A STUDY OF POLYMER-METAL-PROTEIN COMPLEXES AND EFFECTS OF IRRADIATION

by

Betül DİNÇER

B.S. in CH.E., Boğaziçi University, 1993

Submitted to the Institute for Graduate Studies in Science and Engineering in partial fulfilment of the requirements for the degree of

Master of Science

in

Chemical Engineering

Boğaziçi University

1995

ACKNOWLEDGMENTS

I would like to express my sincere gratitudes to Prof. Dr. Fahir Borak for his everlasting encouragement and supervision throughout my studies.

I would also like to express my deepest appreciation to Prof. Dr. Muhammed Mustafaev and Dr. Yılmaz Erkol for their valuable suggestions and helpful attitudes.

I am grateful to Prof. Dr. Bahattin Baysal for his kind help and interest.

Deepest thanks are due to all my friends at Chemistry Department of Çekmece Nuclear Research and Training Center (ÇNAEM).

I would also like to thank the Turkish Atomic Enegy Commission for their financial support without which this research could not have been carried out.

Finally, my deepest gratitude is to my family for their encouragement and everlasting understanding.

ABSTRACT

The lethal and harmful effects of X- and gamma rays have been found to be reduced by the prior administration of certain chemical substances called radioprotectors. In the present work, complex formation of some water soluble polymers (polyacrylic acid, polyacrylamide containing amino acid end group, and polyacrylamide containing no end group) with Bovine Serum Albumin (as a model protein) in the presence of divalent copper ions (Cu^{2+}) was investigated using High Performance Liquid Chromatography (HPLC) before evaluating their possible usage as a radioprotector.

For this purpose, polyacrylamide with and without functional end groups were synthesized. Polymerization of acrylamide was carried out with Cerium (IV)-Methionine redox initiator system. The effect of cerium (IV) concentration, reaction time, and temperature on the molecular weight and polymerization yield were studied by the means of viscosimetric measurements. The molecular weight (MW) distributions of the polymers synthesized at different reaction conditions were analyzed by High Performance Liquid Chromatography (HPLC). In the light of HPLC results, optimum reaction conditions which provide an opportunity to obtain a polymer having a narrow molecular weight distribution (MWD) were determined. In order to facilitiate an understanding of the complex formation mechanism of the triple complexes, the HPLC analysis of polymer-metal and polymer-protein complexes were also performed.

Finally, aqueous solutions of polyacrylic acid (PAA), BSA, PAA-Cu^{2+}, PAA-Cu^{2+}-BSA, and PAA-BSA were irradiated using a Co-60 gamma source for a total dose of 1.2 kGy and ultimately, HPLC and spectrophotometric methods were employed to these samples in order to investigate the effect of irradiation on them.

ÖZET

Radyoprotektör olarak adlandırılan bazı kimyasal maddelerin ışınlanma öncesinde alınması sonucu, X ve gama ışınlarının etkilerini azalttığı bulunmuştur. Bu çalışmada, suda çözülen bazı polimerlerin (fonksiyonel uç gruplu ve grupsuz poliakrilamid ve poliakrilik asidin) Bovin Serum Albumin (BSA) ile iki değerlikli bakır iyonları varlığında oluşan kompleksleri Yüksek Basınçlı Sıvı Kromatografi (HPLC) metotu yardımıyla araştırılmıştır. Daha sonraki çalışmalarda bu komplekslerin radyoprotektör olarak kullanımı değerlendirilecektir.

Bu amaçla, uç gruplu ve grupsuz poliakrilamid sentez edilmiştir. Ce(IV)-Metionin redox başlatıcı sistemi ile akrilamidin polimerizasyonu gerçekleştirilmiştir. Ce (IV) konsantrasyonunun, zamanın, ve sıcaklığın molekül ağırlığına ve verimine olan etkisi viskosimetrik ölçümler vasıtasıyla incelenmiştir. Çeşitli reaksiyon şartlarında sentez edilen polimerlerin molekül ağırlık dağılımları HPLC ile analiz edilerek dar molekül ağırlık dağılımına sahip olan polimerler elde edilmesine imkan sağlıyan optimum reaksiyon şartları tayin edilmiştir. Bu üçlü komplekslerin oluşum mekanizmasının anlaşılmasına kolaylık sağlanması için polimer-metal ve polimer-protein komplekslerinin HPLC analizi yapılmıştır.

Son olarak, poliakrilik asit (PAA), BSA, PAA-Cu^{2+}, PAA-Cu^{2+}-BSA, ve PAA-BSA sulu çözeltileri değişik ışınlama hızında ancak 1.2 kGy toplam doza maruz kalacak şekilde Co-60 gamma kaynağı tarafından ışınlanarak HPLC ve spektrofotometrik metotlarla ışınlamanın bu numuneler üzerindeki etkisi incelenmiştir.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF SYMBOLS

d	Density
k	Reaction Rate Constant
K	Distribution Coefficient
M_n	Number Average Molecular Weight
M_r	Relative Molecular Mass of Solute
M_w	Weight Average Molecular Weight
M_v	Viscosity Average Molecular Weight
M_z	Z Average Molecular Weight
n_{Me}	Number of Moles of Metal
n_p	Number of Moles of Polymer
R	Native Protein Molecule
R_u	Unfolded Molecule
t_o	Flow Times of Solvent
t_c	Flow Times of Polymer
V_o	Void Volume
V_e	Elution Volume
V_i	Interstitial Volume
V_R	Retention Volume
w	Differential Weight Distribution
w_1	Weight Fraction Eluted up to Volume V
X	Labile Atom
η	Solution Viscosity
$[\eta]$	Intrinsic Viscosity
η_o	Solvent Viscosity
η_r	Relative Viscosity
η_{sp}	Specific Viscosity

I. INTRODUCTION

A well-established phenomenon is that extremely small amounts of certain chemicals taken a short interval before exposure to irradiation can provide a significant measure of protection for living beings from the effects of ionizing radiation. The protective chemicals named as radioprotectors (or chemical protectors) must be taken before exposure to irradiation and must be in the cell or in the animal during the time of exposure to irradiaton if it is to give any significant help.

Water-soluble polymers and their various polymer complexes have potential possibility of radioprotective activity since they might bond temporarily to side chains of the protein that are particularly radiation sensitive and absorb damage that would otherwise be sustained by the protein [1-3]. Therefore, it is very important to understand the mechanisms of protein cooperative binding by synthetic polymer for the construction of the new type of radioprotectors based on synthetic polymer.

The aim of this study is to investigate the complex formation of some water soluble polymers (polyacrylamide with and without functional end groups, and also polyacrylic acid) with Bovine Serum Albumin (as a model protein) in the presence of divalent copper ions (Cu^{2+}) using HPLC (High Performance Liquid Chromatography) before evaluating their possible usage as a radioprotector. In addition, HPLC analysis of polymer-metal and polymer-protein complexes were aimed at facilitiating an understanding of the formation mechanism of polymer-metal-protein complexes.

In this work, polymerization of acrylamide containing methionine end group, initiated by cerium ammonium nitrate (Ce IV)-methionine redox initiator system was carried out in aqueous solution. The dependence of molecular weight and yield polymer on the concentration of Cerium (IV), polymerization time, and temperature of was determined.

Ceric salts such as the nitrate, perchlorate and sulfate in aqueous acidic solutions are used as initiators of vinyl polymerization [4]. It is well known that these ceric salts also form very effective redox systems in the presence of organic reducing agents such as alcohols [5], aldehydes [6], ketones [4], carboxylic acids [7], organic acids [7], hydroxy acids [8], polyaminocarboxylic acids [9, 10]. The resulting polymer was suggested to have corresponding chain ends [6].

Also, the synthesis of polyacrylamide containing no functional end groups was achieved by exposing an aqueous solution of acrylamide monomer to heat under nitrogen atmosphere. The molecular weight (MW) distributions of the polymers were examined using the method of HPLC. Fractional precipitation of the polymer with nonsolvent (methanol) addition was carried out for the purpose of collecting a fraction having a narrow molecular weight distribution. Obtaining such a fraction is an essential prerequisite of studying the complex formation of polymers.

One of the main action of water soluble synthetic polyelectrolytes (PE) in biological systems is the cooperative interaction of PE with the biopolymer components of the organism [11]. This allows us to show the potentiality of water soluble PE and their various PE complexes as radioprotective agents.

Cysteine [12] and cyanide [13] were the first radioprotectors to be discovered in 1949. Since then, numerous compounds have been reported to be radioprotective. Sulphydryl compounds, cyanides, nitriles, amines, and antibiotics are examples to some of the protective compounds mentioned in the literature [14-16].

Various mechanisms for this chemical protective action have been proposed since the discovery of the phenomenon. Presently, three main theories involving the mechanism of action of radioprotectors are given. These are the anoxia theory, the free radical scavenger theory, and the mixed disulphide theory. The hypothesis for radioprotection have been reviewed in detail by Bacq and Alexander [17], Brown [18], Eldjarn and Phil [19], Doherty, [20], Bacq [21], and many others.

It is an established fact that molecular oxygen increases the effects of X- or gamma radiation. Protective agents act through the production of cellular anoxia. The increase of radiation damage by oxygen is almost universal in biological systems. The adherents to the theory of indirect action are that oxygen combines with the products of ionization of H_2O to form peroxide radicals, OOH, which in turn attack the vital molecules and cause damage. Some of the most powerful radioprotectors are thiol substances (cysteine, cysteamine, reduced glutathione, and mercaptoethylguanidine) which, in aqueous solution of neutral or slightly alkaline pH, oxidize readily S-S, thus, more or less rapidly consume O_2, and consequently lower the oxygen tension. However, these substances are far from being the ideal protector. They will all be poisonous to some extent, since any chemical which reduces oxygen must interfere with cell respiration.

The second mechanism accepted by many people working in the field of radioprotection is free radical scavenger theory. According to this theory, radioprotectors can act as scavengers of the primary radical species, such as •OH and e_{aq}^-, etc., responsible for the damage induced by radiation on biological targets (e.g. enzyme). Cysteine seems to be almost a perfect scavenger of all the known intermediates in the radiation chemistry of water [22]. The ability of certain thiols and disulphides to protect against irradiation damage has been attributed to their activity in reacting with the free radicals produced by irradiation of water [23]. However, several compounds such as sodium citrate and norepinephrine, which inactivate free radicals in vitro, have no radioprotective value in vivo.

Finally, the experiments done on the mixed disulphide theory have shown that sulfur-containing radioprotectors form complexes with -SH or S-S groups of proteins to form mixed disulphides. For instance, an -SH protector:

2 P-SH	+	R-S-S-R	$\Rightarrow$	2 P-S-S-R
protector		protein		mixed disulphide

The protectors at the time when they exert protection exist largely in the form of mixed disulphides with the proteins of the bloods and tissues. The normal S-S bonds should be more radiosensitive than the rest of the molecule and the "target" modified by reaction with the radioprotector should become more resistant against direct action, subsequent restoration of the S-S protein function would occur.

The interaction of PE with serum proteins was investigated in detail by Kabanov et al. [24]. It was found that the interaction behavior of anionic PE in protein mixtures are dependent on the isoelectric points (pI) of proteins. The pre-existing electrostatic repulsive forces between serum proteins and polyanion (for example, polyacrylic acid) contrary to polycation-protein systems prevent the complex formation in the condition pH > isoelectric point (pI). Recently, a systematic study has been carried out on the formation of water soluble and insoluble ternary complexes of proteins with synthetic PE in the presence of transient metal ions [25]. It is shown that, when protein and PE are incapable of binding to one another, the metal ions can promote the formation of a stable ternary complex. Protein interactions with PE are effectuated through the formation of chelate complexes, in which metal plays the role of the central atom and act as a crosslinking agent. These complexes also reveal high immunogenecity and confer high levels of immunological protection [25].

It is known that the properties of PE complexes are always in some respect different from those of starting components, and this has been used in the studies of their formation [26]. Techniques such as potentiometric-conductometric titrations, turbidimetry, viscometry, calorimetry, light scattering, nuclear magnetic

resonance spectroscopy, chromatography, and even electron microscopy have all been employed to follow the course of complex formation. For example, the formation of the water soluble triple complexes of poly-4-vinylpyridine with bovine serum albumine in the presence of divalent copper ions (Cu^{2+}) was investigated by sedimentation analysis, turbidimetric titration, viscometry, and u.v. spectroscopy in neutral aqueous media according to the method developed by Mustafaev and Kabanov [27].

Among the four modes of HPLC, which are adsorption chromatography, partition chromatography, ion-exchange chromatography, and gel or exclusion chromatography, gel chromatography was selected. The reason for this is that gel chromatography is very suitable for the initial, exploratory separation of the samples. Such a separation quickly provides an overall picture of the total sample. It tells whether one is dealing with a simple or complex mixture, and whether the sample components are of low, intermediate, or high molecular weight. Besides, gel chromatography is a logical first choice for separating high-molecular-species (mol. wt.> 2000), especially for polymers, proteins and nucleic acids.

