy ions (SHI) from Cf-252 and Am- 241 sources.. The radiation graft polymerisation is one of the methods for obtaining the so called INTELLIGENT MATERIALS [4] and [5]. A graft co-polymer may be reached when an active site in a polymer A, initiates the polymerisation of the monomer B [6]. In our work we use two methods to prepare graft co-polymers: in the simultaneous irradiation method or mutual method, the active sites formed during irradiation and contacted with reactive monomer, initiate the polymerisation of the monomer and form graft chains grafted to the polymer substrate. In the peroxide method, the polymer A is at first irradiated in the presence of air which leads to either hydroperoxides or diperoxides. They are stable and can be decomposed at high temperatures. In a second step it is contacted with the monomer to initiate the grafting reaction.

When an ion with atomic number Z and energy per nucleon E/n falls on a polymer film it produces a damage zone around the incident axis. In this way, the charged particle creates a cylindrical region easily attacked by a suitable reagent. The etching solution creates holes along and around the particle path [7]. In this way SSNTD have found widespread applications [8]. The nuclear track technology allows in wide margins the independent choice of pore diameter, shape, inclination and membrane porosity forming the nuclear track membranes (NTM).

An intelligent material is a material with specific characteristics that responds to environmental conditions. In this way NTM can be combined with a polymer that responds to environmental conditions.

2. MATERIALS AND METHODS

2.1 Materials

In our work we use PET films of commercial use, 14 and 20 μm thickness, PVDF film from Solvay (Belgium), 6, 9, 14 and 25 μm thickness; CTA, Triafol TX from Bayer, 60 μm thickness, and prepared films by dissolution in chloroform and evaporation of this material, 27 μm thickness. CTA films from pellets, Aldrich Chemical Company, Inc., were prepared by dissolution in diclorometano and evaporation, 20 and 40 μm thickness. Makrofol E (Bayer AG) foils of 10 μm thickness and 5 μm pore diameter Micron Separation inc. filters were used. Also films of commercial PMMA and PS 150 μm thickness were prepared in the laboratory by dissolution in chloroform. and evaporation. NIPAAm and HEMA was from Aldrich, AAc and styrene from Merck, vinylpirrolidone (VP) from Fluka and MMA from J.T.Baker.

2.2. Irradiations

Irradiations were performed by means of a Californium- 252 fission fragments source, and an Americium-241 α-particles emitter, 25 MeV protons particles from the CAE cyclotron accelerator and gamma rays from an extended Co-60 source. The Cf-252 source is a foil source from ISOTOPE PRODUCTS LABORATORIES USA, Catalog Number FF-252-3 with an active area of 5.04 mm diameter spot containing 0.05 μg of electrodeposited Californium oxide. The activity is fixed to the supporting foil by diffusion bonding at 1000 ^{o}C and it is initially of 925 kBq (25 μCi). The fission spectrum have a heavy fission fragment and a light fission fragment with 160-200 MeV energy divided among them, corresponding to ff energies between 1 and 2 MeV/amu. The Am-241 α source with 5.5 MeV energy, 1.4 MeV/amu was prepared by CNEA Radiochemical Laboratories in a round foil shape containing 15 μCi of the radioisotope emitter.

2.3. Methods

2.3.1. Protein coating procedure

The bombarded foil samples are rinsed in a phosphate buffered saline solution (PBS). They are further reconditioned one hour by immersion in a solution at physiologic pH (7.4) and osmolarity and containing both a protein (type I, collagen, 33 μg/ml) and a non ionic surfactant (Pluronic F68, 0.01 w/v) [1]

2.3.2. Chemical etching procedure I

Etching solution using 6 N KOH + 0.1 F KMnO4 solution at 85 ^{o}C (PVDF).

2.3.3. Chemical etching procedure II

Etching solution using 5N NaOH at 40 ^{o}C or 60 ^{o}C (PET).

2.3.4. Chemical etching procedure III

Etching solution using 6.25N KOH at 60 ^{o}C (CTA).

2.3.5. Cell seeding and cultivation procedure

The V79-4, lung, Chinese hamster, Cricetulus griseus passage number 15–20 from ATCC was used for the cell adhesion experiments. It was kept in F-12 culture medium, supplemented with 10% fetal bovine serum and 50 μg/ml gentamicine in 5% CO_2 atmosphere.

3. EXPERIMENTS

3.1. Cell adhesion

Three experiments were designed to study the cell adhesion capability of the V79-4 cells.

3.1.1.. Exp. a1

Foils of PMMA, 150 μm thickness were irradiated and reconditioned with the protein coating procedure and then inoculated with the V79-4 cells. There are two groups according to the radiation treatment.

Subgroup a1.1: irradiated with 10^{11} ff/cm^2
Subgroup a1.2: irradiated with 3.10^{11} α/cm^2.

3.1.2. Exp. a2

Foils of PS, 150 μm thickness were bombarded with 10^{11} ff/cm^2 [2] and then inoculated with cells according to the cultivation procedures.

3.1.3. Exp. a3

Foils of PVDF, 25 μm thickness were bombarded with ff with exposure time between 2 seconds and 600 seconds and etching times corresponding to track diameters between 1 and 9 μm [3]. Then cell inoculation according to the corresponding procedure.

3.2. Intelligent materials and grafted materials

Foils of polyethylene terephtalate (PET), cellulose triacetate (CTA) and polyvinylidene fluoride (PVDF) were used to obtain intelligent materials. PET, PVDF 14 μm and CTA 20 μm foils were transformed to NTM by bombardment with ff and then etched to get a porous structure with determined porous size. PVDF foils, 25 μm thickness, were grafted with NIPAAm, MMA, HEMA, AAc and styrene using 25 MeV protons particles from the CAE cyclotron accelerator and VP, MMA, HEMA, AAc and styrene using gamma rays from an extended Co-60 source.

Foils of CTA, 60 μm thickness, were grafted with NIPAAm and with AAc monomers without ff bombardment. PET grafting was assayed under mutual and preirradiation grafting methods, while CTA and PVDF only by the preirradiation one.

3.2.1. PET soaked

Two experiments with PET 20 and 14 μm respectively were performed searching etching conditions for different foil thickness:

3.2.2. PET 20 μm, soaked

PET 20μm thickness bombarded 5 s with ff were chemical etched with a 5N NaOH solution at 40°C during 5h to obtain 3μm diameter tracks. The NTM was soaked at room temperature in aqueous solution of 10% wt N-isopropylacrylamide (NIPAAm) monomer and irradiated at 60°C under nitrogen atmosphere for 1 h with gamma rays from a cobalt source at a dose rate of 10kGy/h [5].

3.2.3. PET 14 μm, soaked

PET- NTM filters 14 μm thickness 1 h etch in 5N NaOH at 60°C (3 μm diameter). Irradiation and grafting conditions as in the previous case.

3.2.4. PET 14μm thickness, in solution

PET 14 μm thickness, bombarded 5 s with ff were chemical etched with a 5N NaOH solution at 42°C during 5h to obtain 3μm diameter tracks. The NTM were washed and tested with phenolphthalein until the residual water did not tack, then NTM were washed again using boiling distillate water during an hour. After that NTM were put into test tubes with a solution of NIPAAm at concentrations between 0.1, 0.2, 0.5, 1 and 2.5% and irradiated with gamma rays from the cobalt source at 2.1 and 3.6 kGy.

3.2.5. PET 20μm, preirradiated

PET 20μm thickness bombarded 5 s with ff, chemical etched to obtain 5 μm track diameter NTM were preirradiated by gamma rays from the cobalt source at a dose of 100 kGy and at dose rate of 0.178 kGy/h. After that, they were put into test tubes with aqueous solutions of NIPAAm at concentrations between 7% and 30%, in nitrogen ambient.

3.2.6. CTA, 27 μm

Cellulose triacetate foils 27 μm thickness were extracted in boiling toluene during 24 h and then preirradiated with gamma rays from the cobalt source at a dose of 100 kGy and put into a 6% NIPAAm solution in nitrogen ambient during 1 h at 62°C.

3.2.7. CTA, 60 μm

Cellulose triacetate foils 60 μm thickness were preirradiated with gamma rays from the cobalt source at doses between 10 and 100 kGy and put into test tubes between 0 and 40% acrylic acid solution and different Mohr salt concentrations in nitrogen ambient during 1 h at 62°C [9]. These foils were also extracted in boiling toluene during 24 h.

Some CTA samples were chemically etched using 6.25 N KOH solution during 10 minutes at 60°C.

3.2.8. CTA, 20 and 40 μm

CTA films from pellets, Aldrich Chemical Company, Inc., were prepared by dissolution in diclorometano and evaporation, 20 and 40 μm thickness. CTA foils were irradiated between 10 s and 60 s with ff, etched chemically using a 6.25 N KOH solution at 62°C during different etching times to produce tracks with diameters between 1.5 μm and 8 μm. The foils were washed with distillate water during 24 hour at room temperature and dried in vacuum during a day at 40 °C. After that, irradiated with gamma rays from the cobalt source at between 5 and 60 kGy at a rate of 0.179 kGy/min in pure oxygen ambient to produce peroxides. The foils were put into closed test tubes with aqueous solutions of AA of 15%, bubbled in nitrogen ambient and put into the warming bath at 62°C.

3.2.9. PVDF 14μm

PVDF foils, extracted in boiling toluene 24 h were irradiated between 1 s and 330 s with ff, etched chemically using a 6N KOH + 0.1 F $KMnO_4$ solution at 85°C during different etching times to produce tracks with diameters between 0.5 μm and 6μm. The foils were washed with distillate water and put in an oxygenated water solution of chloridric acid during an hour at room temperature, then washed in distillate water and dried in vacuum during a day at 40 °C. After that, irradiated with gamma rays from the cobalt source at between 10 and 140 kGy at a rate of 0.179 kGy/min in pure oxygen ambient to produce peroxides. The foils were put into closed test tubes with aqueous solutions of NIPAAm between 0% and 16% or acetone solutions of NIPAAm between 0% and 40%, bubbled with nitrogen and put into warming bath at 62 °C or 80 °C during grafting times between 0 and 15 h and 18 min.

PVDF-NTM, 20 sec. ff irradiation time, 3μm pore diameter, were irradiated with gamma rays from the cobalt source at between 10 and 100 kGy at a rate of 0.179 kGy/min in pure oxygen ambient. The foils were put into closed test tubes with aqueous solution of AAc (15%) or with VP.

3.2.10. PVDF 25μm

PVDF foils were irradiated with gamma rays from the cobalt source at between 10 and 100 kGy at a rate of 0.179 kGy/min in pure oxygen ambient to produce peroxides. The foils were put into closed test tubes with styrene, HEMA, MMA, VP and aqueous solutions of AAc (15%), bubbled in nitrogen ambient and put into the warming bath at 62°C.

3.2.11. PVDF 25μm

PVDF foils were irradiated with 25 MeV proton particles from the cyclotron CAE accelerator between 9.2 and 276 kGy at a rate of 69 kGy/min in air ambient to produce peroxides. The foils were put into closed test tubes with styrene, HEMA, MMA, and aqueous solutions of AAc (15%) or NIPAAm (10%), bubbled in nitrogen ambient and put into the warming bath at 62°C.

3.2.12. PVDF 25 μm

PVDF films, 25 μm thickness were irradiated with:

(1) Fission fragments with doses between 0,3 and 200 kGy
(2) Alpha particles with doses from 3 to 50 kGy.

Samples i) and ii) were grafted with styrene between 0 and 22 h at 62°C. Same procedure as above.

3.2.13. PC-NTM and PC foils

Makrofol E (Bayer AG) foils of 10 μm thickness and 5 μm pore diameter Micron Separation inc. filters (NTM) were grafted with HEMA. Thin polycarbonate (PC) foils and NTM, were grafted with hidroxyethylmethacrylate (HEMA) using gamma photons and the simultaneous method. Pc foils were cleaned with destiled water and 2% Extran MAO2 neutral Merck for one day, thoroughly washed in destiled water for ten days and then dried in air for a month. NTM were used as received.

4. RESULTS

4.1. Cell adhesion

Cases a1 and a2 did not show great difference between irradiated and no irradiated zones. with respect to cellular adhesion.

In a3, foils of PVDF were irradiated during different times with fluences between 3.8 10^4 and 2.3 10^7 tracks/cm^2 and chemically etched during different etching times. Foils with track diameters between 0.5 and 9 μm were inoculated with V79-4 cells (hamster Chinese lung line).

The microphotography 1 shows the cell adhesion for:

(a) without tracks
(b) with 2.3 10^6 tracks/cm^2 and
(c) with 2.3 10^7 tracks/cm^2.

The track diameter was 0.5 μm in all the cases.

In the microphotography 2:

(a) without tracks.
(b) Pore diameter of 1 μm and bombarded with 2. 10^5 tracks/cm^2.

The microphotography 3 shows the cell adhesion for:

(a) without tracks
(b) with 1.1 10^5 tracks/cm^2. The track diameter was 2.5 μm in both cases.

It can be observed differences in the cell adhesion among the different cases especially for microphotography 1 and 2. However, the samples show scattering among different zones in the same sample.

4.2. Intelligent materials

The included numbers correspond to experimental samples

4.2.1 PET soaked

4.2.2 (3.2.2) the foil did not grafted.

4.2.3 (3.2.3) It was obtained between 2% and 5% of grafting yield. However, it depended on the form of washing. After washing at room temperature foils showed greater grafting than foils washed at 70 ^{o}C.

4.2.4 (3.2.4) PET, 14 μm in solution. It was obtained 19.5% of grafting yield for 2.1 kGy and 1% NIPAAm. However, the swelling of the foil at 18^{o}C was 0%.

On the other hand for NIPAAm concentration between 0.5 and 2.5% the grafting yield was 0%. In foils irradiated at 3.6 kGy for 1% and for 2.5% NIPAAm concentration it was obtained 39% and 200% graft yield respectively. In both cases there were observed homopolimerization

4.2.5 (3.2.5) PET 20 μm preirradiated. The grafting yield was 0% in every case.

The grafted surfaces obtained were non uniform for 3.2.2, 3.2.3, 3.2.4 and 3.2.5 groups and it was very difficult to control the grafting yield and the homogeneity of the grafted surface. The grafting yield gave different results by stirring and washing three to four times at ambient temperature or by the same procedure plus an extra washing at 70^{o} C In same foils it was obtained 0% grafting yield after the more intensive procedure.

4. 2.6 (3.2.6) CTA, 27 μm. It was obtained 58% grafting yield for cellulose triacetate foils using the peroxide method.

4.2.7 (3.2.7) CTA, 60 μm

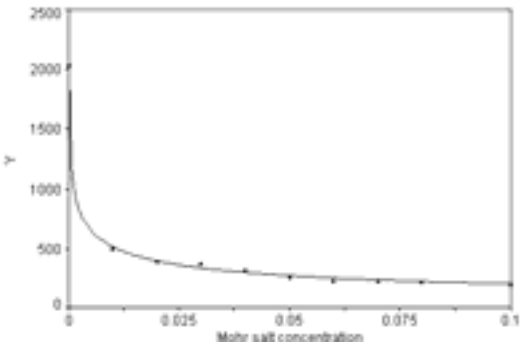


FIG. 1. Grafting yield (Y) as a function of Mohr salt concentration.

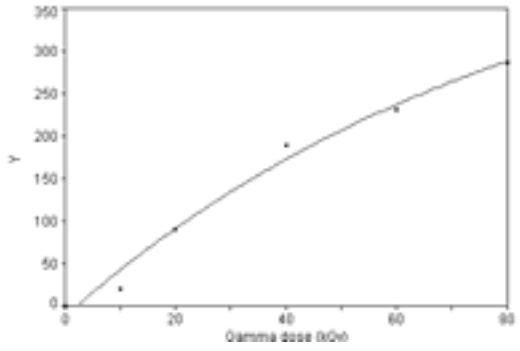


FIG. 2. Y as a function of γ dose.

Fig 1 shows the grafting yield (Y) as a function of Mohr salt percentage for triacetate cellulose foils for 50 kGy of preirradiation dose and 15% of acrylic acid. It can be observed a minimum for 0.1% Mohr salt concentration. For concentrations greater than 0.05% homopolimerization is not observed.

Figs 2 and 3 show Y as a function of dose in kGy for 15% acrylic acid and 0.1% Mohr salt, 2 h 21 min. and 2h of grafting time at 62 ^{o}C and 88 ^{o}C grafting temperature respectively.

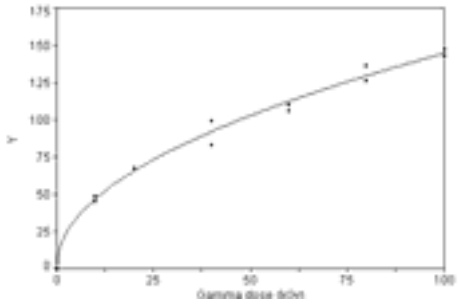


FIG. 3. Y as a function of gamma dose for 88°C grafting temperature.

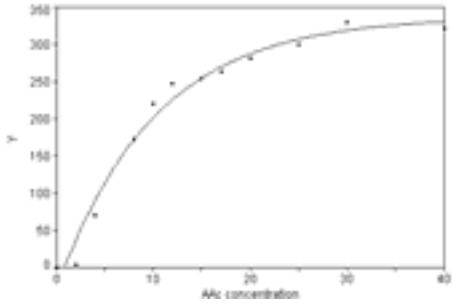


FIG. 4 Y as a function of AAc concentration in water.

Fig. 4 shows Y as a function of AA concentration for 50 kGy gamma dose, 0.1% Mohr salt, 3 h of grafting time at 62 ^{o}C.

Figs 5 and 6 show Y as a function of grafting time for 15% of acrylic acid, 0.1% Mohr salt, 62 ^{o}C grafting temperature for 40 and 80 kGy gamma dose respectively.

4. 2.8 (3.2.8) CTA-NTM.

CTA-NTM were obtained using the method descripted in Experiment- 3.2.8. Fig. 7 shows the calibration curve of CTA- NTM in track number ($N^{o} \times 10^{5}/cm^{2}\cdot\pi$) as a function of the irradiation time with Cf-252 fission fragment ions (sec.), for 40 μm thickness foils. The number of tracks observed after the standard chemical etching procedure was approximated to a rate of T= 1. 10^{4} tracks/cm^{2}.sec.

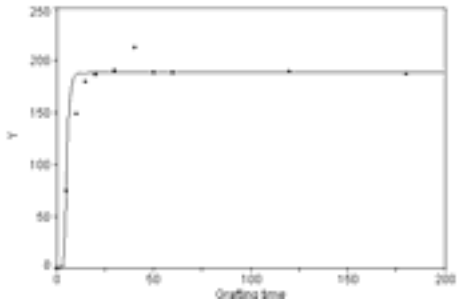


FIG. 5. Y as a function of grafting time for 40 kGy gamma dose.

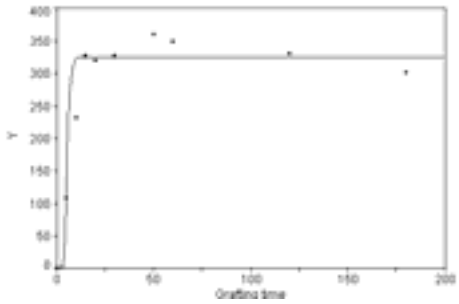


FIG. 6. Y as a function of grafting time for 80 kGy gamma dose.

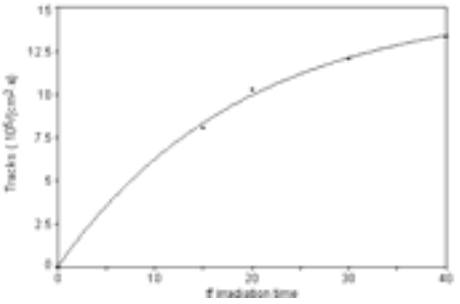


FIG. 7. Calibration curve of CTA- NTM.

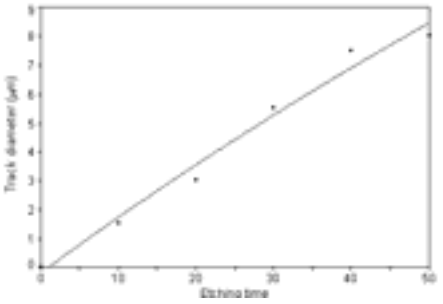


FIG. 8. Track diameter as a function of the etching time.

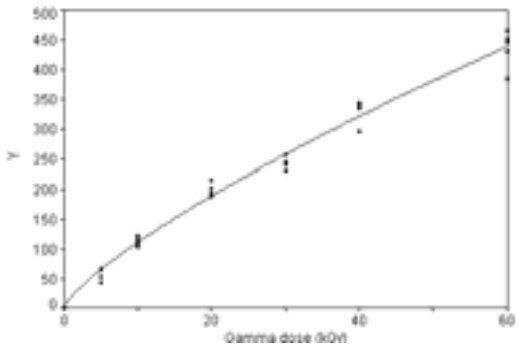


FIG. 9. Y as a function of gamma dose for foils without pores.

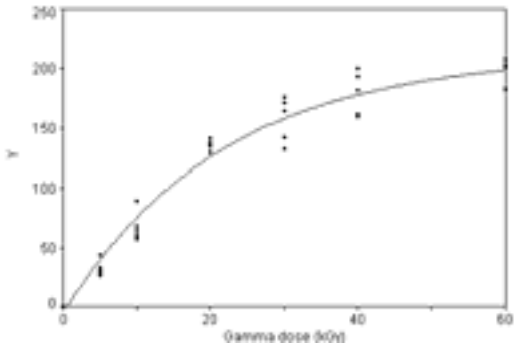


FIG. 10. Y as a function of gamma dose for foils with pores.

Fig. 8 shows the study modification of the track diameter in microns of the CTA films 40 μm thickness, irradiated by 60 seconds and subjected to etching times between 0 and 50 min. post irradiation. The results can be plotted approximately by a lineal function with a slope equal to 0.16 in a μm vs. min. representation. NTM was then irradiated with gamma rays at different doses and grafted with AA.

Fig. 9 shows Y as a function of dose in kGy for 15% acrylic acid and 0.1% Mohr salt, 24 h of grafting time at 62 ^{o}C grafting temperature, for foils without pores.

Fig. 10 shows Y as a function of dose in kGy for 15% acrylic acid and 0.1% Mohr salt, 24 h of grafting time at 62 ^{o}C grafting temperature, for 60 sec. ff irradiation time and 40' etching time.

Fig. 11 shows Y as a function of etching time in min for 15% acrylic acid and 0.1% Mohr salt, 24 h of grafting time at 62 ^{o}C grafting temperature, for 60 sec. ff irradiation time and 20 kGy gamma dose.

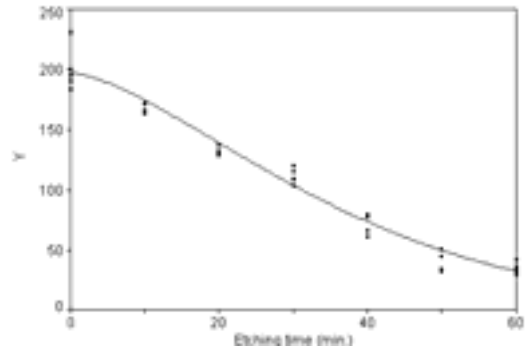


FIG. 11. Y as a function of etching time.

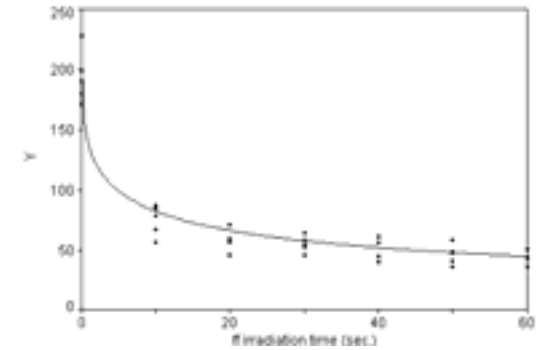


FIG. 12. Y as a function of ff irradiation time.

Fig. 12 shows Y as a function of ff irradiation time in sec. for 15% acrylic acid and 0.1% Mohr salt, 24 h of grafting time at 62 ^{o}C grafting temperature and for 7 μm pore diameter 20 kGy gamma dose.

4.2.9 (3.2.9) PVDF, 14 μm

To study the influence on the grafting yield due to the monomer solutions, water and acetone were used as solvent in different NIPAAm concentrations. Fig. 13 shows the

evolution of the grafting yield in PVDF foils under the following irradiation, polymerization and polymer substrate parameter:

PVDF foils of 14 μm thickness were irradiated with fission fragment at fixed flux of 5, 15 and 120 seconds etched to a 3–4 μm pore diameter and irradiated with gamma rays at 100 (FIG. 13 a and b) and 140 kGy (FIG. 13 c). Grafting times were fixed at 1.75 (FIG. 13 b), 2.5 (FIG. 13 a) and 15 hours (FIG. 13 c).

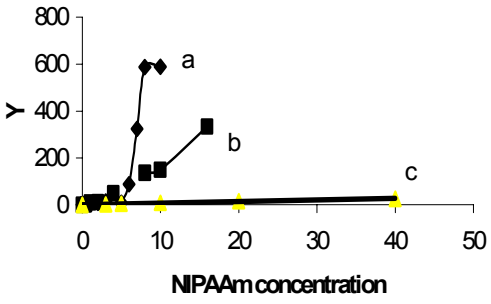


FIG. 13. Y as a function of NIPAAm concentration: a) 15 s ff irradiation time in water solution, b) 5 s ff irradiation time in water solution, c) 120 s ff irradiation time in acetone solution.

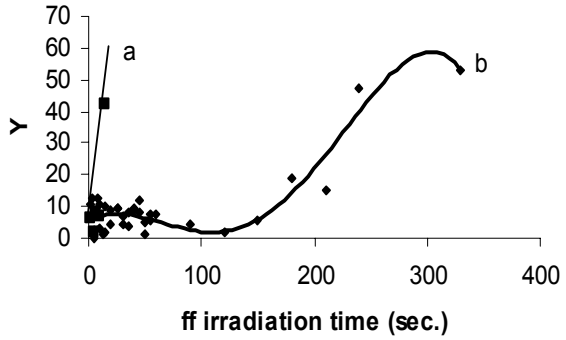


FIG. 14. Y as a function of ff irradiation time: a) 6% NIPAAm, b) 3% NIPAAm.