Since the elimination of high molecular weight polymers from the organism (the liver, kidney, and other organs) is very difficult and their prolonged storage in vivo may cause histocytic reactions, which can produce tumorlike growths that could be carcinogenic, it should be emphasized that selecting appropriate molecular weight range of the polymer is an essential criterion that must be met by the polymer being used as a radioprotector. For this reason, fractional precipitation was applied to the polyacrylamide with no end groups having high molecular weight (868,784), and ultimately, the fraction consisting of 100,000 MW was obtained. The MW range of the other polymers used (polyacrylamide containing methionin end group, polyacrylic acid) is between 50,000 and 150,000.

Some attention has been paid to the effects of high energy radiation on polymers in so far as these can serve as models for the biological effects of radiation, but little information concerning the influence of irradiation on both

double polymer-protein and ternary polymer-metal-protein complexes is known. There is a close parallel between the changes produced in macromolecules whether they are biological or polymeric nature. Since the chemical changes and mechanisms are far easier to study in the simple polymers, this technique opens interesting research avenues which could be adapted and extended in the more complex field of radiobiology. Another aim of this work is to examine the effect of irradiation on aqueous solutions of polymer, protein, polymer-metal, polymer-protein, and polymer-metal-protein complexes. For this purpose, they were irradiated with Co-60 gamma rays and HPLC and spectrophotometric methods were employed to investigate them.

Further investigations in the same field, namely, the intraperitonally injection of the above mentioned polymers and their various complexes to mice, exposure of these mice to a lethal dose of 500 rem gamma-rays by Co-60 and finally, a study on chromosomal aberration of the exposed mice will be carried out in the near future in order to observe radioprotective effect of the polymer and their various complexes. All these studies will be carried out in collaboration with the Departments of Chemistry, Health Physics, Radiopharmacology, and Radiobiology at Çekmece Nuclear Research and Training Center (ÇNAEM).

In Chapter II, some information on the following subjects are given: the synthesis of polyacrylamide with and without functional end groups, the determination of polymer molecular weight by various techniques, the synthesis of polymer-metal and on polymer-metal-protein complexes and radiation chemistry of aqueous solutions of polymer and proteins. The experimental studies carried out are presented in Chapter III and the results are discussed in Chapter IV. Finally, conclusions and recommendations for future work are given in Chapter V.

II. THEORY

In the beginning of this chapter, general information about the synthesis of polyacrylamide with and without functional end groups, and also about the determination of polymer molecular weight by various techniques is given. Since the present work was not only carried out on polyacrylamide but also on polyacrylic acid, which is a typical polyelectrolyte, general information about polyelectrolytes and their complexes are presented. Finally, the synthesis of polymer-metal and polymer-metal-protein complexes and radiation chemistry of water, and aqueous solutions of polymer and proteins are briefly summarized.

2.1. General Information About Polymers

Polymers are based on one or more systematically repeating units (or mers) to form a macro-molecule. Their molecular weight may vary from a few thousand to several million. Monomers generally have molecular weights up to 300. Polymers can have linear, branched linear, lightly cross linked or highly cross linked structure.

Carothers (1940) and Flory (1953) proposed a classification of polymers based on polymerization mechanism: condensation polymerization and addition polymerization or in more precise terminology, step-reaction and chain-reaction polymerization.

Condensation or step-reaction polymerization are usually formed by stepwise intermolecular condensation of reactive groups. For example, a polyester is formed by typical condensation reactions between bifunctional monomers, with the elimination of water:

$$x\text{HO-R-OH} + \text{HOCO-R'-COOH} \Rightarrow \text{HO}\ [\text{-R-OCO-R'-COO-}]_x\ \text{H} + (2x\text{-}1)\ H_2O$$

Addition or chain reaction polymerization ordinarily result from chain reactions involving some sort of active center. Among the several types of active center, three of them have been found experimentally. These are: cation, anion and free radical.The most important group of addition polymers includes those derived from unsaturated vinyl monomers:

$$CH_2\text{=}CHX \Rightarrow \text{--}CH_2\text{-}\underset{\displaystyle X}{\underset{|}{CH}}\text{-}CH_2\text{-}\underset{\displaystyle X}{\underset{|}{CH}}\text{-, etc.}$$

2.2. Free Radical Polymerization

2.2.1. General Nature of Vinyl Type Polymerization

The most commonly encountered monomers are substituted ethylenes of the types CH_2=CHX (e.g. styrene, acrylic acid, acrylamide, acrylonitrile, or the acrylic esters) and CH_2=CXY (e.g. vinylidene chloride, methacrylic acid or its derivatives)

called vinyl monomers. Their conversion to a polymer is referred to vinyl polymerization.

Vinyl polymerization is a chain reaction in which the chain carrier may be an ion or a reactive substance with one unpaired electron called a free radical. A free radical is usually formed by the decomposition of a relatively unstable material called an initiator. The free radical is capable of reacting to open double bond of a vinyl monomer. In a very short time (usually a few second or less) many monomers add successively to the growing chain. Finally, two free radicals react to cease each other's growth activity and form one or more polymer molecules.

2.2.1.1. Initiation. When free radicals are generated in the presence of a vinyl monomer, the radical adds to the double bond with the regeneration of another radical. The radical formed by decomposition of the initiator I is represented by R•

$$I \Rightarrow 2\ R\bullet$$

$$R\bullet + CH_2{=}CHX \Rightarrow RCH_2\overset{H}{\underset{X}{C}}\bullet$$

The regeneration of the radical is characteristic of chain reactions. Evidence for the radical mechanism of addition polymerization comes not only from the capability of radicals to accelerate vinyl polymerization, but also from showing that the polymers formed contain fragments of radicals.

2.2.1.2. Propagation. The chain radical formed in the initiation step is capable of adding successive monomers to propagate the chain:

$$R\text{-}(CH_2CHX\text{-})_xCH_2C\bullet + CH_2{=}CHX \Rightarrow R\text{-}(CH_2CHX\text{-})_{x+1}CH_2C\bullet$$

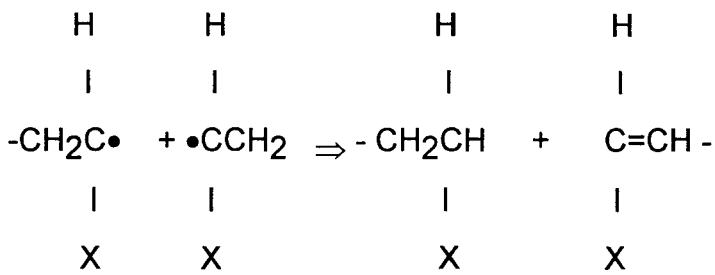
The chain propagation steps consist essentially of free radicals attack at one of the double bonded carbon atoms of monomer. The driving force of the reaction between the odd electron initiator fragment and unsaturation electrons of the double bond in the monomer is the tendency for two electrons of opposite spin to couple and form a covalent bond. The remaining electron of the double bond shifts to the other carbon atom which then becomes a free radical. In this way, the active center shifts uniquely to the newly added monomer, which is capable of adding another monomer etc.

2.2.1.3. Termination. Propagation would continue until the supply of monomer is exhausted by the strong tendency of radicals to react in pairs to form a paired-electron covalent bond with loss of radical activity. The termination step can take place in two ways: combination or coupling:

Combination:

$$-CH_2C\bullet + \bullet CCH_2 \Rightarrow -CH_2C\text{-}C\text{-}CH_2-$$

Disproportionation



in which hydrogen transfer results in the formation of two molecules with one saturated and one unsaturated end group.

2.3. Generation of Free Radicals

Radical generation reactions can be divided into two general types according to the manner in which the first radical species is formed, these are:

-Homolytic decomposition of covalent bonds by energy absorption.

-Electron transfer from ions or atoms containing unpaired electrons followed by bond dissociation in the acceptor molecule.

2.3.1. Homolytic Decomposition of Covalent Bonds

Chemical compounds may be dissociated into two or more free-radical fragments by absorption of energy in almost any form, including thermal, electromagnetic, particulate, electrical, sonic, mechanical and all of these forms of

energy have been used for the initiation of radical, chain growth polymerization reactions at one time or another. The most important of these are the thermal and electromagnetic energies and the latter is generally considered to include two major forms of energy, ultraviolet light and high energy radiation.

2.3.2. Generation of Free Radicals by Electron Transfer

A very efficient method of generating free radicals under mild conditions is by one-electron transfer reactions. The most effective one is redox initiation. This particular technique has found wide application for initiating polymerization reactions, especially in aqueous emulsion systems. Other one electron transfer reactions which have been applied to chain-growth initiation are electrolysis and electron transfer from an alkali metal directly to a monomer.

2.3.2.1. Redox Initiation.

The technique of producing radicals by one-electron oxidation-reduction reactions finds frequent use, since the presence of an unstable initiator is not required and the reactions can be used as source of radicals at low temperatures. Radicals can be produced by either oxidation or reduction. In the Kolbe electrolysis, salts of organic acids are electrolyzed, and the caboxylate anions undergo oxidation to a radical that loses carbon dioxide. The resulting radicals couple.

$$R\text{-}CO_2^- \xrightarrow[-e^-]{\text{Anode}} R\text{-}CO_2\bullet \Rightarrow R\bullet + CO_2$$

$$2R\bullet \Rightarrow R\text{-}R$$

The most important radical-forming redox reactions are those involving a metal ion that can undergo a one-electron transfer. Of these, the reaction of hydrogen peroxide with ferrous ion is one of the oldest and best known. Fenton discovered the reaction in 1894, and in 1932 Haber and Weiss proposed a mechanism which, with only slight modification, is accepted today. The reaction occurring when hydrogen peroxide reacts with ferrous iron is as follows:

$$H_2O_2 + Fe^{2+} \Rightarrow HO\bullet + OH^- + Fe^{3+}$$

Another widely used peroxide-type initiator is persulfate ion. With a reducing agent R the reaction is

$$S_2O_8^{2-} + R \Rightarrow SO_4^{2-} + SO_4^{-}\bullet + R^+$$

the reducing agent is often the thiosulfate ion,

$$S_2O_8^{2-} + S_2O_3^{2-} \Rightarrow SO_4^{2-} + SO_4^{-}\bullet + S_2O_3^{-}\bullet$$

or the bisulfite ion,

$$S_2O_8^{2-} + HSO_3^- \Rightarrow SO_4^{2-} + SO_4^{-}\bullet + HSO_3\bullet$$

Saldick [4] have demonstrated that ceric ions in aqueous solution initiate polymerization in the presence of organic reducing agents. The reducing agent may be side chain of a polymer (e.g. poly(vinyl) alcohol, its partial esters and

ethers, carbohydrates and their partial esters and ethers, poly(hydroxyaklyl acrylates), polyketones, polyvinylamines, polyaldehydes, poly(mercaptoalkyl acrylates) and poly(vinyl acetals)). Radical formation in the presence of a monomer gives rise to graft polymers with the formation of little or no homopolymer [28]. Mino and Kaizerman [29] suggest that radicals are formed on the breakdown of a complex between Ce^{4+} and the reducing agent, e.g.,

$$Ce^{4+} + RCH_2OH \Leftrightarrow Z \Rightarrow Ce^{3+} + H^{+} + RCH_2O\bullet$$

2.4. Radical Polymerization of Acrylamide Iniatiated by Ce (IV)-Methionine Redox System

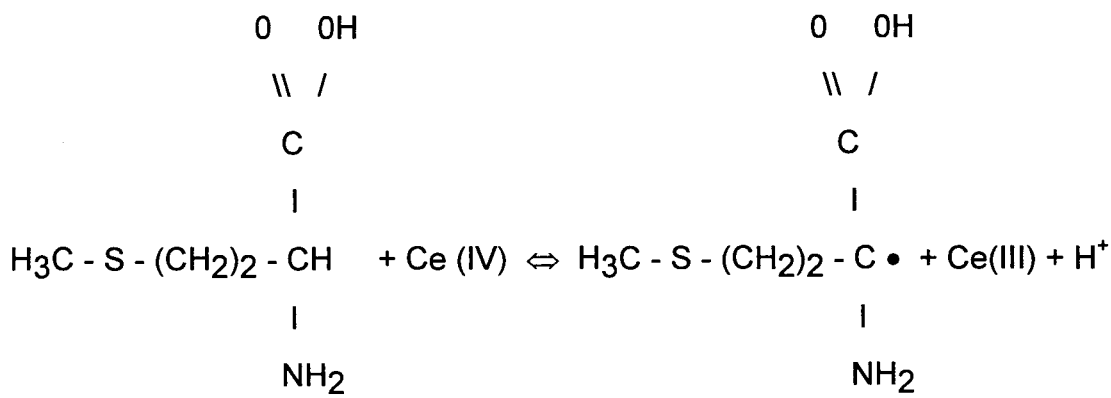
The catalysts used as initiators for free radical polymerization reactions serve as the prime source of functional end groups in vinyl polymers [30]. Cerric salts such as the nitrate, perchlorate and sulfate in aqueous acidic solutions are used as initiators of vinyl polymerization [4]. It is well known that these ceric salts also form very effective redox systems in the presence of organic reducing agents such as alcohols [5], aldehydes [6], ketones [6], carboxylic acids [7], organic acids [7], hydroxy acids [8], polyaminocarboxylic acids [9, 10]. This method was also used for the preparation of graft copolymers of vinyl monomers such as acrylonitrile and acrylamide [31].