The grafting yielding in acetone was observed under the more stringent conditions due to low grafting evolution. Grafting yielding in water solution are shown as curves (a) and (b) for increasing ff fluxes. Grafting yields have increasing rates with increasing monomer concentration. For lower ff values the yield is increasing steadily but gently up to 300% about. For higher ff values the yield is changing suddendly up to a maximun of 600% in a narrow range of NIPAAm concentration between 5–8%. It can be predicted a transition zone for the grafting yield in this monomer concentration values, for the three main parameters increasingly changed, i.e. ff, grafting time and pore diameter. Although, NIPAAm is more soluble in acetone than in water, the yield obtained represented by curve (c) in much higher in this later condition than in the former one, reading 27% yield for monomer concentration of up to 40%.

Fig. 14 shows the evolution of grafting yield as a function of the ff irradiation time, for 100 kGy irradiation dose and 14 μm thickness foil, for two NIPAAm concentration in aqueous solution and 62 ^{o}C grafting temperature (a) is for 6% NIPAAm concentration, for 3 μm pore diameter and 1 h 48 min. grafting time. (b) is for 3% NIPAAm concentration, for 3.5 μm pore diameter and 2 h 30 min. grafting time.

Fig. 15 shows Y as a function of pore diameter for 3% NIPAAm concentration, for 20 s ff irradiation time, for 100 kGy gamma dose, for 2 h 30 min. grafting time and 60 ^{o}C grafting temperature. The grafting yield is practically constant between 0 and 4–5 μm pore diameter and it has a abrupt increase in about 6 μm pore diameter.

Fig. 16 shows the evolution of grafting yield as a function of the grafting time for 100 kGy irradiation dose and 14 μm thickness foil for two NIPAAm concentration in aqueous solution. Fig. 16a is for 3% NIPAAm concentration, for 2 min ff irradiation time, for 3.5 μm pore diameter and for 80 ^{o}C grafting temperature. Fig. 16b is for 6% NIPAAm, for 15 s ff irrad, for 4 μm pore diameter and for 60 ^{o}C grafting temperature. Fig. 16c is for 6% NIPAAm concentration, for 2 min. ff irradiation time, for 3 μm pore diameter and for 60 ^{o}C grafting temperature. After a rapid rise in the initial stage of the grafting, Y reaches a saturation level for grafting time greater than about 100 min. A height dispersion is observed, specially during the initial grafting time.

Fig. 17 shows the evolution of grafting yield as a function of the gamma dose for different grafting temperatures, ff irradiation times and NIPAAm concentrations. Y reaches a maximum at about 110 kGy for 180 sec ff irradiation time (figs 17 b and c) and at 60 kGy for 5 sec. ff irradiation time (figs a and d). Y increases with the NIPAAm concentration (figs a and d) and with the ff irradiation time (figs a and b). Y diminished when the grafting temperature increases (figs b and c)

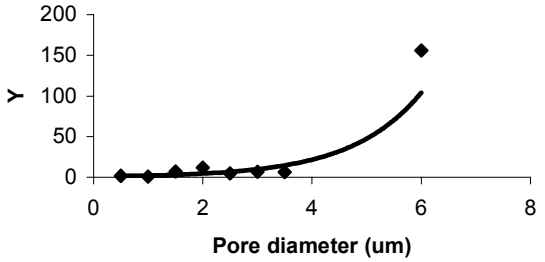


FIG. 15. Pore size grafting dependence.

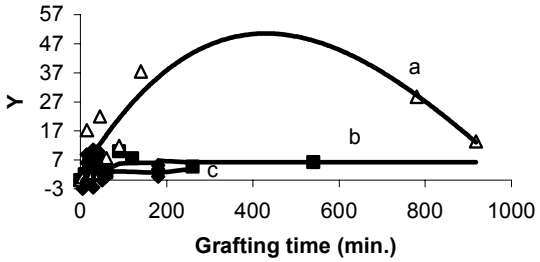


FIG. 16. Grafting time dependence: a) 2 min ff, 3% NIPAAm at 80 ^{o}C, b) 15 sec ff, 6% NIPAAm at 60 ^{o}C, c) 2 min ff, 6% NIPAAm at 60 ^{o}C.

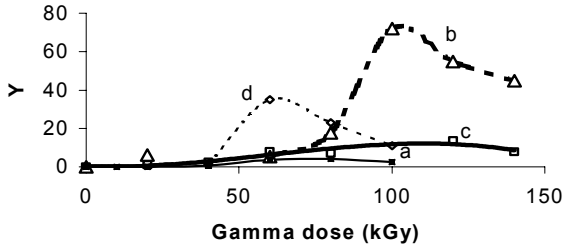


FIG. 17. Gamma dose dependence: a) 5 sec. ff, 3% NIPAAm at 60 ^{o}C, b) 180 sec. ff, 3% NIPAAm at 60 ^{o}C, c) 180 sec. ff, 3% NIPAAm at 80 ^{o}C, d) 5 sec. ff, 6% NIPAAm at 60 ^{o}C.

Assuming a superficial grafting, the track number and the track diameter are related with the grafting yield in the following way [10]:

$$Y(\%) = (S - N\pi r^2 + N\, 2\pi r\, L)\, L' \rho_{Nipaam}/m_i \quad (1)$$

Where:

Y%: grafting yield,
S initial surface of the film without tracks,
N: track number,
r: track radious,
L: thickness foil,
L': grafting thickness,
ρ_{NIPAAm}: NIPAAm density,
m_i: initial mass of the foil.

The limit condition for the pore not to be occluded with the grafted monomer is the pore track radious just near to the grafted thickness.

In such a case

$$L' = r \quad (2)$$

The density of NIPAAm is

$$2 > \rho_{NIPAAm} > 1 \quad (3)$$

We are interested to find the conditions such as the pore track is just closed due to the grafting thickness. So, we must impose L'= r. Supposing 2>ρNIPAAm>1 we obtain from equation (1) a grafting yield about 10%. From Fig. 17d we obtained 10% grafting yield for 40–60 kGy dose for 6% NIPAAm concentration, 5 s irradiation time and 3μm track diameter. Moreover, the grafting yield depend strongly on the track diameter and the ff irradiation time, Figs 15, 14 a and 14 b, for track diameters greater than 5 μm and for ff irradiation time greater than 10 sec. or 200 sec repectively. So, these variables must be controlled carefully to obtain a given grafting yield. On the other hand, Y shows a smooth dependence as a function of the NIPAAm concentration in acetone. In this way NIPAAm acetone solutions can be used to obtain a given grafting yield.

(3.2.9) PVDF, 14μm, 3 μm pore diameter, AAc and VP monomers

Fig 18 shows the grafting yield as a function of gamma dose for PVDF 14 μm thickness foils, with 3um pore diameter and 20 sec ff irradiation time. Fig 18 (a) is for 100% VP and Fig 18 (b) is for 15% AAc in aqueous solution.

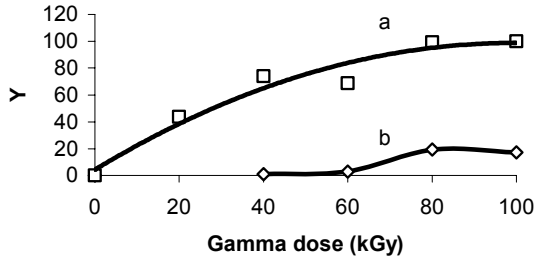


FIG. 18. Gamma dose dependence for PVDF-NTM grafted with: a) VP b) AAc.

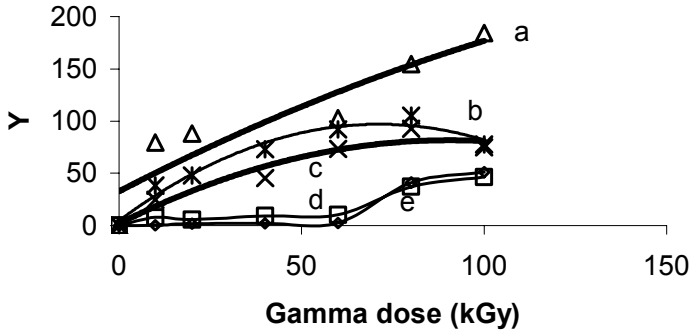


FIG. 19. Gamma dose dependence for PVDF grafted with: a) MMA b) VP c) Styrene d) HEMA and e) Aac.

4.2.10(3.2.10) PVDF 25 μm gamma irradiation

Fig 19, from a to e, shows the grafting yield as a function of the gamma dose, for 25 μm thickness PVDF films grafted with MMA, VP, styrene, HEMA and AAc respectively.

4.2.11 (3.2.11) PVDF 25 μm, protons

Figs from 20 and 21 show the grafting yield as a function of the protons dose, for 25 μ thickness PVDF films grafted with AAc, styrene, HEMA, MMA and NIPAAm respectively.

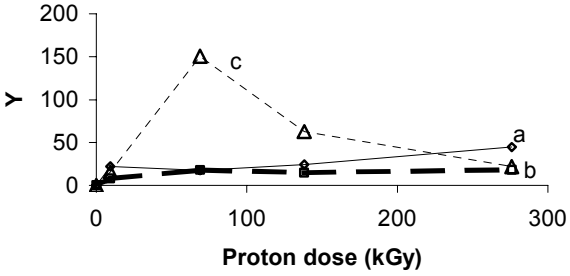


FIG. 20. Proton dose dependence for PVDF grafted with: a) AAc b) Styrene c) HEMA.

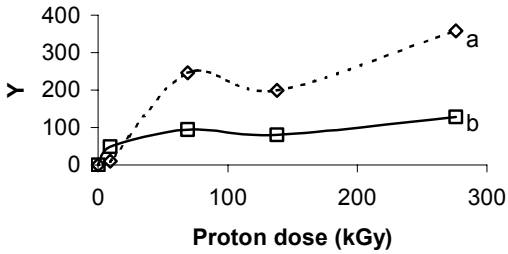


FIG. 21. Proton dose dependence for PVDF grafted with: a) MMA b) NIPAAm.

4.2.12 (3.2.12) PVDF 25 μm, ff and alpha

Foils irradiated with alpha particles from a Am-241 source or with fragment fission from a Cf-252 source at different fluences were grafted with styrene.

Fig 22 shows the grafting yield as a function of alpha and ff dose for 62^{o}C temperature bath.

Fig 23 shows the grafting yield as a function of the grafting time for 3kGy ff dose.

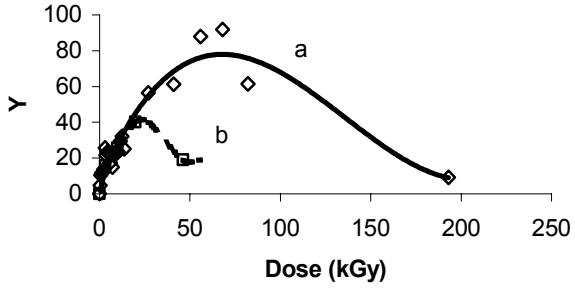


FIG. 22. Dose dependence for PVDF grafted with Styrene: a) ff irradiation, b) alpha irradiation.

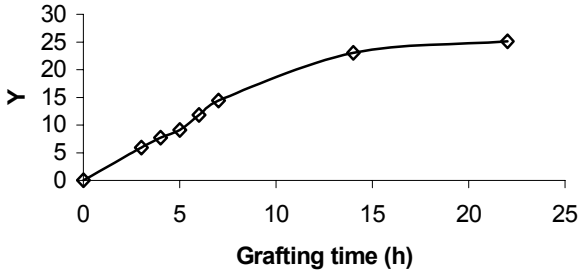


FIG. 23. Y as a function of the grafting time for PVDF and for 3 kGy ff dose.

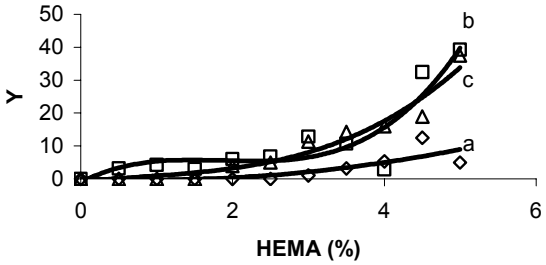


FIG. 24. Y as a function of HEMA concentration, for different gamma dose irradiations in PC-NTM: a) 0.5 kGy b) 2 kGy c) 3 kGy.

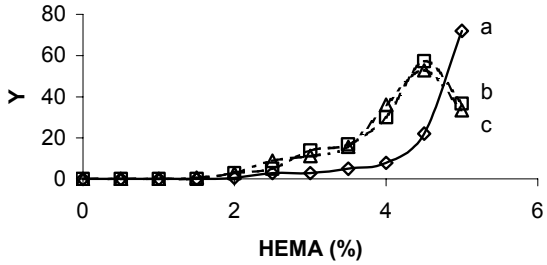


FIG. 25. Y as a function of HEMA conc. for different gamma dose irrad. in PC foils: a) 0.5 kGy b) 2 kGy c) 3 kGy.

4.2.13 (3.2.13) PC-NTM and PC foils

Grafting yield curves as a function of the HEMA percent in aqueous solutions for different gamma doses in PC-NTM (FIG. 24) and PC foils (Fig. 25) were obtained. The method of simultaneous irradiation was used.

c) The target theory applied to swift heavy ions (SHI) track grafting experiments.

The damage induced in polymers by SHI can lead to chain scission, free radicals formation and many excited molecules. The active sites so produced along the polymer chains can be used to initiate grafting reactions. Betz [11] obtained interesting results in SHI track grafting of styrene monomers onto poly (vinylidenefluoride) (PVDF) films. Moreover, Butts and Katz have developed a target theory that allows to predict the response of different substrates to heavy ions irradiations. In our work we postulated a non active zone for grafting around the ion incidence axis. This non grafting zone allows the experimental results obtained by Betz to be approximated by the target theory. In the Saclay's experiments the electronic energy loss of the ions were varied from 2.2 to 72.6 MeV Cm^2 mg^{-1} and the fluency range was varied from 5 10^7 to 510^{10} Cm^{-2}.