The polymerization mechanism involves the generation of free radicals from a complex formed between Ce (IV) salt and reducing agent following the formation of free radicals which initiate the polymerization of vinyl monomer.It is known that, when a carboxylic acid reducing agent is used the same functional group may be present as end group at the end of the polymer chain. Quantitative determination

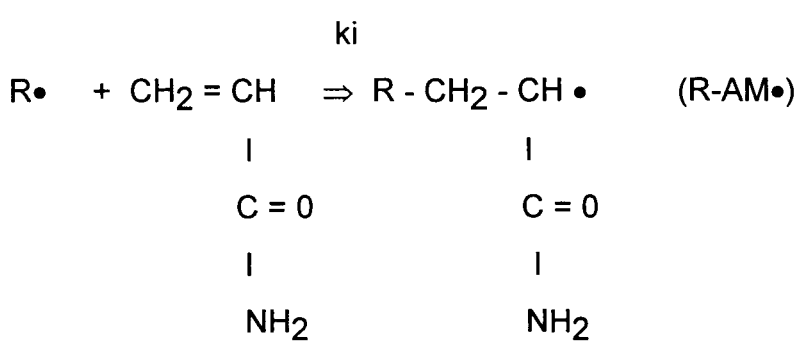
of such functional groups is particularly difficult because of the extremely low level at which they exist in the polymer. However, such analysis may play an integral part in understanding the mechanism of poymer initiation and termination, and the role of the catalyst. Titrimetric methods for determining functional end groups are widely used for number average molecular weight measurements [32, 33].

In this work, the polymerization of acrylamide initiated by the cerric ammonium nitrate - methionine redox system was studied in aqueous solution.

INITIATION



[Methionine - Ce (IV)] ⇒ [Primary Radical (R•)]



[Acrylamide]

PROPOGATION

$$\text{R-AM}\bullet \;+\; n\,\text{AM} \;\xrightarrow{kp}\; \text{R - (AM)}_n\text{ - AM}\bullet$$

TERMINATION

Mutual Termination

$$\text{R - (AM)}_n\text{ - AM } + \text{ R - (AM)}_m\text{ - AM} \;\xrightarrow{k_t}\; \text{R - (AM)}_{n+1}\text{-(AM)}_{m+1}\text{ - R}$$

Termination by Ce(IV)

$$\text{R - (AM)}_n\text{ -CH}_2\text{ -}\overset{\bullet}{\text{CH}}\text{(CONH}_2\text{)} \;+\; \text{Ce (IV)} \;\xrightarrow{k_t}\; \text{R - (AM)}_n\text{ -CH = CH(CONH}_2\text{)} \;+\; \text{Ce (III)} + \text{H}^+$$

(In each case the CH bears the pendant group: CH – C=O – NH_2.)

2.5. Polymerization of Acrylamide without Functional End Group

Acrylamide polymerizes easily in the presence of free-radical initiators to form chains of the structure (- CH_2-CH- $)_n$ where n is 20,000 to 300,000.

$$\left(-\text{CH}_2\text{-}\underset{\text{CONH}_2}{\underset{|}{\text{CH}}}\text{-}\right)_n$$

Solutions of acrylamide in water polymerize at moderate temperatures under the influence of nearly all free-radical sources, including peroxides, persulfates, redox couples, azo compounds. Acrylamide will also undergo polymerization by exposure to heat, high energy radiation, ultrasonic waves, ultraviolet radiation and ionic polymerization catalysts to produce water soluble polymers.

In this study, a fully linear polyacrylamide was synthesized by exposing an aqueous solution of acrylamide monomer to heat under nitrogen atmosphere.

2.6. Molecular Weights of Polymers

2.6.1. Definitions

With the possible exception of certain biological polymers, all polymers consist of a distribution of molecular weights. This is a consequence of the random nature of polymerization reactions. Therefore, the molecular weight of a particular polymer can not be characterized by fully polymeric materials. It is essential to have some means of defining and determining their molecular weights and molecular weight distributions.

The number average molecular weight, M_n is the simple counting average in which the mass of the sample w, expressed in atomic mass units or daltons, is divided by the number of molecules it contains: $M_n = w/N$. Expressing N as the sum over all species of the number N_i of the molecules of i th kind, and similarly as w_i where

$w_i=N_i * M_i$. The M_n usually is defined by the following expression:

$$M_n= \Sigma N_i *M_i / \Sigma N_i \quad (2.1)$$

M_n is characterized by its sensitivity to a small weight fraction of a constituent of low molecular weight and by its relative insensitivity to the changes at the higher molecular weights. Number-average molecular weights of commercial polymers usually lies in the range 10.000-100.000.

The weight average molecular weight (M_W) is defined by any of the following expressions:

$$M_W = \Sigma w_i * M_i = \Sigma N_i * M_i^2 / \Sigma N_i*M_i \quad (2.2)$$

Because heavier molecules contribute more to M_W than light ones, M_W is always greater than M_n except for a hypothetical monodisperse polymer. The value of M_W is greatly influenced by the presence of high molecular-weight species just as M_n is influenced by species of the low end molecular-weight distribution curve.

There are two other molecular weight averages using in describing polymer properties. The first is the z-average:

$$M_Z= \Sigma N_i*M_i^3 / \Sigma N_i*M_i^2 \quad (2.3)$$

The second is the viscosity average:

$$M_V= \Sigma [N_i *M_i ^{1+\alpha} / N_i*M_i]^{1/\alpha} \quad (2.4)$$

where α is an empirical constant for a given polymer, solvent and temperature.

The ratios of molecular weight averages are useful indicators of molecular weight distributions. For monodisperse samples the molecular weight averages are identical so that the ratios are all unity. For those condensation polymerizations in which there is statistically the most probable distribution of molecular sizes then M_Z: M_W: M_n = 3 : 2 : 1. For the chain addition polymerization, particularly at high levels of conversion, the ratio M_W : M_n for example can increase to > 25.

2.6.2. Identification

The molecular weights of polymers can be determined by chemical or physical methods of functional-group analysis by measurement of the colligative properties, light scattering, or ultracentrifugation, or by measurement of dilute solution viscosity. All these methods except the last are in principle absolute. Molecular weights can be calculated without reference to calibration by another method. However, dilute-solution viscosity is not a direct measure of molecular weight. Its value lies in the simplicity of the technique and the fact that it can be related empirically to molecular weight for many systems.

There are several ways of identifying number average molecular weight (M_n). The measurement of the concentration of end groups when one knows the exact number per molecule, is a method of counting the number of molecules. End group analysis can be more sophisticated as in the use of spectroscopic techniques such as infra-red or nuclear magnetic resonance. Another way of determining number average molecular weight are based on measurement of one of the colligative properies; vapor pressure lowering, freezing point depression (cryoscpy), boiling point elevation (ebulliosmetry), and osmotic pressure (osmometry). These colligative properties are used to estimate the molecular weight of the solute. The melting point depression is usually used in organic chemistry for the characterization of small molecules. Osmotic pressure probably is the most popular of the colligative method. according to thermodynamic theory

osmotic pressure, at infinite dilution, is inversely proportional to the molecular weight of polymer. This relationship provides a measure of molcular weight.

Light scattering and equilibrium ultracentrifugation methods are the methods to obtain the weight average molecular weight (M_W). In the light scattering method, measurement is made of the difference in scattered-light intensity between a polymer solution and its solvent. This scattered intensity depends upon both concentration and the angle between the incident and scattered light beams. The second requirement sets the major design features of light scattering photometers.

Viscosity average molecular weight (M_V) may be determined from Eq. 2.4, if the intrinsic viscosity-molecular weight relationship, i.e., the constant α is known. If the constants are not known, the relationship may be established by plotting the intrinsic viscosity vs. molecular weight of a well fractionated sample on a log-log plot. The molecular weights of the fractions may be measured by light scattering. A point in favor of the light scattering method in preference to the osmotic method for this purpose is the fact that the former gives the weight average, which is closer to viscosity average [34].

M_Z and M_{Z+1} may be determined with the ultracentrifuge. In the sedimentation equilibrium experiment the ultracentrifuge is operated at a low speed of rotation for times up to one or two weeks constant operating conditions. A thermodynamic equilibrium is reached in which the polymer is distributed in the cell solely according to its molecular weight and MWD, the force of sedimentation on each species being just balanced by its tendency to diffuse back against the concentration gradient resulting from its movement in the centrifugal field. The method allows also the determination of M_W [35]. The distribution of molecular weights in a typical polymer sample is shown in Fig. 2.1.

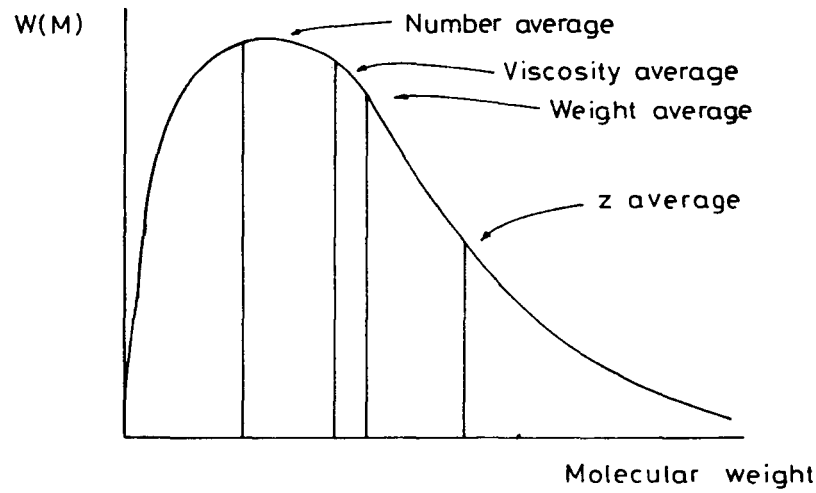


Figure 2.1. Distribution of Molecular Weights in a Typical Polymer

2.6.3. Dilute Solution Viscosity Method

One of the most widely used methods for the determination of polymer molecular weights is based on solution viscosity measurements. Staudinger (1930) was the first to cite the usefulness of solution viscosity as a measure of polymer molecular weight. Solution viscosity is basically a measure of the size or extension in space of polymer molecules. It is empirically related to molecular weight for linear polymers.

Nomenclature of solution viscosity is shown in the Table 2.1.

Relative viscosity, η_r is given by the ratio of efflux time for the solution, t to that of the solvent t_o $\eta_r = \eta / \eta_o$ where the viscosities of the solution and solvent are related to the corresponding efflux times by

$$\eta = c * t * d - E*d / t^2 \qquad (2.5) \qquad \eta_o = c * t_o * d_o - E * d_o / t_o^2 \qquad (2.6)$$

where d is the density and c and E are constants for the particular viscometer used. For the dilute solutions, d and d_o are substantially equal and viscometers

are designed so that, for efflux times greater than 100 sec or so, the second term is negligible [36].

The specific viscosity can be seen to be the relative increment in viscosity of the solution over that of the solvent, and the reduced viscosity is the quantity taken per unit concentration.

The intrinsic viscosity [η] is determined experimentally by measurements of flow times of solvent (t_o) and a series of dilute polymer solutions of known concentration (t_c) in a standard capillary viscometer. The specific viscosity is calculated from the equation [34] :

Table 2.1. Nomenclature of Solution Viscosity

Common Name	Recommended Name	Symbol and Defining Equation
Relative viscosity	Viscosity ratio	$\eta_r = \eta/\eta_o \cong t/t_o$
Specific viscosity	-	$\eta_{sp} = \eta_r - 1 = (\eta - \eta_o)/\eta_o \cong (t - t_o)/t_o$
Reduced viscosity	Viscosity number	$\eta_{red} = \eta_{sp} / c$
Inherent viscosity	Logarithmic viscosity number	$\eta_{inh} = (\ln \eta_r) / c$
Intrinsic viscosity	Limiting viscosity number	$[\eta] = (\eta_{sp} / c)_{c=0} = [(\ln \eta_r) / c]_{c=0}$

$$\eta_{sp} = (\eta_c - \eta_o)/\eta_o = (t_c - t_o)/t_o \quad (2.7)$$

and from the Huggins equation [37]

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c \quad (2.8)$$

or alternatively from Kraemer equation [38]

$$\ln \eta_r / c = [\eta] + k''[\eta]^2 c \quad (2.9)$$

where k' and k'' are positive constants. The intrinsic viscosity is obtained by plotting η_{sp}/c versus c and extrapolating to zero concentration.

Staudinger's equation implies that the reduced viscosity of a polymer is independent of its concentration and it is proportional to its molecular weight. Later work has demonstrated that Staudinger's equation must be slightly modified by substituting the intrinsic viscosity for the reduced viscosity and the proportionality is to a power of the molecular weight less than 1. The relation is expressed in the following equation:

$$[\eta] = K' * M^a \quad (2.10)$$

where K' and a are constants determined respectively by the intercept and the slope of a plot. This equation is generally known as the Mark-Houwink relationship [34].

2.6.4. Fractionation

Phase separation by solubility differences forms the basis of the most widely applicable methods of polymer fractionation. The separation of a polymer into fractions of different molecular weights is a laborious and difficult process. Most of the methods commonly used depend on the fact that, as the molecular weight of a polymer increases, its solubility in a particular solvent decreases. Two rather different types of procedure employed are as follows:

*Fractional Precipitation
*Elution Fractionation

2.6.4.1. Fractional Precipitation.

The method involves the fractional precipitation from solution of a series of fractions, the first member of which has the highest molecular weight and the succeeding ones progressively have decreasing molecular weights. The precipitation is carried out by a stepwise decrease in the solvent power of the system. This may achieved by any of the following three methods:

(1) Addition of nonsolvent (or precipitation).
(2) Elimination of solvent by evaporation.
(3) Lowering the temperature of the system.

In this study, the first method mentioned above was applied.

Fractionation by Nonsolvent Addition: First of all, the polymer sample is dissolved in some suitable solvent. To dilute this solution, a nonsolvent is added dropwise with vigorous stirring at constant temperature to cause separation of the mixture into two liquids called the precipitated and supernatant phases until a slight turbidity develops at the temperature of fractionation. The precipitated phase consist mainly of solvent, some nonsolvent and no more than 10-20% of the

original polymer if the precipitation conditions have been chosen correctly. Each polymer species is distributed between the two phases but the distribution is such that the precipitated phase contains a greater proportion of the high molecular weight compound. The temperature of the system is then raised until the two-phase system becomes one and the temperature is allowed to fall slowly to its original value. The precipitated phase is allowed to settle to a coherent layer, and the supernatant phase is removed. After decanting off the supernatant phase, the first fraction of polymer is recovered from the precipitated phase, weighed and its molecular weight measured by viscosimetric method. The supernatant liquid was treated with a further volume of nonsolvent, using the same procedure described above to obtain next fractionation. The last fraction was obtained by adding a large volume of nonsolvent to the remaining solution in order to precipitate all the polymer.

2.6.4.2. Elution Method.

Fractionation of a polymer by elution method involves preparing the polymer in an appropriate physical state and then extracting fractions of increasing molecular weight by use of a series of eluents of increasing solvent power. In contrast to fractional precipitation, the lowest molecular weight fraction is the first, and the highest is the last to be obtained. There are four main experimental arrangements that have been employed: direct extraction in which finely divided polymer itself is extracted, film extraction in which the polymer may be deposited on a support (e.g., sand) in a column, coacervate extraction in which a concentrated solution countering an appreciable quantity of polymer may be selectively extracted. Gradient elution is the most efficient of the molecular weight fractionation methods based on fractional solution. The coacervation method is quite similar to the conventional precipitation method.

2.6.5. High Performance (Pressure) Liquid Chromatography

Chromatography basically involves separation due to differences in the equilibrium distribution of sample components between two immiscible phases. One of these phases is a moving or mobile phase, and the other is a stationary phase.The velocity of migration is a function of equilibrium distribution. So, the components having distributions favoring the stationary phase migrates slower than those having distributions favoring the mobile phase. Separation then results from different velocities of migration as a consequence of differences in equilibrium distributions.

Chromatographic methods can be classified according to the type of mobile and stationary phases selected. Liquid chromatography (LC) refers to any chromatographic process in which the moving phase is a liquid, in contrast to the moving gas phase of gas chromatography (GC). The different stationary phases give rise to the names liquid-solid chromatography (LSC) and liquid-liquid chromatography (LLC). Liquid chromatography can also be classified according to the mechanism of retention: adsorption chromatography, partition chromatography, ion-exchange chromatography, and gel or exclusion chromatography, as shown in Figure 2.2.

Liquid-solid or adsorption chromatography (LSC) is the oldest of the four basic LC methods. Its principle is known from classical column and thin-layer chromatography A relatively polar material with a high specific surface area is used as the stationary phase, silica gel being the most popular but alumina and magnesium oxide are also often used. The mobile phase is relatively non-polar. The different rates at which the various types of molecules in the mixture are adsorbed on the stationary phase provide the separation effect. Polar compounds are eluted later than nonpolar compounds.

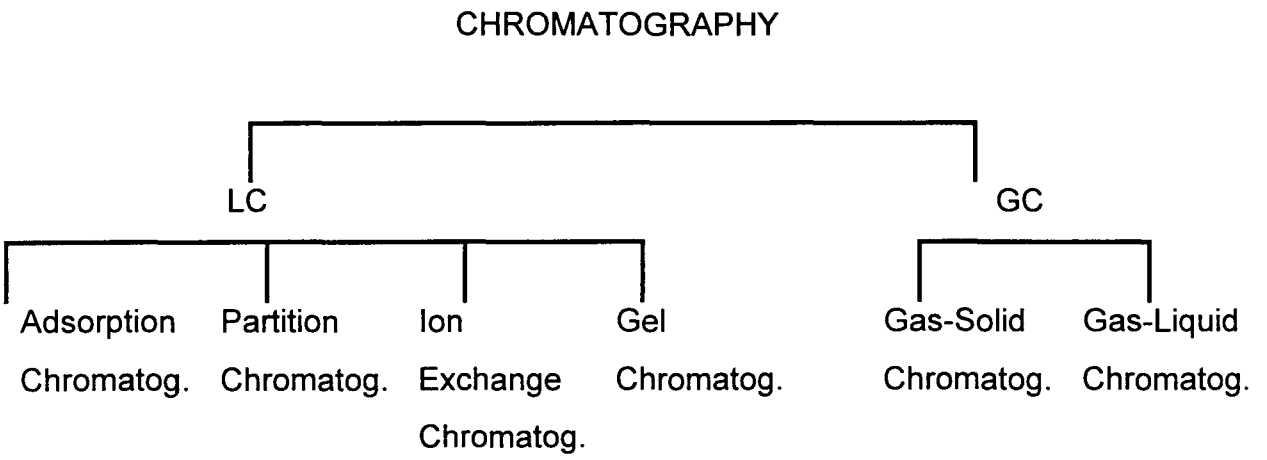


Figure 2.2. Classification of Chromatographic Methods

Liquid-liquid or partition chromatography involves a liquid stationary phase whose composition is different from that of moving liquid phase. Sample molecules distribute between the moving and stationary liquid phases, just as in liquid-liquid extraction within a separatory funnel. The moving and stationary phases can be chemically bonded to the particle or support.

In ion-exchange chromatography the column packing contains fixed ionic groups such as $-SO3^-$ along with counter ions of opposite charge (e.g., Na^+). The counter ions are also present in the moving phase in the form of a salt (e.g., NaCl). Ionic sample molecules of the same charge as counter ion (e.g., X^+) are retained by ion-exchange:

$$X^+ + -SO3^-Na \Leftrightarrow Na^+ + -SO3^-X^+$$

Finally, in gel or exclusion chromatography, the column packing is a porous material with pores of different sizes. Large molecules are excluded from all the pores, because they are too large to enter, while small molecules penetrates most

of the pores. Thus, large molecules are retained by the packing. Usually separation in gel chromatography is determined strictly by molecular size. he developments of chromatographic techniques, i.e. partition and paper chromatography (1940's), gas and thin layer chromatography (1950's), improved the speed and resolution of LC. However, there were still serious limitations compared to modern LC methods since analysis times were long, resolution was poor and quantitative analysis, preparative separations and automation were difficult.

In 1941, Martin and Syringe [36] who were the "inventors" of modern chromatography suggested that efficiency could be improved if the particle size of the stationary phase materials used in LC could be reduced. However, the development of modern high performance liquid chromatography (HPLC) had to wait until the mid-1960's when several workers who had advanced the development of gas chromatography turned their attention to the use of liquid chromatography. Using the lessons learnt in gas chromatography (GC), the technology required to produce high speed, high efficiency liquid columns was developed.

Therefore, high performance liquid chromatography (HPLC) is a technique that has arisen from the application to liquid chromatography (LC) of theories and instrumentation that were originally developed for gas chromatography (GC). The HPLC is also referred to as a high pressure liquid chromatography since a high pressure is essential for forcing the mobile phase through the column.

2.6.5.1. Gel Chromatograhy

Although gel chromatography is not a primary method for determining molecular weights, it has developed into one of the most useful methods for

routine determination of average molecular weights and molecular weight distributions of polymers.

Gel chromatography is the newest of the four LC methods, and it is also referred to as exclusion chromatography, gel filtration, or gel permeation chromatography (GPC). It is the easiest of the various modern LC methods to understand and to use. Despite this simplicity, the technique is very powerful. It has been applied to a broad variety of sample types to solve widely different problems.

Gel chromatography is a logical first choice for the following kinds of sample and the separation problems:

First, gel chromatography is uniquely useful for separating high-molecular-species (mol. wt. > 2000), particularly those that are non-ionic. Aside from the resolution of individual macromolecules such as proteins and nucleic acids, gel chromatography is often used to obtain the molecular weight distribution of a synthetic polymer.

Second, simple mixtures can be separated easily and conveniently by gel chromatography, particularly when the components of mixture are of widely differing molecular weights. In such cases, it is often possible to handle larger sample sizes than by other LC methods.

Third, gel chromatography is very suitable for the initial, exploratory separation of the unknown samples. Such a separation quickly provides us an overall picture of the total sample. It tells us whether we are dealing with a simple or complex mixture, and whether the sample components are of low, intermediate, or high molecular weight. Initial separations by gel chromatography often define which LC method or combination of the methods will be required for a given sample. Also, an initial gel separation is often an essential first step in the

resolution of a complex sample by the successive application of more than one LC technique.

The stationary phase used in gel chromatography are porous particles with a closely controlled pore size. Unlike other chromatographic modes, in gel chromatography there should be no interaction between solute and surface of stationary phase. Separation results through differences in the sizes of sample molecules. Those that are too large to enter any of the pores in the matrix are totally excluded. They therefore pass directly down through the column in the interstitial regions between the porous particles and are eluted first in the chromatogram. Molecules of intermediate size can enter some pores; such molecules consequently suffer retardations in their progress down the column. Molecules in the sample that are so small can enter all the gel pores and permeate the entire particle. These compounds are retained to the greatest degree, move through the column most slowly, and appear last in the chromatogram. The process is illustrated in Fig. 2.3.

In gel chromatography, the total volume of mobile phase in the column is the sum of the total volume external to the stationary phase particles (the void volume, V_O) and the volume within the pores of particles (the interstitial volume, V_i). Large molecules that are excluded from the pores must have a retention volume V_O, small molecules that can completely permeate the porous network will have a retention volume of $(V_O + V_i)$. Molecules of intermediate size that can enter some, but not all, of the pore space will have a retention volume between $V_O$and $(V_O + V_i)$. In Fig. 2.4. the relative molecular mass of the solute, M_r, is plotted on a log scale against the retention volume and the size range of solutes depend on the sort of material that is used for the stationary phase. Because, for a given separation, V_O and V_i are constant, we can reliably predict the total volume of solvent or the time taken for a particular analysis. The calibration curve is established by determining the retention volume for standards of known M_r.

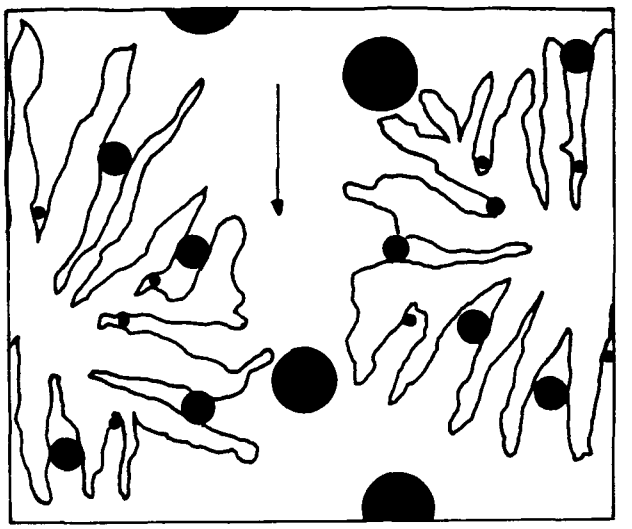


Figure 2.3. Separation by Exclusion

The retention volume V_R for a given solute is given by

$$V_R = V_o + K * V_i \qquad (2.11)$$

where K is a distribution coefficient which indicates the relative ease of penetration of the solute molecules into pore structure. When K=0 there is no penetration and when K=1 there is total unrestricted penetration. In principle, K values could be associated directly with molecular size but it is common practice to relate V_R to molecular weight empirically.

The essential requirements for a GPC chromatograph are as follows:

1. Solvent delivery system: capable of maintaining a constant linear velocity flow;

2. Column(s) containing suitable microporous gel particles to produce the necessary size separation;

3. Injection system: capable of delivering accurately small volumes of sample solutions without disturbing the solvent flow;

4. Detection system to monitor output from the columns and to provide continuous quantitative and possibly qualitative data on the fractions being eluted;

5. Recorder to give continuous output traces.

In addition, most modern GPC systems include an automatic data handling facility to convert output data into useful average values.

The retention times show a logarithmic relationship with molecular weight so it is essential that the flow rate remains constant. Typical flow rates are in the range 0.01 to 10.00 ml/min and commercial instruments are reproducible to about 0.3%.