When the grafting yield Y is ploted as a function of the fluency F for ions with different dE/dX a set of maximun can be observed such that “when dE/dX increases the yield and the fluency corresponding to the maximun are decreased”.

We consider:

One hit processes: a hit, in a target embedded in a passive matrix, is responsible for creating an active site for the grafting. We postulate a cylindrical deactivated zone (non activated zone for grafting), with radius ro, around the ion incidence axis where very high dose can be delivered and where the target don’t contribute to the grafting yield. Moreover, we have taken into account the intersection among tracks where deactivated zones can intersect and disable activated zones. From the above considerations we have obtained the net number of activated targets and then the grafting yield as a function of the fluency.

Fig. 26 shows the experimental results from Betz et. al. and the theoretical one obtained using our equations [12].

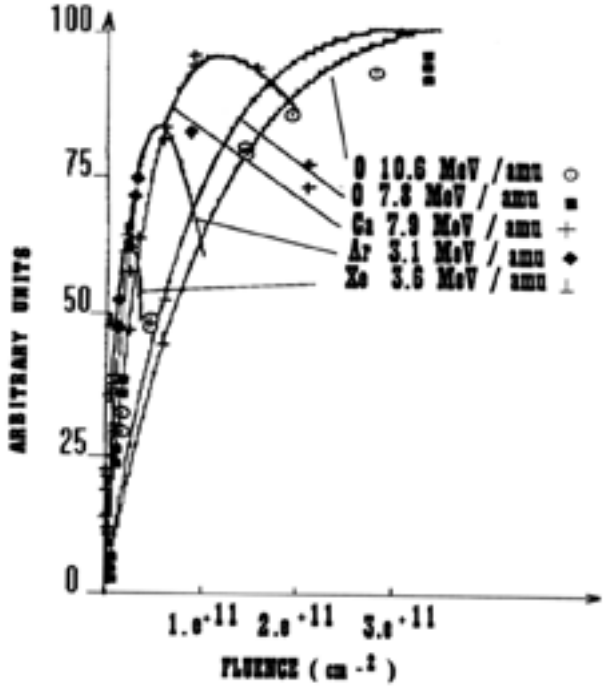


FIG. 26. Comparison between Betz's experimental points and theoretical curves obtained from the target theory.

5. CONCLUSIONS

The nuclear track technology allows in wide margins the independent choice of pore diameter, shape, inclination and film porosity. The etching solution creates holes along and around the particle track creating widespread possibilities for the cell to grow. Although we can observe differences between the tracked zone and the no tracked one, it is necessary to increase the variation between the two zones combining, different irradiation times and track diameter to observe improvements in cell adhesion. With track diameter between 0.5 and 1 μ and flux of up to 10^7 tracks/cm^2 PVDF films showed improving results for the cultivation of the V79-4 cells (exp a3) [13].

CTA-NTM can be produced using 6.25N KOH at 60 ^{o}C at different etching time to obtain NTM with different pore diameter. Moreover, CTA 27 μm thickness (4.2.6) and grafted acrylic acid substrates can be grafted with NIPAAm [14]. In this way, we can produce grafted NIPAAm-NTM using CTA. So, it can be used to make intelligent materials. CTA-NTM using different irradiation time and different alkali solutions are in course.

The grafting yield of NIPAAm monomer onto PVDF films was studied under various experimental conditions. Different radiation sources, mainly ff and γ irradiation and polymerization variables such as pore etching conditions, solvents and grafting times, were assayed. Although the main purpose of the work considered the evolution of grafting under different conditions the study is of importance for the opening and closing of the pores mechanisms in the development of slow and intelligent release of drugs and medicaments. In our approach the grafting yield percentage as given by the formula:

$$Y(\%) = (S - N\pi r^2 + N\, 2\pi r\, L)\, L' \rho_{Nipaam}/m_i \qquad (1)$$

was considered of outermost utility, for the study of the switch on-off mechanism.

The course control of the grafting yield was obtained by varying the monomer concentration in water solution, as shown in Fig. 13. The values of the grafting yield as a function of NIPAAm concentration taking ff as a parameter, showed a clear cut in the shape of the curves. Under general conditions, for NIPAAm concentrations up to 3–5%, the yield response shows moderate values of a few percent. There from the accelerate increment of the grafting, detected for low and high ff dose (curves a and b in Fig. 13) corresponding to higher monomer concentration, respectively, has been accompanied to a visible change in size or volume of the treated foils. This finding could be described as a "suspected" function of the active sites life time. In this way, higher monomer concentration allows the diffusion of the available monomers into the bulk material before the active sites decay. So, it give place to a transition from a superficial to a volumetric polymerization. The same kind of transition is observed when the grafting yield is represented as a function of ff dose taking the NIPAAm concentration as a parameter as shown in Fig. 14. For low NIPAAm concentration of the order of 3%, and when the ff dose is increasing a clear-cut in the shape of the curve is observed. In it the transformation from superficial to volumetric polymerization also occurs. In higher NIPAAm concentration, this transformation was observed at lower ff doses. The grafting yield evolution by changing the grafting time showed a wide dispersion of the values for grafting times in the first two hours. In this case the temperature of the solution could provide a mean for a fine control of the polymerization reaction. The dependence of the yield with respect to the γ irradiation showed in Fig. 17, is a very clear and simple way to study the influence of the following parameters:

(a) variation of the ff dose
(b) variation of concentration
(c) variation of grafting temperature,

taken by comparison curves (a) and (b), (a) and (d) and (b) and (c) respectively.

In general the grafting yield evolution as a function of the γ- dose gave well defined bell-shape curves. The maximum observed for PVDF-NTM as a function of gamma dose can be due to the competition between the grafting and the grafting termination. For PVDF and

CTA-NTM, swelling and conductivity measurements as a function of the temperature are projected.

PVDF-NTM can be grafted with AA, NIPAAm and VP, so it can be used to make intelligent materials with selectivity response to pH, temperature and humidity. PVDF was grafted with alpha particles from Am-241 source and with fragment fissions from Cf-252 source. PVDF-foils were grafted using protons from the CAE cyclotron accelerator We have observed similar behaviour that Betz [11] have been observed using foils irradiated with other accelerator particles.

ACKNOWLEDGEMENT

We thank Lic. J. Rocco for the proton irradiation in the CAE Cyclotron facility.

REFERENCES

[1] LHOEST J.B., DEWES J. L., BERTRAND P., NIMB, 105 (1995) 322–327.
[2] SUZUKI Y., KUSAKABE M., KAIBARA M., IWAKI M., SASABE M., NISHISAKA T., NIMB 91 (1994) 588–592.
[3] DAUBRESSE C., SERGENT-ENGELEN T., FERAIN E., SCHNEIDER I. J., LEGRAS R., NIMB, 105 (1995) 126–129
[4] OMICHI H., NIMB, 105 (1995) 302–307.
[5] REBER N., OMICHI H., SPOHR R., TAMADA M., WOLF A., YOSHIDA M. NIMB, 105 (1995) 275-277
[6] CHAPIRO A., Radiat. Phys. Chem. (1977), Vol. 9, pp. 55–67.
[7] MAZZEI R., Radiation Measurements, Vol. 26, No 4, pp. 577–583, (1996).
[8] FLEISCHER R. L., PRICE P. B. AND WALKER R. M., Nuclear Tracks in Solids. Principles and Applications (University of California Press, Berkeley, 1975).
[9] MONNIN M. AND BLANFORD G. F JR., Science vol. 181,pp. 743–744, (1973).
[10] Mazzei R., SMOLKO E., TADEY D. AND GIZZI L., submitted to NIMB.
[11] BETZ N., Nucl. Instr. and Meth. B 105 (1995) 55.
[12] MAZZEI R.AND SMOLKO E. Nucl. Instr. and Meth. B 132 (1997) 452–456.
[13] SMOLKO E., MAZZEI R., MICHELIN S. AND TADEY D., 15th. European Conference on Biomaterials (Abstract) Bordeaux-Arcachon, France, Sept. 8–12 (1999).
[14] KONDO T., KOYAMA M., KUBOTA H., KATAKAI R., Journal of Applied Polymer Science, Vol. 67, 2057-2064 (1998).

PREPARATION OF PATTERNED SURFACES AND MICROSPHERES USING RADIATION PROCESSING TECHNIQUES

A. Safrany

Radiation Chemistry Department, Research Center for Chemistry,
Hungarian Academy of Sciences,
Budapest, Hungary

1. INTRODUCTION

The Biomaterials group of the Department of Radiation Chemistry uses radiation for synthesis and modification of polymers to create new biomaterials. The advantages of this method are: it is a simple and additive-free process and can be used at all temperatures, can be limited to the surface only and in certain cases the synthesis/modification can be combined with sterilization. In our case, tipical examples are: synthesis of hydrogels, preparation of monodisperse functional micro- and nanoshperes, modification of surfaces and immobilization of bioactive materials. Potential biomedical applications of these materials include: topical dressings, diagnostic and treatment devices and drug delivery materials.

In this report, the work carried out during the period of the Co-ordinated Research Programme in collaboration mainly with argentine and dutch laboratories and involves synthesis of functional microspheres and surface modifications is described, and future plans identified.

2. SYNTHESIS OF FUNCTIONAL MICROSPHERES

Polymeric micro- and nanospheres are one of the most useful materials for many of the current technology[1]: they are used in optical sensors, as fuel containers in nuclear fusion experiments or as antistatic coatings for spacecrafts, to mention but a few. Besides, they are increasingly utilized as functional supports in various biomedical applications ranging from purification procedures to diagnostics and therapy.

The main polymerization methods[2] available to prepare such spheres are the nonaqueous dispersion polymerization, aqueous emulsion polymerization, seeded suspension polymerization and precipitation polymerization. Precipitation polymerization is unique from the point of view that it can lead to monodisperse microspheres without added surfactant or stabilizer[3-5]. We are synthesizing them by a radiation initiated precipitation polymerization of a monomer (usually diethyleneglycol dimethacrylate) solution[6]. The characteristics and the advantages of this method are that not only could it be done free of surfactants, but also no initiator or cathalyst is necessary (usually toxic materials that could contaminate the product), the synthesis can be done at any temperature, and the particles have narrow size distribution. The size is mainly controlled by the properties of the monomer and the solvent, the use of co-monomers and their ratio. For immobilization of biomolecules a desired functional group can be added either in an additional activation step, or by direct incorporation of a suitable co-monomer during the polymerization itself. Since the activation process requires an additional step and use of highly toxic materials, we prefer to use the incorporation of the adequate functional group by co-polymerization.

Here we report the incorporation of carboxy (AAc) and epoxy (GMA) functionality by this method. The carboxy groups could provide bioadhesion, while the epoxy groups are suficiently stable in aqueous solutions and react readily with a number of functional groups, thus enabling a covalent bind.ing of a vide range of bioactive materials to the microspheres.

3. MATERIALS AND METHODS

Several solutions containing different concentrations of diethyleneglycoldimethacrylate (DEGDMA-Aldrich) and glycidylmethacrylate (GMA-Sigma) in ethylpropionate (EP-Aldrich) were prepared, degassed with nitrogen and irradiated on a ^{6o}Co gamma source at room temperature without stirring, with a dose rate of 15 kGy/h and with total doses from 1 to 50 kGy.

The obtained microspheres were washed several times with the solvent, centrifuged and freeze dried. The characterization was performed by spectroscopy (ATI Mattson RS-1 FTIR spectrometer, equipped with an MTEC 300 photoacoustic detector) and electron microscopy (JEOL JSM 5600LV).

The amount of epoxy group incorporated in microspheres was visualized by copper staining, and their bioactive material binding capacity was tested with histidine and lysosyme in the following way:

Microsphere samples were immersed in 1 M IDA dissolved in dimethyl sulfoxide/water (1:1). The reaction was performed at 80^{O}C for 8 h. After washing with water, the microspheres were dried until constant weight. Measured quantities were immersed in 0.2 M CuSO4. After washing with deionized water, copper was released from the microspheres by shaking them with 0.1 M EDTA, pH7.0, at room temperature for 8 h. Copper content was determined spectroscopically comparing the absorbance of the supernatant at 715 nm with that of 0.1 M EDTA with Cu(II) at various concentrations. For the reaction with histidine, a total volume of 1–10 mg of microspheres was immersed for 24 h in an excess of histidine in 20 mM sodium phosphate buffer, pH7.0, 250 mM NaCl, in a f nal volume of 1 ml. The concentration was caiculated from the measured absorption at 220 nm.

The microspheres were also tested for affnity purification of peroxidase from *A.rusticana* roots and *Gycine max* seed coats in aqueous two-phase system. In this method, the microspheres were mixed with several different PEG/phosphatase aqueous two-phase system and the crude extract, equilibrated, then recovered and washed. The peroxidase was eluted with a-D mannopiranose and the amount of high-puriti peroxidase was determined by SDS-PAGE analysis.

4. RESULTS AND DISCUSSION

When a solution of DEGDMA is irradiated in an adequate solution, due to the homogeneous initiation, monodisperse co-polymer spheres are formed. An adequate solution is not only a good solvent for the monomer, but it also has to allow a streched-out configuration for the monomer in order to allow independent solvation of the two methyl groups, thus enhancing the intermolecular over intramolecular crosslinking[7]. The monomer concentration is another important factor for microsphere formation, and Fig.1 presents the products obtained from irradiation of several DEGDMA solutions of different concentration in ethyl propionate.

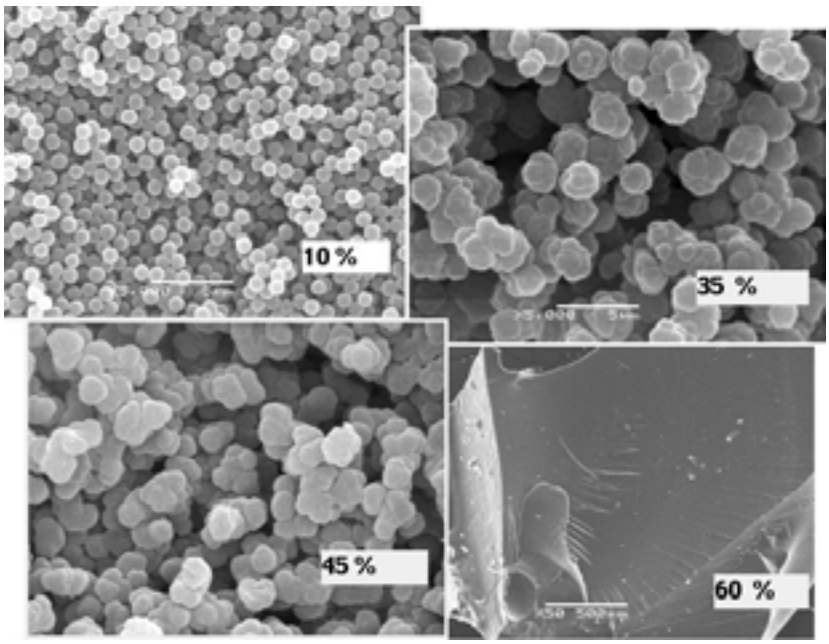


FIG. 1. SEM photographs of microspheres prepared by irradiation of a 10% (upper left); 35% (upper right); 45% (lower left); and 60% (lower right) DEGDMA solution in ethyl propionate.

The pictures illustrate the fact, that for microsphere formation only monomer concentrations below 30% are suitable. When the monomer concentration lies between 30 and 50%, so called "monolith" is formed. (Such monoliths are useful for affinity separation and microfiltration, and we are now investigating the effect of various parameters on the pore size and flux trough such monoliths[8]. This work is beong carried out in collaboration with Argentina.) When the monomer concentration was increased further, glassy homopolymer formed.

To enable covalent binding of variety of bioactive molecules to the microspheres, we introduced epoxy groups by compolymerization of DEGDMA with glycidylmethacrylate (GMA) in their common solvent, ethyl propionate. The investigated factors of influence on the microsphere yield and size were: irradiation temperature, absorbed dose and dose rate, and co-monomer ratio in the feed solution.

Table I and Fig. 2 illustrate the effect of GMA content on the yield and size of microspheres for a 10% monomer solution, irradiated at room temperature, with dose of 20 kGy.

The increase in the size of the microspheres with increasing GMA content was expected. Since the growth of a particle is influenced by competition of polymerization and crosslinking reactions, with the increasing content of a monofunctional monomex, the the probability of propagation over crosslinking will also increase, leading to bigger particles. Further increase of the GMA content caused the particles to aggregate. When only GMA is irradiated, no precipitate formed.

The size of the microspheres does not depend on the absorbed dose, as shown on Fig. 3. The microspheres irradiated with 3 and with 25 kGy have the same size (10% DEGDMA solution in EP), but the size distribution is slightly broader for lower dose.

TABLE I. PROPERTIES OF GMA/DEGDMA MICROSPHERES

GMA content (%)	Yield (%)	Diameter (μm)
0	98.7	0.94
20	68.8	1.04
40	56.1	1.32
60	51.2	2.91

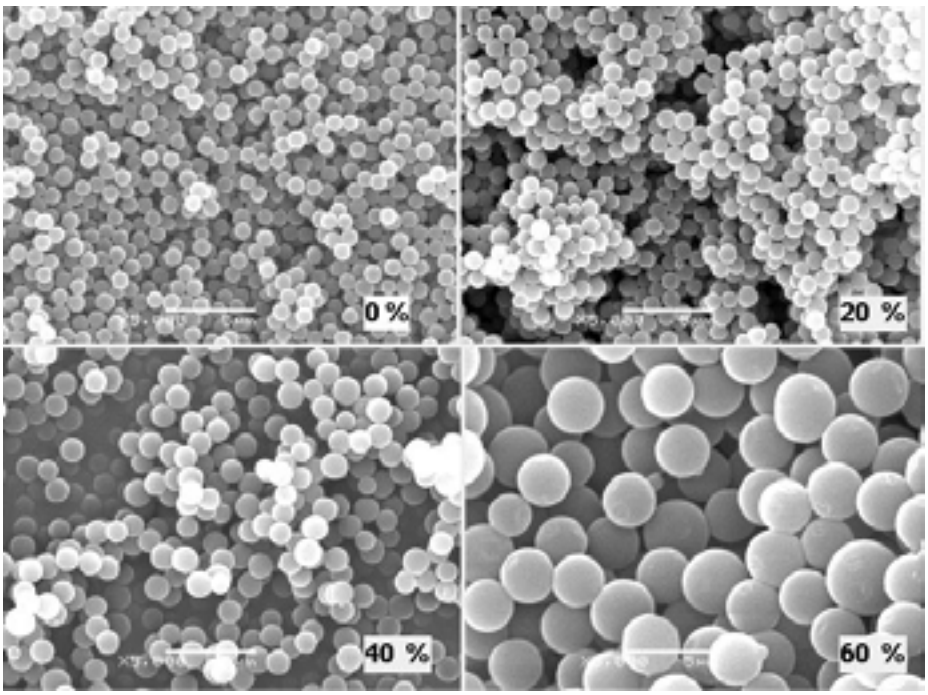


FIG. 2. SEM photographs of microspheres prepared by irradiation of a 10% co-monomer solution with different GMA contents of 0% (upper left); 20% (upper right); 40% (lower left); and 60% (lower right), with 20 kGy.

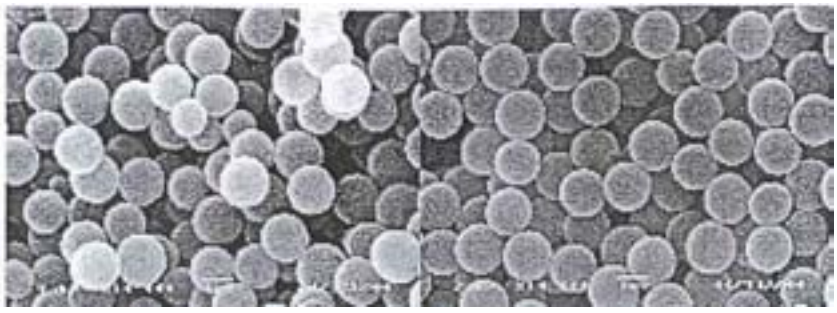


FIG. 3. SEM photographs of microspheres irradiated with 3 kGy (left) and 25 kGy (right).

Due to their similar chemical structure, the infrared spectra of DEGDMA and GMA are almost identical. The incorporation of GMA in the microspheres is seen only from the appearance of a new peak at around 910 cm-l, assígned to epoxy groups (Figs 4. and 5).

It is also possible to confirm this incorporation by the reaction with copper, both visually and spectroscopically (Fig. 6). The usefulness of the microspheres for immobilization of various bioactive molecules was tested with histidine and lysosyme[9], also shown on Fig. 6.

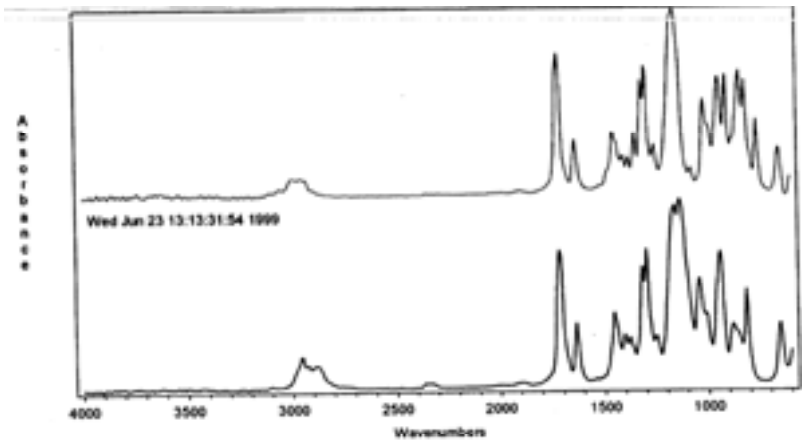


FIG. 4. FTIR spectra of pure monomers, DEGDMA (lower spectrum) and GMA (upper spectrum).

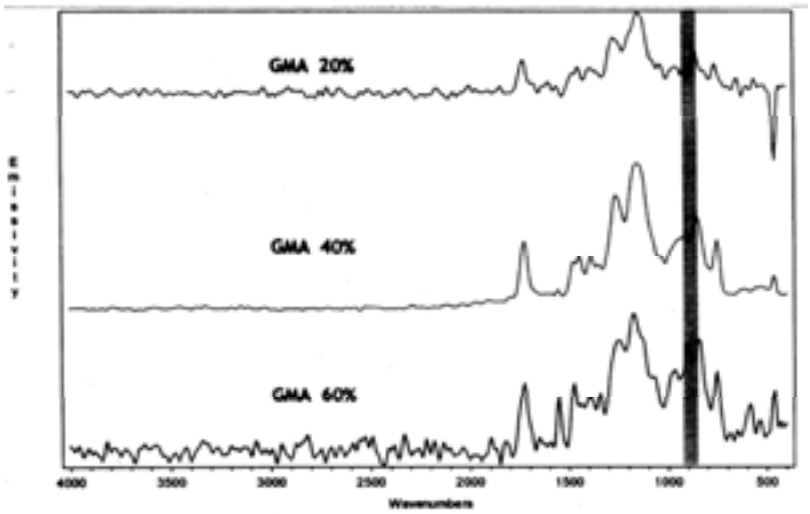


FIG. 5. FTIR/PAS spectra of microspheres with different GMA content.

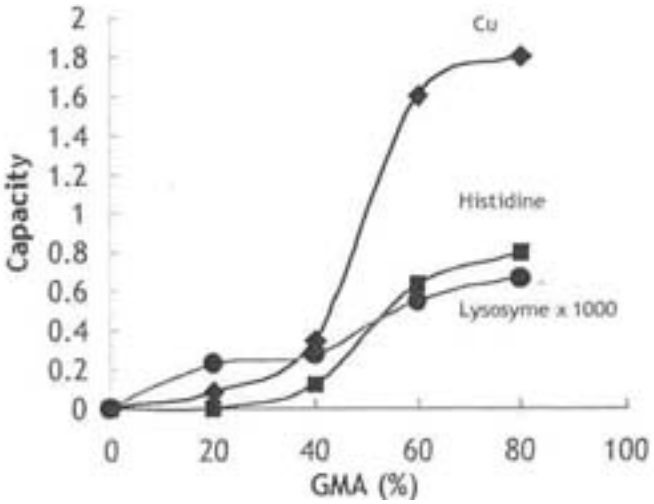


FIG. 6. Binding capacity of GMA microspheres.

Aqueous two-phase systems are formed when PEG salts and inorganic salts are dissolved above certain concentrations. The characteristic feature of such a system is that it allows the partitioning of biomolecules and cell particles of diverse origin under nondenaturing conditions, therefore it is specially suited for enzyme extraction and purification from biological media. When the microspheres were added to this system, they distribution depended on the molecular weights of the PEG, as shown on Table II.

TABLE II. PARTITION OF *A.RUSTICANA* CRUDE EXTRACT PROTEINS AND MICROSPHERES IN PEG-PHOSPHATE SYSTEMS AT PH7

	PEG M.W.			
Material	20.000	6.000	1.540	600
Microspheres	Whole system	Top phase	Interphase	Interphase
K HRP	0.02	0.03	0.60	37.70
K Total Proteins	0.79	0.63	0.63	5.20

For the affinity separation, PEG 600 system was chosen, as there the peroxidase was completely in the top phase[10]. After addition of microspheres, only 10–15% of peroxidase remained soluble. By elution, 75–80% of high purity enzyme was obtained. These results showed that by using the functional microspheres, it was possible to achieve a selective recovery of enzymes with good yield and high purification level.

The microspheres, beside the narrow size distribution, have another advantage: they are stable in dry state and can be used in both aqueous and organic solvents. Presently, immobilization of several proteins for purification purposes and monoclonal antibodies of schistosoma for immunodiagnosis are under way.

5. SURFACE MODIFICATIONS

It is widely accepted that the surface properties of polymeric biomaterials (wettability, biocompatibility, adhesion, electrical charge, etc.) are vital for their successful applications. Therefore, the need for the control of surface characteristics is increasing.

Several techniques have been used to tailor the properties of polymer surfaces. These include surface functionalization by physical deposition/adsorption, chemical modif cations, gamma-, electron- and ion irradiation, and glow discharge techniques.

Here we report the results of the work carned out in collaboration with The Netherlands. We used the method of surface oxidation by electron beam irradiation to prepare ELISA plates with improved sensitivity for early detection of tropical diseases [11].