The columns are packed with porous gels having a range of pore sizes. GPC columns are available with a range of pore sizes from 0.5 to 10^5 nm, enabling molecular weights from < 100 to approximately $4 * 10^{-7}$ to be discriminated. The columns are about 30 or 60 cm long and 7.5 mm in diameter, constructed of stainless steel.

The detector is required to monitor the concentration of the solute molecules in the solvent continuously and ideally to permit qualitative analysis of various constituents in the compound. The most common types of detector are differential refractometers, which may be used for all types of molecules, and ultraviolet/visible absorption instruments having fixed or variable wavelengths which find particular application for detecting additives which absorb in the ultraviolet or visible spectral regions. Infra-red absorption detection systems are also available. Fourier transform infra-red spectroscopy is particularly useful since it makes possible the determination of complete spectra in very short times.

Although GPC separates molecules according to their molecular size, the technique does not give absolute values of molecular weight and there is need to

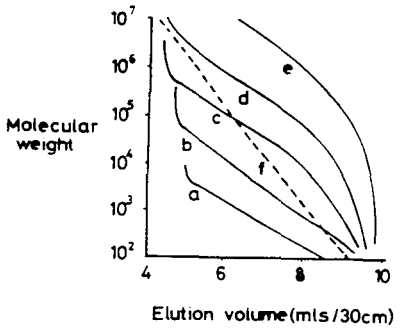


Figure 2.4. Calibration Curves For Different Pore Size Gels; a: 10 nm; b. 10^2; c. 10^3; d. 10^4 nm; e. 10^5 nm; and f. mixed pore size.

calibrate with polymer standards of known molecular weight. There is one of the major limitations of the technique since only a limited number of standards are available. Polystyrene standards are most commonly used. Monodisperse samples of the polymer having molecular weights in the range 500 to $15*10^6$ with Mw / Mn $\approx$ 1.05 are available from suppliers. Other polymer standards including poly (methylmethacrylate), poly(ethylene oxide), poly(ethylene glycol) and polyethylene may also be supplied for calibration purposes.

Various methods have been employed to construct GPC calibration curves. Most are based on direct calibration using polymer standards having known narrow (monodisperse) or broad (polydisperse) molecular weight distributions. the molecular weights of the standards are determined using absolute methods such as light scattering and osmotic pressure measurements, or by solution viscosity measurements.

For narrow molecular distribution standards the elution volume for each of the standards is determined and a calibration curve of log M versus V_e is constituted. Curve fitting procedures permit the determination of the molecular weight at any elution volume within the range covered. Fig. 2-4 shows the use of a mixed pore sized gel to give an essentially linear calibration curve such that

$$\log M = A - B * V_e \qquad (2.12)$$

However, the use of calibration curve based upon linear polystyrene standards is not likely to be strictly applicable to other polymer types and errors will undoubtedly arise in some cases. For instance, since GPC separates molecules according to their effective size, it may not adequately distinguish between branched and linear chain polymers of the same molecular weight. Polymer-solvent interactions also influence the effective size or hydrodynamic volume of polymer molecules, and since these interactions are also concentration dependent, they constitute possible sources of error.

The most widely used calibration method is termed the universal calibration procedure. This relies on the dependence of the effective molecular size or more correctly the hydrodynamic volume on the product of the intrinsic solution viscosity [η] and the molar volume of the solute M which is proportional to the molecular weight. A calibration curve produced for one polymer, usually polystyrene, can then be used to construct the calibration curves for other polymers.

A universal calibration curve of log ([η] M) versus V_e is constructed from solution viscosity and GPC measurements for standard monodisperse polystyrene samples. Typically the curve is linear over most of the range so that

$$\log ([\eta] M) = C - D * V_e \qquad (2.13)$$

where C and D are empirical constants. [36]

By making use of the Mark-Houwink equation (2.10)

$$[\eta] = K * M^{\alpha}$$

so that

$$[\eta] * M = K * M^{\alpha+1} \qquad (2.14)$$

where K and α are empirical constants, so that knowledge of α and K for the particular polymer and solvent allows the molecular weights to be readily determined.

For polydisperse samples, the GPC data may be used directly to determine the various molecular weight averages. This may be shown by the following. The GPC chromatogram is a record of the concentration of the solute at a particular elution time or volume. This data can be summed to give the integral or cumulative weight distribution curve which is conveniently normalized to give a total weight of unity. The differential of this curve gives the differential weight distribution W(M), which may be expressed by

$$W(M) = \frac{dW_1}{dM} = \frac{dW_1 * dV * d(\log M)}{dV * d(\log M) * dM} = \frac{dW_V * dV * 1}{dV * d(\log M) * M} \qquad (2.15)$$

where W_1 = weight fraction eluted up to volume V, i.e. with molecular weights < M

$$\frac{dW_V}{dV} = \text{height of the chromatogram} \qquad (2.16)$$

$$\frac{d(\log M)}{dV} = \text{gradient of calibration curve at volume V} \qquad (2.17)$$

The various molecular weight averages are then related to W(M) in the following manner:

$$M_n = \frac{1}{\int (1/M)\, W(M)\, dM} \qquad (2.18)$$

$$M_W = \int MW\,(M)\, dM \qquad (2.19)$$

$$M_Z = \frac{\int M^2\, W(M) dM}{\int W(M)\, dM} \qquad (2.20)$$

$$M_V = [\int M^2\, W(M) dM]^{1/2} \qquad (2.21)$$

Computer software is available to perform the necessary calculations on the chromatographic data to yield the various molecular weight averages. Commercial equipment is now supplied with the automatic data processing capability built-in.

The ability of GPC to produce molecular weight distribution curves directly and to enable calculation of average molecular weights, makes this an invaluable technique for polymer characterization. The ability to separate and identify low molecular weight fractions such as monomers, oligomers and additives such as

stabilizers etc. finds applications in a number of areas of polymer science and technology. This technique is finding increased uses for quality control purposes as well as detailed analysis in polymer syntheses and polymer processing.

2.7. Polyelectrolytes

Polyelectrolytes are defined as linear macromolecular chains bearing a large number of charged or chargeable groups when dissolved in suitable polar solvents.

Poly(acrylic acid) is a typical synthetic polyelectrolyte. Like propionic acid, its molecular weight analog, poly(acrylic acid) is soluble in several common basic organic solvents such as dioxane and dimethylformamide. Ionization of the carboxylic acid groups does not occur in these solvents; the resulting solutions exhibit physicochemical properties typical of those observed for solutions of neutral polymers in general. In sharp contrast, its aqueous solutions display unique and remarkable properties characteristics of solutions containing charged macroions.

Solutions of polyelectrolytes exhibit a behavior that may differ considerably from that of either uncharged macromolecules or low-molar-mass electrolytes. The origin of this specificity lies in the combination of properties derived from those of long-chain molecules with properties that result from charge interactions. This combination is not a simple superposition, as there is a mutual influence of the characteristics of both types of properties.

The dimensions of a polyelectrolyte in saltfree aqueous solutions are strongly dependent on the concentration of the polyelectrolyte. At very low concentrations, macroions appear to be highly extended and probably adopt rodlike configurations in the limit of infinite dilution. The addition of simple salt results in a pronounced

contraction of the polyion; in the presence of a large excess of simple electrolyte; the macroion is probably coiled in a manner which resembles the configuration of nonionic polymers in organic solvents. In short, viscometric evidence indicates that a realistic model of polyelectrolyte solutions must provide for changes in polyelectrolyte dimensions with at least three variables; polyelectrolyte concentration, concentration of added salt, and degree of neutralization.

Polyelectrolytes may be classified in different ways. As for neutral macromolecules, it is possible to distinguish between natural macromolecules, synthetic macromolecules and chemically modified biopolymers. Examples are DNA, poly(acrylic acid), and carbomethylcellulose, respectively. In analogous way, polyelectrolytes may be grouped into linear, branched and cross-linked chains. On the other hand, electrochemically, they can be classified as either polyanions (negative charges), polybases (positive charges) and polyampholytes (both positive and negative charge) depending upon the nature of its ionization in aqueous solution.

An essential criterion that must be met by biologically useful polyelectrolytes is the absence of lethal toxicity in both the polymer itself and its metabolites. Consequently, there must be an appreciable difference between toxic and therapeutic doses. Also, the polymer should be completely eliminated from the organism since retention may cause histocytic reactions, which can produce tumorlike growths that could be carcinogenic.

For example, water soluble polyelectrolytes have been successfully employed [40] clinically as plasma extenders. Their main role is to maintain osmotic pressure to increase or restore the effective circulating blood volume. Molecular weight is important here, since blood volume restoration must be retained for several hours. Therefore, a polymer of sufficient molecular weight (greater than 30,000) must be employed so as to delay clearance, but still be below the kidney threshold which is approximately 50,000. Consequently, the

molecular-weight distribution is more critical than the average molecular weight since very large molecules can also cause erythrocyte aggregation [40], and changes in morphology. Prolonged storage in vivo can be avoided by selecting polymers with appropriate molecular dimensions so as to facilitate their clearance of liver, kidneys, and other organs. another approach to this problem is to fabricate biodegradable polymers that can retain their biologically active structure long enough to form their task, and then are converted to harmless by-products that are readily eliminated.

2.7.1. Polyanions

Polyanions are polyelectrolytes with negative charges that generally reside on pendant groups attached to the backbone of the polymer chain. Both natural (e.g. heparin, heparinoids) and synthetic (e.g., poly(acrylic acid) and pyran) polyanions produce a variety biological activities. They enhance host resistance to bacteria and fungi, and enhance the immune response.

Polyanions that enter into biological functions through distribution throughout the host are similar to certain proteins, glycoproteins or polynucleotides that modulate a variety of biological responses related to host-defence reactions, including resistance to viral, bacterial and fungal infection.

2.7.2. Polycations

Polycations are a class of polyelectrolytes that derive their unique properties from the density and distribution of positive charges along a macromolecular backbone. Chain conformation and solubility depend on the extent of ionization

and interaction with water. Cationic functional groups can strongly interact with suspended, negatively charged particles or oil droplets and are useful for many applications including waste treatment and paper making.

Polycations have several biomedical applications which include antiviral, and antibacterial activities. These activities are primarily related to their ability to bind to surfaces with negative electrostatic charges. Cell surfaces have a large excess of negative charges due to the acid anion moieties on the cell membrane. Examples of polycations are poly (vinylamine), poly (ethylenimine), poly (4-vinylpyridine), poly(4-vinyl-N-alkylpyridinium halides).

2.7.3. Polyampholytes

Polyampholytes are polymeric systems containing both anionic and cationic charges. Such materials can be polymeric zwitterions with positive and negative charges on the same pendant group (betaines) or on the same backbone (ampholytes). Alternatively, they can be complexes of positively charged chains and negatively charged chains (interpolymer complexes). The betaines necessarily have an equal balance of anionic and cationic groups. The ampholytes can be charge-balanced or charge-imbalanced. The interpolymer complexes must be charge-imbalanced to maintain insolubility. However, all amphoteric water-soluble polymers have a high charge density to ensure water solubility.

Proteins and nucleic acids are the examples of polyampholytes. Proteins are polypeptides whose side may contain both ionizable acidic and basic groups. The net macromolecular charge on a protein is thus a function of the pH of its aqueous environment. Strong polyacidic behaviour is displayed by the nucleic acids in their native state owing to ionization of phosphate groups, but the amino groups of the heterocyclic bases can become positively charged at low pH. A few synthetic

polyampholytes have been prepared, for example, the copolymer of acrylic acid and vinylpyridine.

2.8. Polyelectrolyte Complexes (PEC)

Polyelectrolyte complexes which are generally called as inter-polymer complexes are formed when macromolecules of opposite charge are allowed to interact. The interaction usually involves a polymeric acid or its salt with a polymeric base or its salt. Thus the interaction between the ionizable groups of macromolecules [PA] and [PB] in aqueous solutions can be expressed very simply as:

$$PA^{+} + PB^{-} \Rightarrow [\,PA\text{-}PB\,]$$

Polyelectrolyte complexes may arise from electrostatic forces, hydrophobic interactions, hydrogen bonding, van der Waals forces or combinations of these interactions [41]. The formation of complexes may strongly effect the polymer solubility, rheology, conductivity and turbidity of polymer solutions. Similarly, the mechanical properties, permeability and conductivity of the polymeric systems are greatly affected by complexation.

The properties of polyelectrolyte complexes are always in some respect different from those of starting components, and this has been used in the studies of their formation. Techniques such as potentiometric-conductometric titrations, turbidimetry, viscometry, calorimetry, sedimentation, dynamic flow birefringence, light scattering, nuclear magnetic resonance spectroscopy, chromatography, and even electron microscopy have all been employed to follow the course of complex formation.

PEC can be considered as a special class of polymeric compounds. The methods of preparation of such compounds are based on direct mixing of the corresponding solutions of polycationic and polyanionic components. Then PEC are formed as a result of fast ion exchange reaction. If polyions with opposite charges interact in equimolar ratios the resulting compounds usually precipitate. Stoichiometric (1:1) PEC insoluble in water are used as materials for producing different articles operating in contact with aqueous media, such as, for example, semipermeable membranes, battery and biomedical materials. Examples are the systems polyvinylbenzyltrimethylammonium with poly(styrene sulfonate) or polyacrylate, quaternized poly(4-vinylpyridine) with polystyrene [42].