6. MATERIALS AND METHODS

Commercial polystyrene ELISA plates (Greiner Labortechnik- Germany and Nunc MaxisorpDenmark) were irradiated either in argon or in air, with or without previous washing in 96% ethanol on a linear electron accelerator in scanned beam regime combined with a conveyor. The conveyor speed and sample distance from the beam window was adjusted to obtain the specified dose, determined by ethanol-monochlorbenzene (ECB) dosimeter.

Surface analysis of the inner bottom of the well was performed on a Kratos Analytical System XPS with Al and Mg X-ray sources; SEM photos were taken on a JEOL LV-5600 microscope.

The two-site immunoassay for detection *of Schistosoma* CAA was used to evaluate the effect of irradiation. Briefly, the microtitration plates were coated with 100 l anti CAA mouse monoclonal antibody of different concentraions in phosphate buffered saline, pH7.8 for 3 hours at 37 ^{O}C. Plates were then washed and postcoated with bovine seru.m albumin. After washing, dilution series of the trichloroacetic acid soluble fraction *of Schistosoma mansoni* adult worm antigens (AWA-TCA) in sodium phosphate buffer was added, and plates incubated. After washing, biotin conjugate was added, plates were incubated 1 h at 37 ^{O}C, washed again and finally streptavidin/peroxidase was added and plates again incubated at 37 ^{O}C. After a last washing step, substrate was added, plates were incubated at room temperature and staining was measured with an ELISA reader at 630 nm.

7. RESULTS AND DISCUSSION

Several series of irradiated plates were tested (Fig. 7), and they all showed better performance as compared to the unirradiated, control plates, both in regard to lower detection level of the assay and to optimal antibody coating concentration.

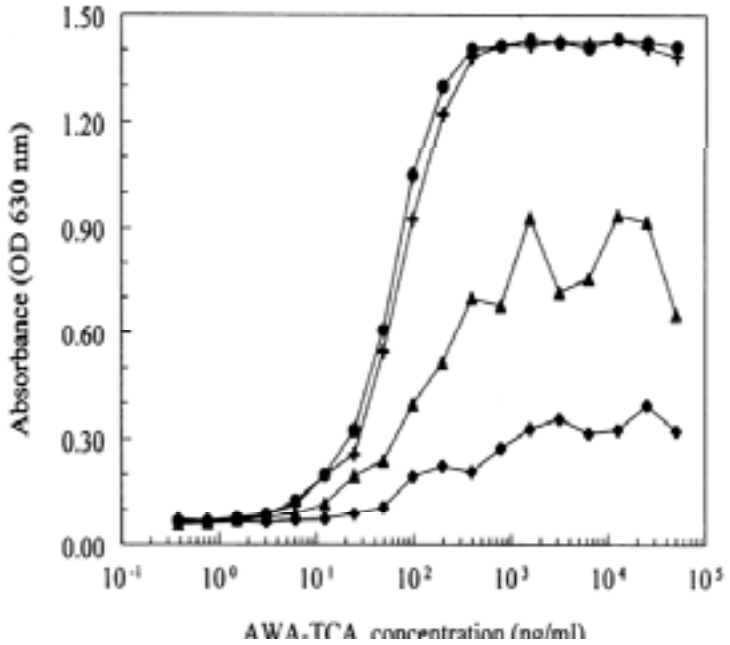


FIG. 7. Antigen dilution curves as a function of absorbed dose.

This effect was not only observed for "standard" Greiner microtitration trays, but also for high absorption (Maxisorp) Nunc microtitration trays. The assay performance was found to increase with the increase in the absorbed dose up to around 15 kGy (Fig. 6). Plates irradiated with higher doses generally tended to result in a somewhat decreased lower detection level, especially at very low antibody coating concentration. Not only was the lower detection level of the assay in 15 kGy treated plates 2–4 fold lower than in untreated plates, but also the absorbance values were much stronger particularly at low antibody coating concentrations.

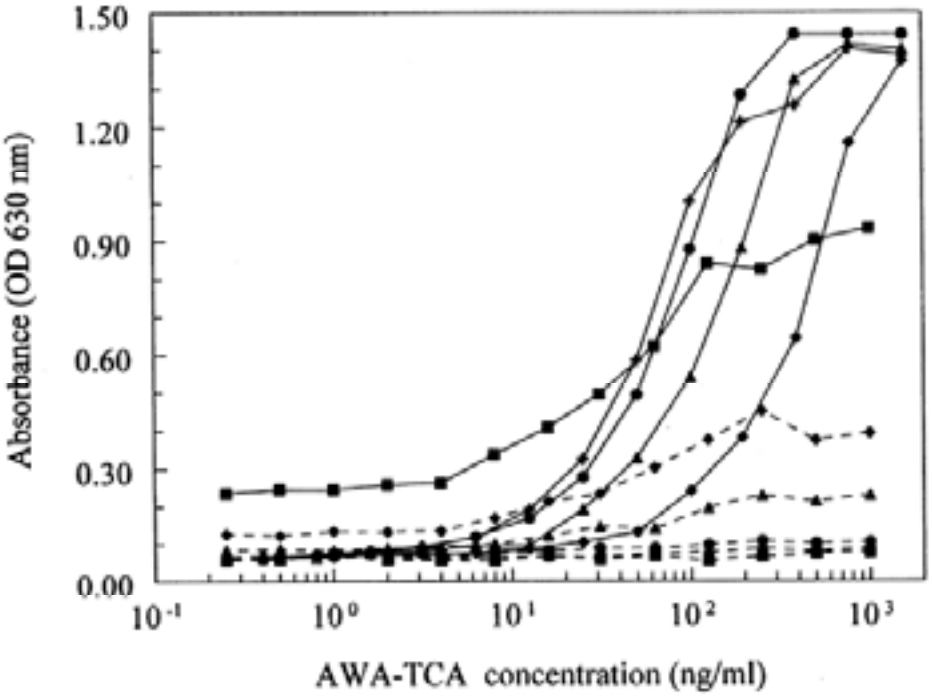


FIG. 8. Antigen dilution curves as a function of coating concentration using Greiner ELISA plates irradiated by 15 kGy.

Results in Fig. 8, clearly show that optimal results are obtained with coating concentrations down to 0.4 g/ml, and that those with a coating of 0.16 g/ml are slightly lower. However, even with coating concentrations as low as 64 ng/ml acceptable performance is still obtained.

In an attempt to evaluate whether this increased performance at low coating concentrations was primaryly due to increased binding of the antibody to the radiation processed plates or due to better steric presentation of the antibody, 15 kGy-treated and untreated plates were coated with different concentrations of antibody and then stained in a direct assay with a rabbit anti mouse peroxidase labeled conjugate. It was found that up to ten-fold lower concentration of coating antibody was still detectable on 15 kGy treated plates.

Finally, in a number of experiments the stability of the pretreated plates was tested on plates which had been sealed in air and then stored for periods up to two years at room temperature. No decrease in assay performance was observed in these plates.

In order to understand the strongly improved performance of the irradiated plates, surface characterizations were done by XPS and SEM. On Fig. 9. the C 1 s and Ol s scans are shown:

The Cl s envelope of polystyrene typically consist of two peaks with binding energies of 285 eV (C-C) and 292 eV (from π-π* shake-up satellite). After irradiation, the oxygen content goes up to 6–8%, and the C 1 s peak is broadening suggesting a series of oxide group. This clearly indicates the oxidation of the surface during irradiation. The SEM photos taken from the bottom of the wells in both low and high vacuum mode, without surface coating, showed no distinctive features that were introduced by irradiation.

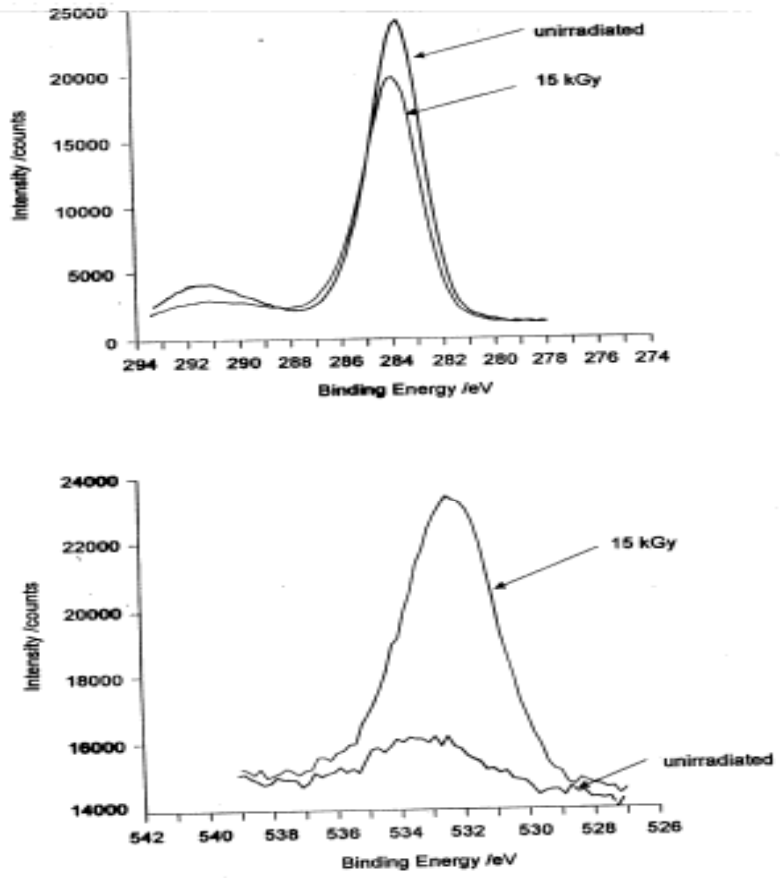


FIG. 9. XPS C 1 s and O 1 s scans for unirradiated and 15 kGy irradiated plates.

We are now planning to investigate the effect of surface oxidation achieved by different methods (gamma, electron irradiations and RFGD) on the interaction between various proteins and those oxidized surfaces.

8. PREPARATION OF PATTERNED SURFACES

Micropatterning of small molecules, macromolecules and cells on matrix surfaces has a wide range of potential applications in molecular electronics, biosensing, diagnostics, tissue engineering and micromachining. To achieve this micropatterning several methods are in use such as photolitography, ion implantation, electron beam and ion beam irradiation, electrochemical methods and patterning with the tip of the probe of atomíc force microscope.

Our objective was to achieve domain-separated surface by radiation grafting, using an electron accelerator. In the work reported here, a thermoresponsive monomer, N-isopropylacrylamide; a pH-sensitive monomer, acrylic acid, and a functional monomer, glycydilmethacrylate were grafted on low density polyethylene.

9. MATERIALS AND METHODS

N-isopropylacrylamide (NIPAAm), acrylic acid (AAc), and glycidylmethacrylate (GMA) were used after standard purification methods. All solvents were reagent grade and used as received. The base polymer was low density polyethylene that was cleaned ultrasonically with methanol three times before use.

A series of irradiations were carried out with the 4 MeV linear accelerator in both single pulse and scanned beam regime combined with a conveyor. In the scanned beam regime the conveyor speed and sample distance from the beam window was adjusted to obtain the specified dose, that varied between 50 and 200 kGy, while in single beam operation mode a stationary sample position and a pulse length of 2.6 μs was used.

The absorbed dose was determined by ethanol-monochlorbenzene (ECB) dosimeter. The conductivity of the irradiated ECB solutions was measured by the K-302/2 type oscillotitrator (Radelkis Electrochemical Instruments, Budapest, Hungary)12

To achieve a domain-separated surface, aluminum and copper masks were prepared to partially cover the surface of the samples.

Before the irradiation of the samples, a radiochromic dye film dosimeter (PVB/pararosaniline) was placed under the mask for dose mapping. After irradiation, the dosimeter film develops color with a maximum adsorption at 554 nm. The relationship between the absorbed dose and optical density is linear up to 100 kGy [13].

After the irradiation, the surfaces were thoroughly washed to remove the physically adsorbed but not grafted monomer and homopolymer, and dried. The grafted polymer samples were then evaluated by FTIR spectroscopy, (ATI Mattson Model RS-1 equipped with ATR and PAS detectors), and the chemical composition was also determined by taking XPS survey and high resolution C 1 s and O 1 s spectra. The modified surfaces were also visualized by scanning electron microscopy (JEOL Model 5600 L V).

10. RESULTS AND DISCUSSION

Polyethylene films were irradiated by various doses in the range of 1-200 kGy, under the masks and in the presence of a monomer. (As reference, the following samples were compared: irradiated PE without a monomer, monomer adsorbed on PE but not irradiated, monomer adsorbed on PE and irradiated without mask.)

On Fig. 10. a representative FTIR spectra of base PE and GMA-grafted PE are shown. (The grafting was obtained by irradiation with absorbed dose of 35 kGy.) On the lower spectrum, that shows the GMA-g-PE, the grafting could be clearly identified from the new peaks that appear at 1151 cm-1 and 1730 cm-1 and belong to ester C-O and C=O groups, respectively, as well as the peak at 904–910 nm assigned to the epoxy groups.

The results of the XPS measurements are shown on Figs 11 and 12. The survey spectra of PE shows only one peak that corresponds to C 1 s but that of the GMA-g-PE has also a huge O 1 s peak. (The grafting shown here corresponds to the absorbed dose of 70 kGy.)

The peak fitting of the C 1s showrs only the existence of CH-type bonds in the base polymer (278 eV binding energy in our case — not corrected). When the C 1s peak of the GMA-g-PE is fitted, the shifts to the higher binding energies of about 2 eV from the hydrocarbon binding energy belongs to ether type C-O bonds. The C=C type bond usually shows another shift to still higher binding energies of about another 2 e V.

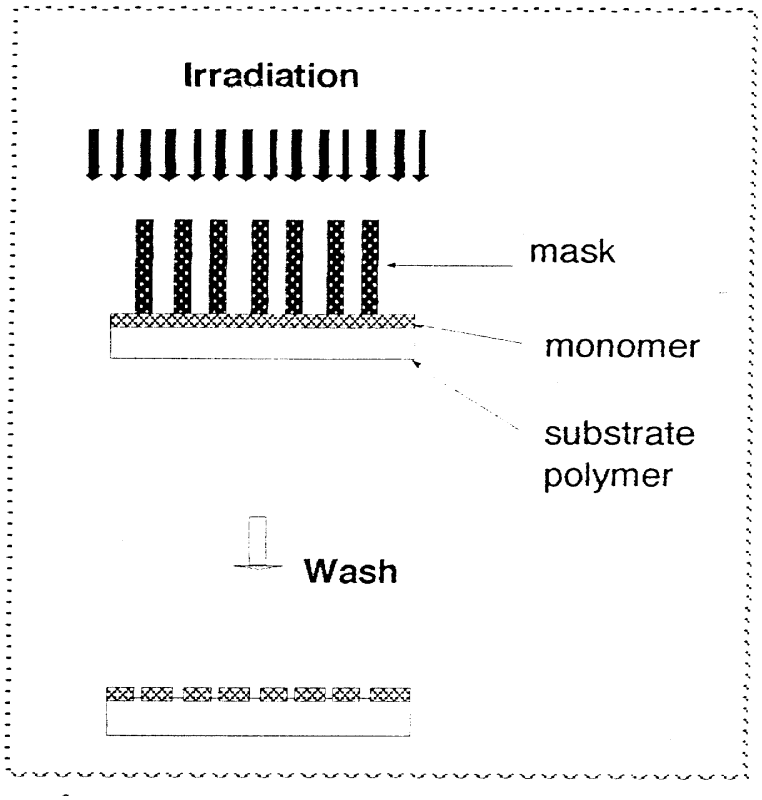


FIG. 10. Scheme for the preparation of domain-separated surfaces.

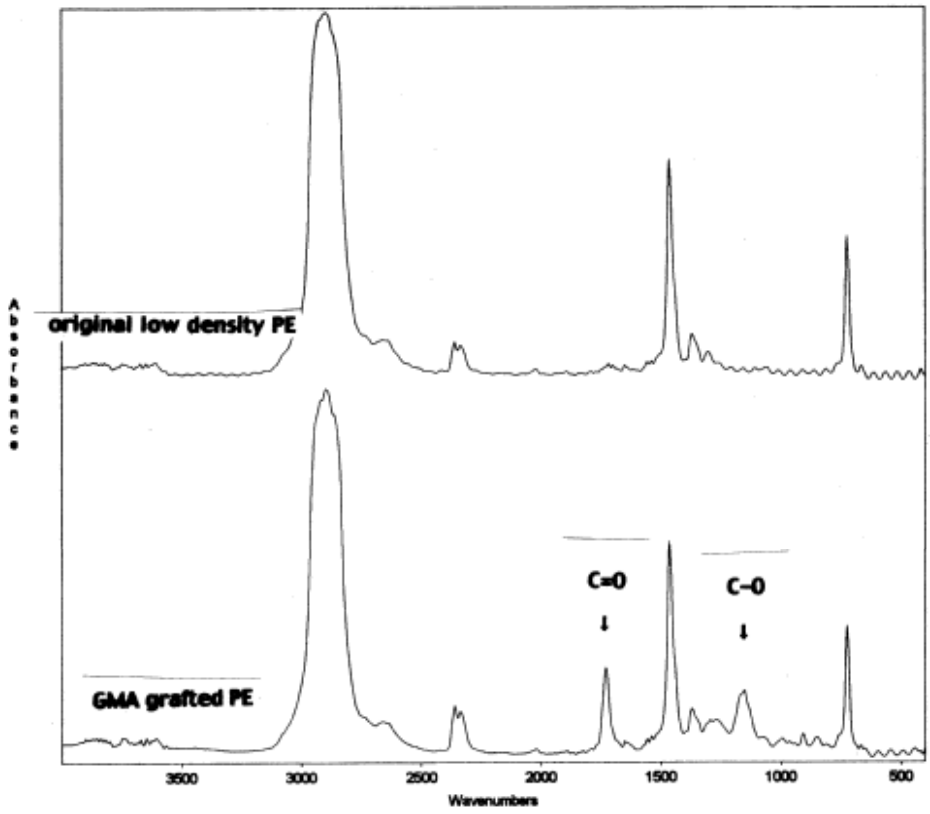


FIG. 11. FTIR spectra of virgin PE (upper spectrum) and GMA-grafted PE (lower spectrum).

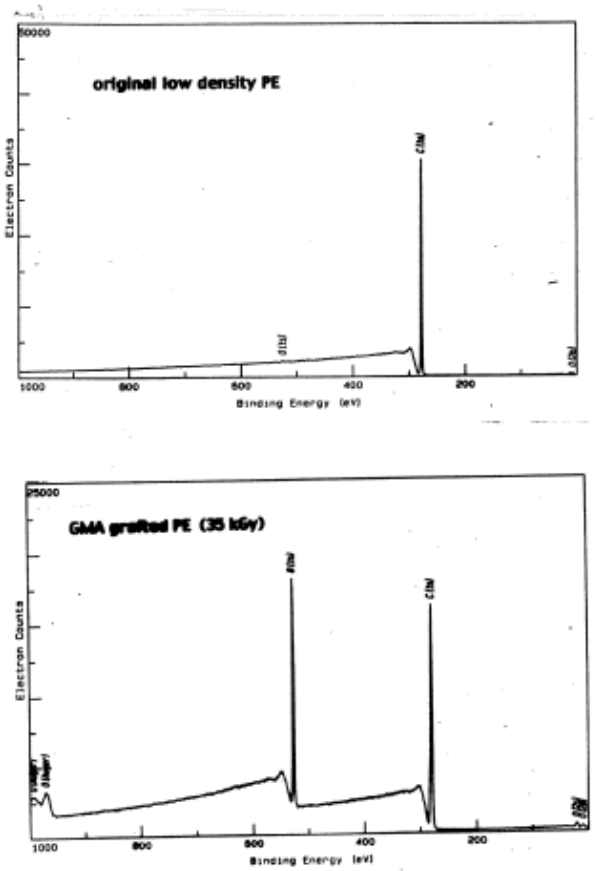


FIG. 12. XPS spectra of virgin and GMA-grafted PE.

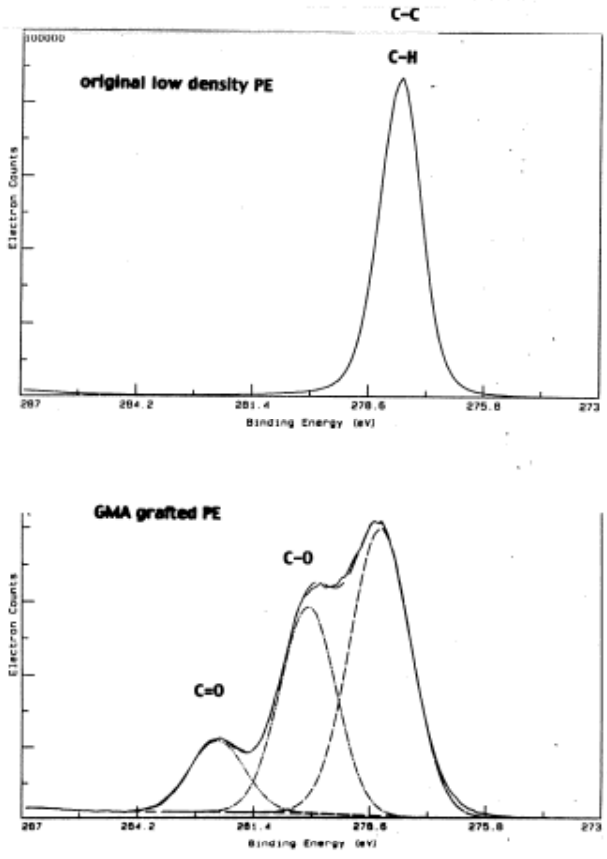


FIG. 13. XPS survey spectra of virgin and GMA-grafted PE.

Similar results were obtained witn other monomers, AAc and NIPAAm too. Besides the guided cell growth, grafting with NIPAAm had also another objective. Since this polymer shows typical soluble-ínsoluble changes in response to temperature changes across a lower critical solution temperature (LCST) at around 32–34 ^{O}C in aqueous solution, the polyNIPAAm-grafted surfaces will be hydrophilic below, and hydrophobic above this temperature. Cells generally adhere and grow on hydrophobic surfaces but not on hydrophilic ones, thus cells grown on hydrophobic polyNIPAAm surfaces at 37 ^{O}C could be detached and harwested without chemicals by lowering the temperature of the surface below the LCST.

The grafted samples were used for cell culture experiments, but unfortnately, the cells could not be grown satisfactortly neither on reference nor on the grafted surfaces. We are now planning to do alI cell culturing experiments in collaboration witn The Netherlands, as this laboratory has long experience in this field.

ACKNOWLEDGEMENT

The work reported here includes contributions from Mariano Grasselli and Eduardo Smolko {Argentina) and André Deelder (Netherlands). I would like to thank them for their interest and enthusiastic and diligent work. I am also indebted to Katalin Gonter and Peter Hargittai (Hungary) for their excellent assistance. The partial financial support from the International Atomic Energy Agency as well as from the Hungarian Research Foundation {OTKA) and Hungarian-Argentine Bilateral Foundation is gratefully aknowledged.

REFERENCES

[1] DAGANI, R., *Science,* Dec. 19, 33 (1994)
[2] STOVER, H.D.H.; LI, K. in *Polymer Materials Encyclopedia;* Salamone, J.G., Ed. CRC Press: New York 1996, 4519
[3] BAMFORD, C.H., LEDWITH, A., SEN GUPTA, P.K., *J. Appl. Polymer.,Sci.,* 25, 2559 { 1980)
[4] LI, K., STOVER, H.D.H., *J.Polym.Sci; PartA: Polym. Chem.* 36,1543 (1998)
[5] STOVER, H.D.H.; LI, K. in *PolymerMaterials Encyclopedia;* Salamone, J.C., Ed. CRC Press: New York 1996, 7237
[6] SAFRANY, A., et al. *Radiat.Phys. Chem.* 46, 203 (l 995)
[7] NAKA, Y., YAMAMOTO, Y., HAYASHI, K., *Radiat.Phys.Chem.* 40, 83 (1992)
[8] GRASSELLI, M., HARGÍTTAI, P., SMOLKO, E., SAFRANY, A., lRaP 200l (4th International Symposium on Irradiation and Polymers), Gouvieux-Chantilly, France, September 24-28, 2000
[9] SAFRANY, A., GRASSELLI, M., HARGITTAI, P., SMOLKO, E., 5th World Biomaterials Congress, Kamuela, Hawaü, USA, May 15-20, 2000
[10] MIRANDA, M. V., FERNANDEZ LAHORE H.M., GRASSELLI, M., CASCONE. O., SMOLKO, E., AND SAFRANY, A., 8th European Congress on Biotechnology, Budapest, 17-21 August 1997
[11] SAFRANY, A., DEELDER, A., "Surface modification by electron beam irradiation for improved immunoassay", *Radiat. Phys. Chem.* 55, 681 (1999)
[12] KOVACS, A., AND MILLER, A., Radiat. *Phys. Chem.* 36(6), 709 (1990)
[13] MILLER, A., BATSBERG, W. KARMAN, W., Radiat. *Phys. Chem.* 31(4-b), 491 (1988)

AFFINITY PATTERNING OF BIOMATERIALS USING PLASMA GAS DISCHARGE

A. Goessl, L. Jung, D. Bowen-Pope, A.S. Hoffman

Department of Bioengineering,
University of Washington, Seattle, Washington, United States of America

Abstract. Patterned surfaces were prepared by combination of gas discharge and photolitographic processes. First a protein-repellant surface was prepared by glow discharge deposition of tri- and tetraglyme vapor on poly (ethylene terephtalate) surface, then on top of it fluorocarbon was deposited on selected domains by photolithography. Cell attachment was shown to be dependent of the surface hydrophilicity.

1. INTRODUCTION

One of the most frequent modes of failure of vascular implants (grafts, stents, etc.) is the occlusion of the lumen due to anastomotic hyperplasia, where an excessive proliferative response of the medial smooth muscle cells (SMC) leads to a thickening of the tissue at the joint of the vessel and a synthetic implant. The activation of SMC by the events occurring at the wound site, the reversion from the contractile to the proliferative phenotype, is hypothesized to result from changes in the chemical and mechanical environment of the cells. In this paper we present a method to manufacture cell culture substrates for the patterned immobilization of cell receptor ligands on a non-fouling background.

We have developed a novel technique that allows the patterned immobilization of peptide or protein ligands on polymeric cell culture substrates. We first deposit a thin, protein and cell resistant coating from a triglyme or tetraglyme vapor onto a clean PET (poly[ethylene terephthalate]) cell culture substrate. The gas discharge source is mostly made of high energy excited molecules and free radicals and short wavelength UV. The deposited polyether polymer film resists the adsorption of proteins or cells. Then, using a photolithographic process we deposit on the nonfouling surface small domains of a thin, hydrophobic fluorocarbon plasma polymer. The domain sizes range from 5–100 microns.