Kabanov and collaborators reported that under suitable conditions soluble non-stoichiometric complexes (NPEC) can be derived from strong or weak polyelectrolytes. In their study, they chose salts of different polymeric amines such as polydimethylaminethyl methacrylate (PDMAEMA), linear polyethyleneimine etc. and also the polymer quaternary ammonium salts such as alphatic ionens, quaternized poly-4-vinylpyridine (Q-PVP) as polycations and salts of polymeric carboxylic acids such as polyacrylic (PAA) and polymethacrylic carboxylic acids and polyphosphates (PP) as polyanions. It has been found that their solubility is not related to the pecularities of the chemical structure of the polyelectrolytes. The only requirement for their formation is that the degree of the polymerization, DP of polyelectrolyte being in NPEC in excess would not be lower than that of the deficient NPEC component [27, 45]. The polyelectrolyte incorporated in NPEC composition in excess is called a host polyelectroyte (HPE) and the second component being deficient in NPEC - guest polyelectrolyte (GPE). Both polyanions and polycations can serve as HPE and GPE. When DP(HPE) < DP(GPE), the result is an insoluble stoichiometric 1:1 complex (NPEC), an excess HPE remains in solution. For DP (HPE) > DP(GPE), the complex remains soluble (NPEC) as long as the initial ratio z= GPE/ HPE, of the two polyelectrolytes remains below the critical composition Φc of the NPEC complex. For $z>\Phi c$, SPEC

coexist with NPEC. Depending on the polymer structures and other factors Φc usually varies between 0.2 and 0.5 [27, 45].

2.8.1. Synthesis of Polymer-Metal Complexes (PMC)

The increasing interest of investigators in PMC is not only due to the crucial role of metal ions in biological processes but also to the unique capabilities of the PMC whose physical and chemical characteristics differ noticeably from those of the original components, polymer and metal (Me) [24]. For example, $CuSO_4$ is not soluble at pH 7 but its mixture with PE is soluble in neutral water systems. This gives an idea the formation of soluble polymer-metal complex.

The use of such models allows us to understand the mechanisms of action of many natural polymers and their behaviour in the presence of transient metal ions. PMC were used as a basis for the construction of a vast variety of biomedical preparation and drugs [25]. Transient metal ions (e.g., Cu, Zn, Fe) as well as other biphilic low molecular weight compounds (e.g., surfactant) possess the ability to bind to neutral or weakly charged water soluble polymers and they confer them adhesive properties and the capacity to form complexes with complementary surfaces. The use of this mediators promotes the polymer binding to the protein without causing any appreciable changes in the chemical structure of polymer and correspondingly its biological activity.

It is known that PMC formed from imidazole-containing PE and Me are convenient models of hemoproteins and hemochromes, etc. Polyanions form ion-coordinate complexes with proteins by crosslinks through metal ions. Recent studies demonstrated the important role of some metals (Cu, Zn, Fe) in the functional activity of immunocompetent cells Prohaska and Zukasewyez, 1981; Hart, 1982). Lithium ions considerably enhance the mitogenic effect of

lymphocytes on lipopolysaccharieds. Zinc salts injected to mice increase the immune response to sheep red blood cells (SRBC), stimulate the migration and proliferation of stem cells. $FeCl_3$ markedly increases the cooperation of T- and B-lymphocytes.

Some publications in the current literature deal with the description of original method for obtaining drugs on the basis of natural PE an Me [16]. These authors succeeded in demonstrating that polysaccharide-protein mixtures supplemented with Me are effective means of prophylaxis and treatment of some microbial infections in animal and man.

Water-soluble PMC were obtained by simple mixing of polymer with metal in neutral aqueous media. The composition and structure of PMC were controlled by adding variable concentrations of metal to aqueous solutions of polymer at a constant concentration of polymer. This approach promoted tight binding of all metal ions added to polymer and eventually formed stable PMC both soluble and insoluble in H_2O.

The chemical structure and composition of PMC depend on both metal concentration in solution and the chemical nature of PE. At relatively low concentrations of Cu^{2+} these cations are randomly distributed between the adsorbing polymers. With an increase in metal concentration the distribution changes considerably. The systems consist of two fractions: part of PE binds the maximal amount of metal whereas the other part is in free state. This complex formation occurs as a result of non-random distribution of Cu^{2+} within polymeric molecule. With a further increase in metal concentration the whole bulk of the free polymer is transformed into PMC as the same mechanism before. At relatively high concentrations of Cu^{2+}, the crosslinking of the macromolecule occurs so that the system loses its homogeneity. The structure of the PMC formed and its change resulting from the alteration of metal concentration are shown in Figure 2.6.

Mustafaev and collaborators [24] studied two types of polymers. PMC based on polyvinylimidazole (PVI) and copolymer (CP) of N-vinylimidazole (VI) with N-vinylpyrolidone (VPD). The mechanism of PMC formation in PVI-Cu^{2+} and CP (VI-VPD)-Cu^{2+} differ by a number of features. In the case of PVI-Cu^{2+} copper ions are unevenly distributed between the adsorbing polycations at very low $[Cu^{2+}]$ / [PE] ratios (<1). Such an uneven distribution may provide a satisfactory explanation for the existence of two fractions, i.e. free PE and PE fully loaded with Cu^{2+} with an increase in Cu^{2+} concentration in the system the free polymer is transformed into PMC which contains the whole bulk of exogeneously added Me and has a compact structure. The situation is quite different in the case of CP (VI-

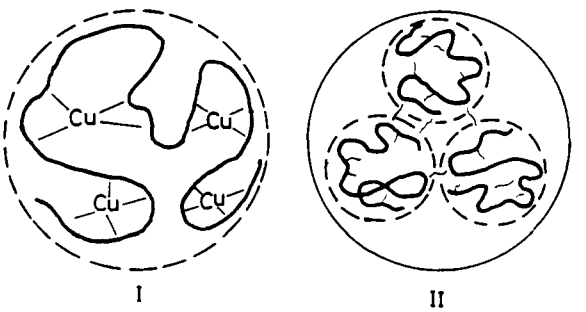


Figure 2.5. The Structure of Polymer (P)-Metal (Me) Complexes Relative to Metal Content, [Me]/[[P]<1 (I) and [Me]/[[P]≥1 (II)

VPD)-Cu^{2+}. In this system the complex formation occurs by a random distribution of copper ions between the adsorbing CP molecules. Such a random binding ensures the involvement of all CP molecules in the PMC. With a rise in the in Me concentration in the reaction medium the Cu^{2+} content in one macromolecule increases proportionally. As a result, polyion acquires different conformations. It changes from a stochastic coil at low values of metal concentration to a compact structure at high concentrations of Me.

It has been shown that in solutions of a high degree of dilution ($c_{PE} \approx 0.001$ g/dl) Me ions coordinate mostly intramolecularly both the adjacent and distant fragments of the polymeric chain (intramolecular crosslinks) which leads to the compactization of the PE coils (Figure 2.7.). With a rise in the initial concentrations of the polymer and Me an intermolecular crosslinking of PE also takes place. This process terminates in the agregation of macromolecules and the formation of a spatial network, in which the role of the crosslinking agent is also played by Me (Fig. 2.7., structure II).

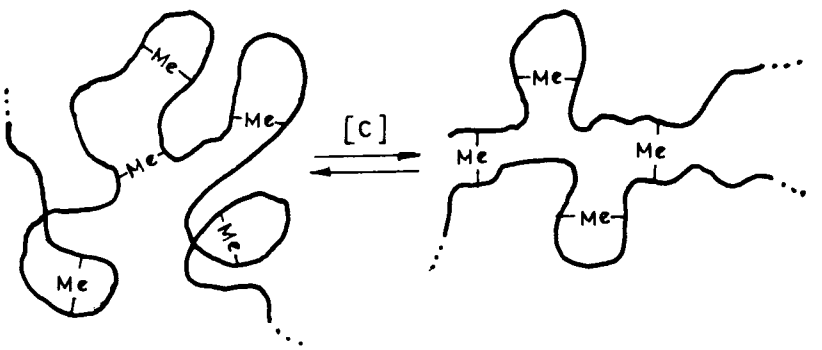


Figure 2.6. A Schematic Presentation of Complex Formation in PMC

2.8.2. Synthesis of Ternary Polymer-Metal-Protein Complexes

Soluble non-stoichiometric polyelectrolyte complexes are obtained not only as a result of a reaction between linear polyelectrolytes. They are also formed at an interaction of linear polyelectrolytes with globular protein such as polymeric quaternary ammonium salts and bovine serum albumin (BSA) [24]. Indeed, when pH of the solution exceeds the isoelectric point of the protein a globule acquires a negative charge due to disassociation of the carboxyl groups being at the periphery of globular species and able to form salt bonds with polycation chain.

Studies of mechanisms of protein cooperative binding by synthetic polyelectrolytes is of interest for immobilization of enzymes, stabilization and destabilization of their tertiary structure, specific sorption of proteins on surfaces and elucidation of the mechanism of polyelectrolyte physiological activity. In addition, such reactions may stimulate the processes of assembling of viruses, chromatine, ribosome strands and other cell components in complicated biological systems.

The formation of water soluble polymer-protein complexes of quaternized poly-4-vinylpyridine (Q-PVP) with Bovine Serum Albumin (BSA) was investigated by sedimentation analysis, turbidimetric titration, viscometry, and u.v. spectroscopy in neutral aqueous media according to the method developed by Mustafaev and Kabanov [27].

Mustafaev et al. also studied the complex formation of synthetic PE with proteins in the presence of transient metal ions (e.g., Cu^{2+}) [25] and showed that in the conditions when PE and protein systems bear the like (positive or negative) charge and are incapable of binding to each other in the absence of metal ions, they begin to play the role of " fasteners " between protein globules and PE chains by promoting the formation of ternary complexes that are stable under physiological conditions. Protein interactions with PE are effectuated through the formation of chelate complexes, in which Me play the role of the central atom.

The results of physico-chemical studies led to propose a hypothetical structural scheme of ternary PEC (Fig. 2.8.). In each molecule of PEC the protein globules interact with one another through copper ions crosslinked to a linear PE. Parts of Cu^{2+} ions form chelate complexes thus promote the aggregation of ternary complex molecules, whereas others form intramolecular crosslinks in the free sites of PE, thus stabilizing the overall structure. Fragments of PE that are not directly involved in the complex formation form free loops accessible to H_2O.

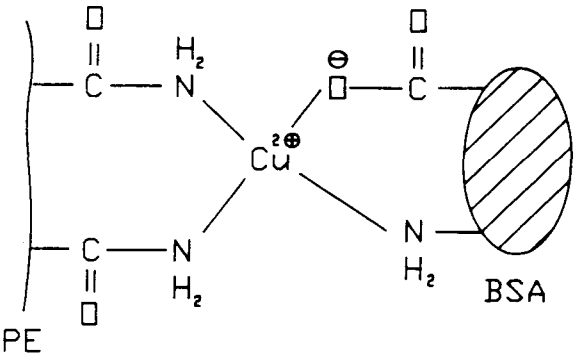


Figure 2.7. Schematic Illustration of the Hypothetical Structure of the Ternary Polyacrylamide-Cu^{2+}-BSA Complex

2.9. Radiation Chemistry

Radiation chemistry is the study of chemical effects of high energy, ionizing radiation. High energy radiation includes electromagnetic radiation (x-rays and γ-rays), particles (α-particles, β-particles or electrons, protons and neutrons) and fission fragments.

The first radiation chemistry effect studied was the blackening of photographic emulsions observed with X-rays by Roentgen in 1895 and with natural radioactivity by Becquerel in 1896. This was followed by the famous studies of Madame Curie, which included the discovery and isolation of polonium and radium. The chemical effects of radiation were investigated with increasing interest. Debernie studied water, Kailan organic compounds, Lind gases, Mund polymerization, while Fricke was interested in aqueous solutions. Because ions were observed in gases subjected to high-energy radiation, it was proposed that the chemical effects resulted from the reactions of these ions.

Since X-rays have more penetrating power than α-particles, they are preferred in the study of the kinetics of radiation-chemical processes taking place in liquid and solid systems. Radiation chemistry therefore made rapid advances with the advent of reliable X-ray apparatus. Fricke et al. [44] irradiated a large variety of aqueous solutions with X-rays, and suggested that both oxidation and reduction takes place during the irradiation. They also introduced a new idea of activated water, in which the solute molecules react indirectly with some third species, rather than directly with water. Since the advent of nuclear reactors, the ready availability of radio-isotopes has led to their use as radiation sources. ^{60}Co being very intense and relatively cheap source of radiation, is now largely used for gamma irradiation.

Radiation chemistry is now at an exciting stage of development. The discoveries that are being made will contribute to advances in many other fields, and more industrial processes utilizing radiation will become established.

2.9.1. Radiolysis of Water and Aqueous Solutions

Radiation-chemical reactions taking place in water and in aqueous solutions have been intensively studied because of their importance in chemical processes, biological systems, and the development of nuclear technology. As a result of all the work done, many of the aspects of the radiation chemistry of water and aqueous solutions are now reasonably well understood [45].