These patterned surfaces were characterized using AFM (atomic force microscopy) to assess topographical properties and pattern fidelity. We also used ToF-SIMS (time of flight secondary ion mass spectroscopy) to assay the chemical composition of the different domains on the surface. Both methods show excellent pattern fidelity and the desired chemical compositions in the different regions. We can adsorb various cell adhesion proteins of interest (eg, laminin, fibronectin, or gelatin) onto the fluorocarbon domains. We demonstrated that by adsorbing fluorescently labeled BSA (bovine serum albumin) to these surfaces; they were then imaged using epifluorescent microscopy.

2. MATERIALS AND METHODS

2.1. Glow discharge and lithography process

Cleaned PET substrates (Lux Thermanox coverslips) were treated with an Ar plasma, then a thin polymer film of tetraglyme (tetra(ethylene oxide)-dimethyl ether) was deposited by plasma polymerization. The thickness was measured by ellipsometry (Rudolph EL, n_f=1.5) the

films were chemically characterized by ESCA. To assess the non-fouling properties of the glyme film, SMC (Fisher Rat 344) were seeded onto the films in the presence of 10% calf serum and incubated for 24h. Onto this non-fouling polymer a positive photoresist (Hoechst AZ 1512) was applied and exposed to UV light through a photomask. The exposed areas of the resist were washed away with an aqueous developer (Hoechst AZ 3510), exposing these areas of the tetraglyme layer for the subsequent plasma deposition of a thin fluorocarbon polymer film (process gas tetrafluoroethylene). Again, the thickness was measured by ellipsometry (n_f=1.395). Subsequently the remaining photoresist was removed by treatment with EtOH, aqueous developer (2x) and water (2x), leaving behind the pattern of a fluorocarbon polymer on a non-fouling background. These substrates were chemically characterized using ToF-SIMS imaging.

2.2. Surfactant adsorption

A fluorocarbon-PEG-peptide amphiphile has been synthesized; the surfactant will be used to bind peptide ligands to the hydrophobic flurocarbon pattern on the substrate. Here we used surface plasmon resonance (SPR, custom buit instrument) to study the adsorption of a FC(9)-PEG(11) surfactant (no peptide attached) to homogeneous FC films deposited on SPR substrates (gold deposited on glass slides). The critical micell concentration (CMC) was measured using the surface tension method. An adsorption isotherm was recorded by measuring the adsorption from different concentrations under stop-flow conditions. SMC were seeded on substrates perpared in the same way to assess the effect of varying surface densities of PEG on attachment and spreading of cells in serum-free media.

3. RESULTS AND DISCUSSION

As written above, the PET substrates were first cleaned with an Ar plasma, then a thin polymer film of tetraglyme was deposited by glow discharge polymerization. On Fig. 1. SEM photomicrographs of pristine PET control surface and a tetraglyme-deposited surface is shown.

The thickness of the tetraglyme plasma films was linearly dependent on the treatment time, a film thickness of 115nm was used for further processing. Elemental analysis by ESCA showed the expected C:O ratio of ~2:1. The high resolution C_{1s} spectrum shows the C-O bond (286.5eV) as the predominant bond. SMC seeded onto these substrates were not able to attach or spread even in the presence of 10% calf serum.

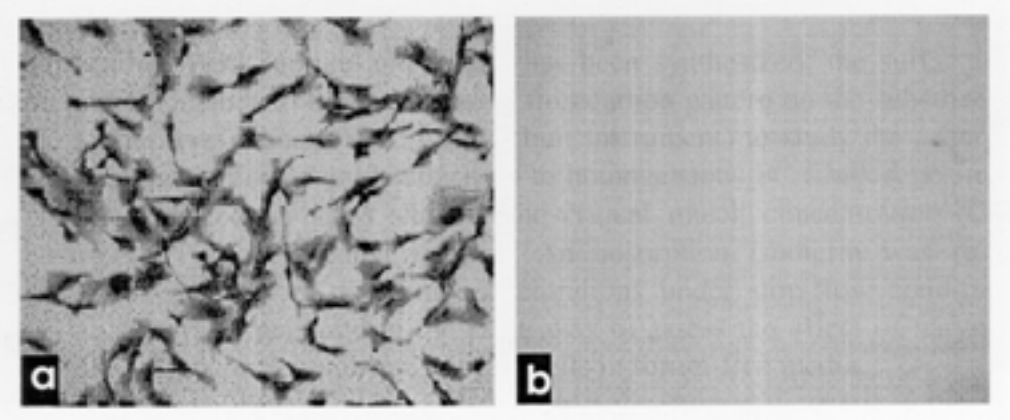


FIG. 1. Photomicrographs of: (a) untreated PET, and (b) tetraglyme-deposited PET surface.

The spatial resolution of the photolithography was found to be almost exclusively dependent on the quality of the photomask; with an appropriate mask feature sizes of <5µm can easily be obtained. The thickness of the FC plasma film, which also increases linearly with treatment time, was found to be critical for the clean removal of the photoresist. Films with a thickness of 10–30nm allow a quick removal of the underlying photoresist; thicker films significantly reduce the edge definition of the pattern. Given a homogeneous tetraglyme film, the photoresist can be completely removed using the above washing protocol. The complete removal was monitored using ToF-SIMS by the absence of resist-typical peaks from the cresol novolak (+121Da, -107Da). The glyme/FC patterned surfaces were analysed using ToF-SIMS in the imaging mode. On Fig. 2 we show the ion maps of a: CF_3^+ (typical for the FC pattern) and of b: ($C_3H_8O^+$), characteristic of the glyme background. The line width is 20µm.

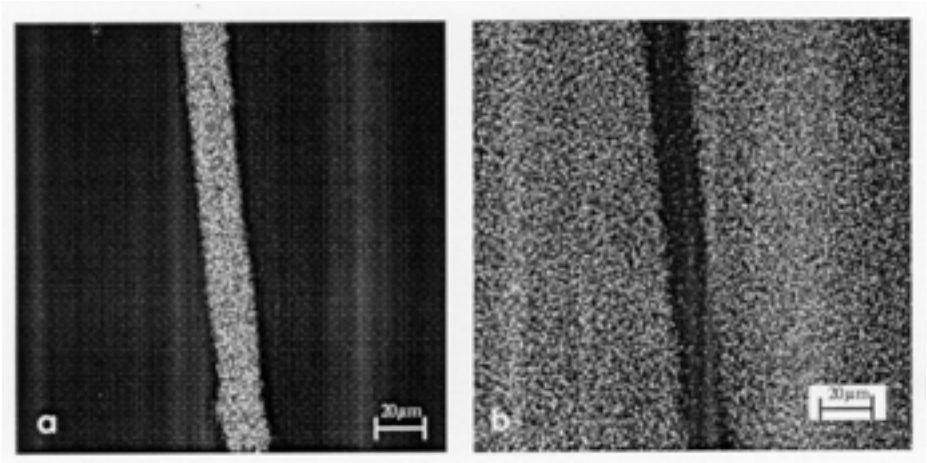


FIG. 2. Ion maps of: (a) CF_3^+ (typical for the FC pattern); (b) ($C3H8O^+$), characteristic of the glyme background.

On Fig. 3. we show the adsorption isoterm of the FC-PEG onto the gold-covered glass, as well as the cell attachment on surfaces treated with various amounts of FC-PEG surfactant. Cells seeded onto a sub-monolayer of the FC-PEG surfactant were severely impaired in attachment and spreading due to the "non-fouling" properties of even relatively short-chained, surface-bound PEG. On substrates covered with a dense monolayer cell attachment was completely inhibited.

We have also synthesized fluorocarbon-PEG-peptide surfactant conjugates that can be used to anchor peptide ligands to the fluorocarbon domains through the hydrophobic interactions between the fluorocarbon tail of the surfactant and the patterned FC domains.

These maps, showing the spatial distribution of the typical ionic fragments, demonstrate clearly that the FC polymer is exclusively found on the 20 microns wide line, whereas the tetraglyme polymer is masked underneath the line and found only on the background. A surfactant concentration of >200 microgram/ml results in the formation of a monolayer within minutes of the exposure to the surface. The adsorption kinetic is dependent on the solution concentration. The monolayer surface concentration was calculated to be ~400 nanograms/cm^2, which corresponds to a specific area of 38Å^2/molecule. By controlling the ligand pattern and the density within the pattern, we can control the size and the shape of cells and study the influence of these parameters on SMC physiology [1].

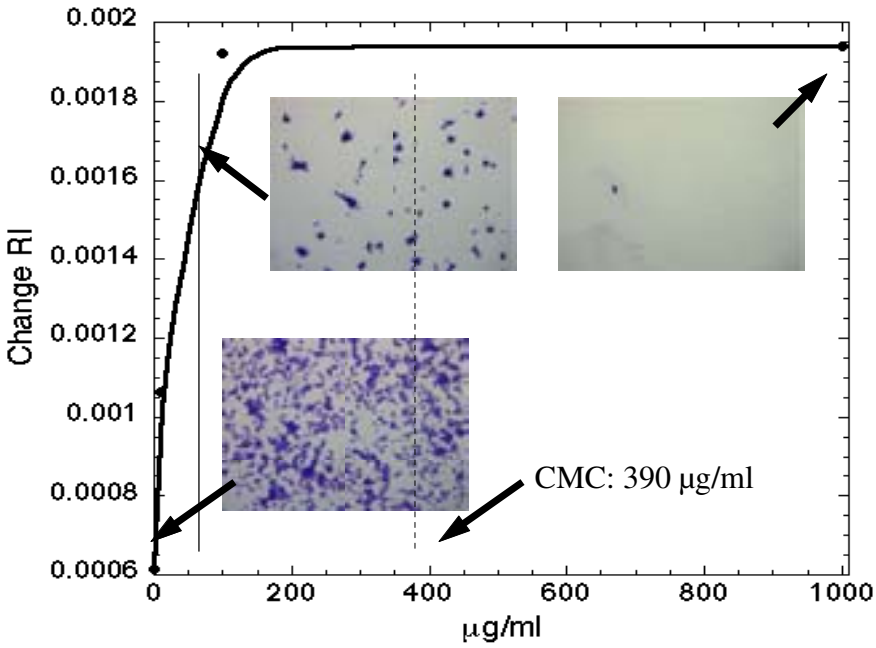


FIG. 3. Photomicrographs of cell attachment onto bare, and FC-PEG covered surfaces superimposed on the FC-PEG adsorption isoterm to the SPR surface.

ACKNOWLEDGEMENTS

NSF provided the funding for this research through the University of Washington Engineered Biomaterials (UWEB) Engineering Research Center Grant ERC 9529161.

REFERENCE

[1] INGBER EL AL, Science 276 (1997) 1425.

BIBLIOGRAPHY

INTERNATIONAL ATOMIC ENERGY AGENCY, Radiation Technology for Immobilization of Bioactive Materials, IAEA-TECDOC-486 (1988).

INTERNATIONAL ATOMIC ENERGY AGENCY, Biomedical Applications for Radiation Technology (1994).

INTERNATIONAL ATOMIC ENERGY AGENCY, Biomedical Applications of Radiation Chemistry (1995).

INTERNATIONAL ATOMIC ENERGY AGENCY, Radiation Synthesis of Intelligent Hydrogels and Membranes for Separation Purposes (1999).

INTERNATIONAL ATOMIC ENERGY AGENCY, Post-Irradiation Stability of Radiation Sterilized Medical Implants (2000).

LIST OF PARTICIPANTS

Deelder, A.	State University of Leiden, Schistosomiasis Research Department, Laboratory of Parasitology, Wassenaarseweg 62, Postbus 9605, 2300 RC Leiden, Netherlands
Güven, O.	International Atomic Energy Agency, Wagramer Strasse 5, P.O. Box 100, A-1400 Vienna, Austria
Ha, H.	Beijing University, Department of Technical Physics, Hai Dian District, Beijing 100871, China
Hegazy, E.-S.	National Centre of Radiation Research and Technology, Polymer Chemistry Department, P.O. Box 29, Nasr City, Cairo, Egypt
Hoffman, A.S.	University of Washington, Center of Bioengineering, Box 35-2255, Seattle, Washington 98195, United States of America
Kaetsu, I.	Kinki University, Department of Nuclear Engineering, Faculty of Science and Technology, Kowakae, Higashiosaka, Osaka 577, Japan
Mazzei, R.O.	Comisión Nacional de Energía Atomica, Centro Atomico Ezeiza, Unidad de Actividade de Aplicaciones Tecnologicas, Avenida del Libertador 8250, 1429 Buenos Aires, Argentina
Nurkeeva, Z.	Al Farabi Kazakh State National University, Department of High Molecular Compounds, Faculty of Chemistry, Karasai Batyr Str. 95a, Almaty 480012, Kazakhstan
Razzak, M.T.	National Atomic Energy Agency, Centre for the Application of Isotopes and Radiation, Jl. Kunigan Barat, Mampang Prapatan, Jakarta 12710, Indonesia
Rosiak, J.M.	Lodz Technical University, Institute of Applied Radiation Chemistry, Wroblewskiego Str. 15, PL-93-590 Lodz, Poland
Safrany, A.	Hungarian Academy of Sciences, Institute of Isotopes, Radiation Chemistry Department, H-1525 Budapest, Hungary
Sen, Murat	Hacettepe University, Department of Chemistry, Beytepe, 06532 Ankara, Turkey