The principal experimental facts have already begun to be acquired by the early days of the twentieth century. Liquid water itself, when highly purified and irradiated under conditions where gas cannot escape from solution, resembles water vapour in that it does not decompose significantly under irradiation with low Linear Energy Transfer (LET) radiation such as X-rays. On the other hand water

decomposes into hydrogen, hydrogen peroxide, and oxygen on irradiation with high LET radiation like α-particles. Among the other facts, many of which were confirmed by the radiation chemists of the United States atomic bomb project [46], is that decomposition under low LET irradiation is very much enhanced by impurities. Oxygen is one such impurity: it causes water to give hydrogen peroxide and some hydrogen on irradiation with low LET radiation. Hydrogen peroxide itself enhances radiolysis by low LET radiation, but hydrogen tends to suppress radiolysis. Correspondingly if water is irradiated under conditions where hydrogen can escape, for example, in contact with a large evacuated space, or while boiling, then it undergoes decomposition.

The radiolysis of water has been explained in terms of ionization by Weiss [47]. He suggested that two unstable ions of opposite charge formed which dissociate to give H and OH radicals:

$$H_2O \Rightarrow H_2O^+ + e^-$$
$$H_2O^+ + H_2O \Rightarrow \bullet OH + H_3O^+$$
$$e^- + H_2O \Rightarrow H\bullet + OH^-$$

It was suggested that energy is lost heterogeneously to the water, and the clusters of ions produced were called "spurs". Recombination of the radicals occured in the spurs to form molecular hydrogen, and hydrogen peroxide.

$$H\bullet + H\bullet \Rightarrow H_2$$
$$\bullet OH + \bullet OH \Rightarrow H_2O_2$$

From a very large number of experiments, the action of radiation on aqueous solutions consists of reduction or oxidation. This could be understood if the radiation acts on the water to form H atoms (reducing) and OH radicals (oxidizing) [48]. Now if the effect of radiation on water is simply to produce H atoms and OH radicals which may then react with solute it might be expected that quite low

concentrations of suitable solutes and radicals would be sufficient to react with all atoms and radicals, so that H atoms would then not be able to react with each other to give molecular hydrogen, and OH radicals could not give hydrogen peroxide. Several aqueous solutions are found to give a certain yield of hydrogen on irradiation whatever the solute concentration. An approximately equivalent amount of hydrogen peroxide also appears to be formed. This can be understood if in addition to decomposition of water into H atoms and OH radicals the radiation also gives some molecular hydrogen and hydrogen peroxide as primary products [48].

The radiation chemistry of pure water may be represented by the general equation:

$$H_2O \Rightarrow e^-_{aq},\ H\bullet,\ \bullet OH,\ H_2O_2,\ H_3O^+_{aq},\ OH^-_{aq} \quad [49]$$

Once the primary species (right side of above equation) have been introduced into a solution by means of radiation, they will begin to react chemically. Molecular hydrogen, hydrogen peroxide, and hydrogen ions are relatively inert, but hydrated electrons, hydroxyl radicals and hydrogen atoms are highly reactive.

When dose-rate is low and solute concentration not too small, the concentration of hydrated electrons, hydroxyl radicals and hydrogen atoms will be kept to a low level, and there will be little reaction of these primary species with each other. At high dose-rates the concentration of primary species can be high, so that interactions between primary species become more important.

The radiation chemistry of water and dilute aqueous solutions thus reduces essentially to the chemistry of hydrated electrons, hydroxyl radicals and hydrogen atoms.

2.9.1.1. Hydrated electrons

Altough they were the last to be discovered, hyrated electrons are perhaps the best understood of the reactive primary species [50]. They can be produced without using radiation. One way is by photolysis of iodide, ferricyanide, phenols and so on, in aqueous solution. Another way is by reaction of hydrogen atoms with hydroxide ions:

$$H + OH^- \Rightarrow e^-_{aq} + H_2O$$

They also appear to be formed during the reaction of metallic sodium with water, and during photo-induced electron emission from metallic cathodes. Irradiation however is the most convenient way of producing them.

The existence of hydrated electrons was postulated by Stein and Platzman in 1950. Stein was studying the effect of CO_2 on the bleaching of methylene blue solutions, and he suggested that both hydrogen atoms and electrons might take part in this reaction [51]. Platzman [52] investigated in detail the hydration of secondary electrons and their chemical and physical properties. He also predicted that e^-_{aq} would absorb in the visible and near infrared regions to yield a blue solution. Hart and Boag [53] were the scientists who first measured and identified a transient absorption at 600 nm. In the following years, a large amount of data has been collected concerning its reactions. Some of the properties of the hydrated electron have been determined [50] by using pulse radiolysis.

Hydrated electrons behave in their chemical properties like the solvated electrons produced when alkali metals are dissolved in liquid ammonia. The hydrated electron may be pictured as being surrounded by four molecules. It is a highly reactive reducing agent and in liquid water its mobility is close to that of H^+ and OH^- ions. In 1962, Rabani and Stein [54] assumed that the half-life of the hydrated electron in pure water is shorter than 10^{-6} s. It is now generally accepted

that the hydrated electron reacts with water extremely slowly, to give H^+ and OH^-_{aq} ·

Hydrated electrons undergo electron attachment reactions with solutes if they have a vacant electron orbital. Their reactions with aqueous, radiolytic and transient species are diffusion controlled. In many cases, the primary products of e^-_{aq} reactions are unstable and these undergo disproportionation, disassociation or protonation reactions.

Nitrous oxide is one of the most efficient e^-_{aq} scavengers and it is specific for electrons. Reaction rate is fast ($k= 8.7 \times 10^9\ M^{-1}s^{-1}$) [48] and N_2 and • OH radicals are final products.

$$e^-_{aq} + N_2O \xrightarrow{H^+} N_2 + \bullet OH$$

2.9.1.2. Hydroxyl Radical

The hydroxyl (• OH) radical is a powerful oxidizing species. It has a redox potential of 2.8, [50] and will oxidize any reduced form above this potential.Since it absorbs only weakly in the 200-300 nm range, it is not easy to follow its disappearance in pulse radiolysis.

In general, • OH radicals (and also H• atoms) react more slowly than e^-_{aq}. Its reaction rate constants are usually in the range of 10^7-$10^9\ M^{-1}s^{-1}$, except for reactions with unsaturated organic compounds ($> 10^9\ M^{-1}s^{-1}$). Hydroxyl radicals undergo several types of reactions in which abstraction, addition, radical combination or electron transfer are involved.

2.9.1.3. Hydrogen Atoms

It may be considered that H• atoms are the acid forms of hyrated electrons. However, they have very little in common with e^-_{aq}. They are not as strongly reducing as the hydrated electrons, the types of reactions with H• atoms undergo being addition, abstraction, charge transfer and radical combination. In many respects their reactions are closer to those of hydroxyl radicals, altough they are distinctly less reactive. With organic compounds containing no double bonds for example, they often abstract hydrogen:

$$H + CH_3CH_2OH \Rightarrow CH_3\overset{\bullet}{C}HOH + H_2$$

2.9.1.4. Radical Scavengers

The use of scavengers to inhibit free-radical reactions is very common. Scavenging is the deliberate addition to a radical reaction system of a compound which reacts preferably with the reactive species present in the solution. Resulting species usually have longer energies and dissipate their energy by recombination, or as thermal energy. The purpose of using radical scavengers is to find out which radicals take part in the reaction by removing other primary species.

As already discussed above N_2O is a very efficient hydrated electron scavenger. Electrons can also be readily scavenged e.g. by carbon dioxide and sulphur hexafluoride. Sodium formate (or formic acid) is an efficient scavenger for hydroxyl radicals and hydrogen atoms. The formate ion reacts with them to give the reducing species CO_2^-

$$H + HCOO^- (HCOOH) \Rightarrow H_2 + CO_2^- \quad (k = 2.3 \times 10^8\ M^{-1}s^{-1}) \quad [49]$$

$$OH + HCOO^- (HCOOH) \Rightarrow H_2O + CO_2^- \ (k = 3.5 \times 10^9 \ M^{-1}s^{-1}) \quad [49]$$

Oxygen is known to be a very efficient scavenger of the hydrated electron and the hydrogen atom. The presence of oxygen drastically changes the character of the radiation chemistry of dilute solutions.

2.9.1.5. Fricke Dosimetry.

The Fricke dosimeter is the most widely used and most reliable chemical dosimeter that has been developed to date. It is based on the oxidation of ferrous ions to ferric on exposure of acidic aqueous solutions to ionising radiation. The oxidation of ferrous sulphate was first suggested by Fricke and Morse [55] and has been extensively studied since that time.

Radiation chemists, working with condensed systems (liquids or solids) almost universally relate the dose adsorbed to that measured in the Frickie dosimeter. The dose adsorbed is proportional to the electron density of the system. This dosimeter has been so universally accepted that dosimetry in the gas phase is often referred to it.

In order to determine the absorbed dose and the dose rate, a sample of the dosimeter solution is irradiated in a glass or plastic (e.g. polymethylmethacrylate) container, the irradiation exposure time being accurately measured. The containing vessel should be thick enough (at least 1mm) to ensure electronic equilibrium and large enough (internal dimensions greater than 8 mm) to avoid wall effects.

The determination of the ferric ion produced can be made by any suitable method but a convenient and rapid method is spectrophotometric analysis. In this

method, the optical density of the irradiated solution is measured at 304nm, using the unirradiated solution as a reference. The molar extinction coefficient of ferric ions may be taken as 2205 $M^{-1}s^{-1}$ at 25°C and it increases by 0.7% per degree centigrade between 20°C and 30°C. The molar extinction coefficient also depends on the concentration of sulphuric acid.

Scharf and Lee [56] have recommended to use 220 nm, since the molar extiction coefficient is greater viz. 4565 $M^{-1}s^{-1}$, than at 304nm enabling higher sensivity. They also reported that the temperature dependence (0.10%) and the acidity dependence are much smaller than at 304nm. Its performance is such that it has been accepted as a secondary standard dosimeter and a precision of 1-2% is readily obtainable on repeated samples. For air-saturated solutions the useful range of the dosimeter is 40-400 J/kg. In oxygen saturated solution, the range can be extended to $2x10^3$ J/kg if the concentration of ferrous ion is increased to 4 x 10^{-3} $mol.dm^{-3}$. The range can also be extended by dearating the solution or by adding cupric ion before irradiation.

The lower limit of the Fricke dosimeter is about 4 krad, but this may be extended by using longer spectrophometer cells. The upper limit for an air-saturated solution is 40 krad. The optimum is 15-20 krad. The range can be extended to 200 krad if solution is oxygenated and the ferrous ion concentration increased to 4 mM. The range can also be extended if the solution is dearated. Another modification to the Fricke dosimeter is the addition of cupric ion immediately before irradiation, which extends to the range 10 Mrad. When the sample is to be given a high radiation dose, the dose rate may be measured with the dosimeter, and the sample subsequently irradiated in the same position, the dose being proportional to the time of exposure.

Other chemical dosimeters have been proposed from time to time, but none have been accepted widely, although some have advantages in specific situations.

2.9.2. Radiolysis of Polymers

The effects of radiation on polymeric systems can be classified in those in aqueous solution and those in solid state. The scope of this section is mainly focused on radiation chemistry of polymeric solutions.

Altough the earliest report on the effects of radiation on polymer solutions dates back to 1941, when Khenokh [57] subjected gelatine solutions to gamma rays, this field of the radiation chemistry of polymers has received much less attention than irradiation of polymers in the solid state. The reason for this apparently lies in the fact that the study of the radiolysis of solid polymers has led to a number of results of great practical importance, and these have stimulated a considerable number of further studies. The pioneering work in the radiolysis of polymer solutions was undertaken with the aim of finding models for investigating the radiation effects in biological systems. Thus, gelatine [57] and starch solutions [59] were irradiated in order to gain experimental information on the effects of radiation on proteins and carbohydrates. A drop in the viscosities was noticed in both systems, together with a more pronounced denaturation of the polymers, leading to oxidized degradation products. A number of authors noticed that the viscosity of these solutions steadily decreased with radiation dose and that polymer molecule was denatured while free ammonia and phosphate ions were liberated.

Alexander and Charlesby [58] showed that water-soluble polymers either degrade or cross-link under irradiation, depending on their chemical structure and on the irradiation conditions. Solutions of polymers of the cross-linking type could be gelled when irradiated at a concentration above a critical value; below this concentration, cross-linked gels were never reached. More recently, a number of detailed studies have become available on the radiolysis of poly(methyl methacrylate) and polyisobutylene, which degrade at all concentrations, and of polystyrene, poly(vinyl chloride), poly(vinyl alcohol) and rubber, which cross-link

under certain conditions. Most investigators in this field have used the viscosity of the polymer solutions as a method for studying changes in molecular weights.

The most important effects of radiation on polymer solutions are crosslinking, which is analogous to dimerization, and degradation, which is analogous to main-chain scission. In most polymers one of these processes predominates. If crosslinking predominates, the ultimate effect of irradiation will be to produce a network polymer in which all molecules are joined to each other. If degradation predominates, then the molecules become smaller and smaller as the irradiation proceeds, and the material loses its polymeric properties. The main response for several polymers is shown in Table 2.2.

A number of theories have been advanced in order to account for the fact that some polymers cross-link whereas others degrade; none of these appears to be entirely satisfactory. A general empirical rule can be derived when examining the structure of polymers of group I and II. It can be seen that those vinyl polymers which have the following structure:

$$(- CH_2 - CRH-)n$$

all belong to group I, whereas polymers of structure:

$$(- CH_2 - CR_1R_2-)n$$

all belong to group II.

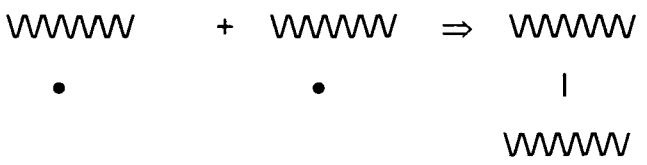
It follows that when the structure of a vinyl polymer is such that each carbon atom of the main chain carries at least one hydrogen atom, the polymer crosslinks, wheras if a tetrasubstituted carbon atom is present in the monomer unit, the polymer degrades. The formation of intermolecular cross-links is one of the most important chemical changes brought about by radiations in polymers of group I. This group comprises those vinyl polymers in which each carbon atom of

the main chain carries at least one hydrogen atom and also a number of condensation polymers such as silicones, polyamides, polyesters, etc. Since cross-linking of many polymers leads to beneficial changes in some of their properties, such as heat resistance, tensile strength, cold flow, etc., the study of radiation effects in polymers of this group has attracted a large number of research workers and the literature concerning cross-linking is much more abundant than that of degradation processes. The formation of cross-linked gels under irradiation of polymer solutions was first studied in the case of water-soluble polymers by Alexander and Charlesby [58]. These authors found that polymers belonging to the cross-linking type could also be cross-linked when irradiated in aqueous solutions above a critical concentration. Thus, when the concentration during irradiation exceeded 1 %, the formation of a gel occurred in polyvinylpyrrolidone, poly(vinyl alcohol), poly(acrylic acid) in its free acid form. In contrast, poly(acrylic acid) neutralized to 60%, poly(methacrylic acid either in the acid or in the neutralized form, and poly(styrene sulphonate) did not lead to any gel. Below 0.3% polymer, "degradation" was observed in all cases as judged from the observed drop in viscosity.

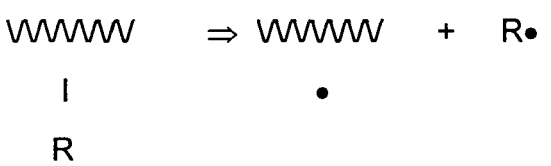
The mechanism of the radiation-induced cross-linking of polymers in solution was discussed by Alexander and Charlesby [58, 59] and Henglein [60]. The detailed mechanism of this process is not definitely established as yet. Several reaction steps have been suggested in order to account for the various observations. The inhibition of the cross-linking process of polystyrene in solution by free radical scavengers strongly suggests that the reaction proceeds via free radical intermediates. The most likely reaction leading to the formation of cross-links is the combination of two polymeric free radicals resulting from side-group abstraction from the polymer chain.

Table 2.2. Effect of Radiation on Polymers

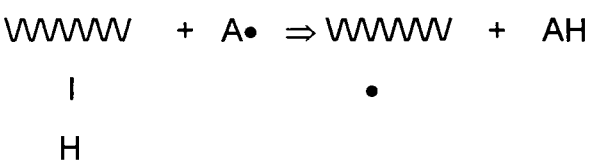
Predominant crosslinking	Predominant degradation
Polyethylene	Polyisobutylene
Polypropylene	
Poly(vinylchloride)	Poly(vinylidine chloride)
Chlorinated polyethylene	Polychlorotrifluoroethylene
Polyacrylonitrile	Polymethacrylonitrile
Poly(acrylic acid)	Poly(methacrylic acid)
Polyacrylates	Polymethacrylates
Polyacrylamide	
Polyvinylpyrrolidone	
Poly(vinyl alkyl ethers)	
Poly(vinyl methyl ketone)	
Polystyrene	Polyα-methylstyrene
Sulphonated polystyrene)	
Natural rubber	Cellulose plastics
Polysiloxanes	
Poyamides	
Poly(ethylene oxide)	
Polyesters	



The formation of the polymeric radicals involved in the above reaction can either result from the splitting off of side-groups from primary excited polymer molecules:

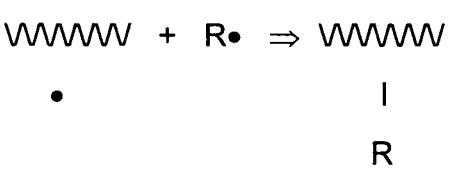


or from the attack of the polymer by "active" free radicals such as H•, Cl•, OH•, etc.

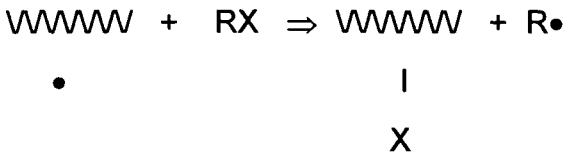


Here A• is an active free radical.

Once such a polymeric radical is formed, it may become involved in several competitive reaction steps. These include cross-linking via reaction and other processes in which polymeric radical is saturated without cross-link formation, such as combination with low-molecular-weight radicals:



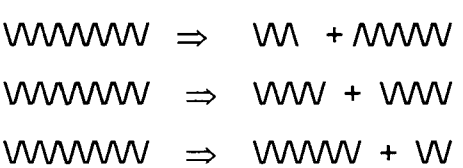
or transfer to solvent



where X is a labile atom.

The radiolysis of polymers in group II, i.e. of polymers which degrade under irradiation, has been investigated much less extensively than cross-linking polymers. The reason for this situation is undoubtedly related to the fact that the cross-linking process has a much greater practical importance than degradation. The most important representatives of degrading polymers which have been studied so far include polyisobutylene, poly(methyl methacrylate), perhalocarbon polymers (particularly Teflon) and cellulose.

Radiation degradation is a process in which polymer suffers random chain scissions. Thus, the molecular weight steadily decreases with radiation dose and in some cases the final product is a low-molecular-weight liquid. For most polymers, chain depolymerization does not occur at room temperature. It follows that radiation degradation usually proceeds through a single-step process in a manner similar to cross-linking and that yields of degradation are generally low. The reaction can be represented schematically:



2.9.3. Radiation Chemistry of Proteins

A considerable number of papers have been published on changes in the physicochemical properties of protein solutions following exposure to ionizing radiation. The importance of the subject derives from the need to understand the basic processes taking place in radiotherapy and also those processes responsible for the harmful effects associated with undesired exposure to nuclear and other radiations. This subject is also important in connection with the use of radiation to sterilize pharmaceutical products and to preserve food.

Indirect action plays a very important role in wet systems and anything which can influence it, as for example, temperature, is apt to be important in the results of irradiation. Unlike with dry or solid pure proteins, there is excellent opportunity for admixture with other substances, and therefore, for intermolecular reactions. Differentiation must be made among simple two-component systems (e.g. a pure protein dissolved in pure water) and more complex systems, such as a natural biological system or a multi-solute solution. The fate of a given substance in the two kinds of systems, simple and complex, might be greatly different upon irradiation.

In broad terms radiation may bring about several kinds of changes in a protein associated with water [61]. There may be rupture of hydrogen bonds with subsequent unfolding of the molecule. There may be aggregation or dissociation into smaller units or there may be fragmentation. In some of these changes, chemically-active groups may be made more available for reaction or they may be altered so as to disappear effectively. In all these changes oppurtunity for reaction with other substances which are part of the protein's environment may exist and there may be other compounds formed in this manner. These kinds of changes may result in the disappearance of the normal properties of the protein. Enzymes, for example, may undergo loss of activity. Chromoproteins may become changed

in their colour. Nucleoproteins may lose their function in biological processes with consequent damage to the host organism, including possibly death.

The following reactions have been proposed to account for aqueous protein solutions:

$$H_2O \Rightarrow H + OH$$

$$R + {}^{\bullet}OH \Rightarrow R_u + H_2O$$

$$R_u + {}^{\bullet}OH \Rightarrow R_u + H_2O$$

$$R_u + R_u \Rightarrow R_u - R_u$$

where R is the native protein molecule and R_u is the unfolded molecule. The increase in viscosity is probably associated with crosslinking to form $R_u - R_u$.

The analytical techniques, such as gas chromatography combined with mass spectrometry which are sufficiently sensitive to detect and identify very small quantities of radiolytic products, is of great value in determining the end products. Other techniques such as electron spin resonance (ESR) for measuring free radical content are also useful, as are usual chemical methods. Liquid phase chromatography (thin-layer, paper, gel, etc.) and standard techniques such as electrophoresis, ultracentrifugation and all types of spectrometry used regularly in protein analyses have also been contributed to the knowledge of radiolytic products in protein.

III. EXPERIMENTAL WORK

In the first sections of this chapter, the synthesis of polyacrylamide with and without functional end groups are described. Next, a summary on the determination of molecular weights of polymers by viscosity measurements, on quantitative determination of carboxyl end groups by conductometric titration method, and also on fractional precipitation of polymers are given. Finally, experimental studies on the synthesis of polymer-metal, polymer-metal-protein, and polymer-protein complexes and their irradiation conditions are presented.

3.1. Materials

L-Methionine, Nitric Acid (%65), $CuSO_4.5H_2O$, Acetone and Methanol were purchased from Merck. Acrylamide (AA) was supplied from Fluka Chemical Company. Bovine Serum Albumin (BSA, MW=70,000) used was a Sigma Chemical Company product and finally Polyacrylic Acid (PAA) was obtained from the Physical Chemistry Department of İstanbul Technical University. The molecular weight of the PAA fraction used in this study was 100,000.

3.2. Equipment

In this work, High Performance Liquid Chromatograph (HPLC), UV-Spectrophotometer, Fourier Transform Infrared (FT-IR), Atomic Absorption Spectrophotometer (AAS), Conductometer, and Viscometer were used. The specifications of the equipment mentioned above are as follows:

- **High Performance Liquid Chromatography (HPLC)**

In this work, HPLC was performed throughout using a Waters Associates Model 501 pump and a Waters Associates Model U6K Universal Liquid Chromatograph injector; a Lamda-Max Model 481 LC spectrophotometer; Waters 746 Data Module integrator and 300 mm x 7.8 mm I.D. stainless steel column packed with Protein Pak (20 μm particle size, Waters, Nortwich, U.K.). Figure 3.1. is a block diagram showing the way in which these different components are arranged in a high performance liquid chromatograph.

The mobile phase consisted of 1/15 M KH_2P0_4 and 1/15 M $Na_2HPO_4.2H_2O$, pH 7. Elution was isocratic at a flow-rate of 1 ml/min. The mobile phase and samples were filtered (0.45 μm filter pore size, Waters) and degassed by ultrasonic bath. A 25-μl volume of the samples was injected to chromatograph. All the samples were monitored at the wavelength of 280 nm where BSA has a maximum peak . The column was maintained at ambient room temperature.

- **Spectrophotometer**

Spectrophotometric analysis was carried out using a Shimadzu Model UV-160A spectrophometer.

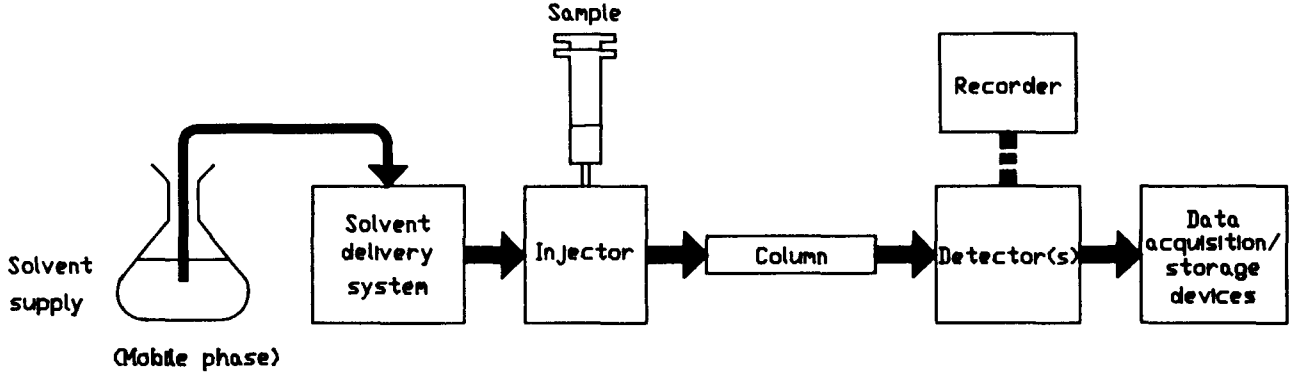


Figure 3.1. A Schematic Diagram of a HPLC Chromatograph

Fourier Transform Infrared (FT-IR) Spectroscopy

The IR spectra of the polymer samples were recorded using a Jasko 5300 Model FT-IR spectrophotometer.

Atomic Absorption Spectrophotometer (AAS)

The metal (copper) content of the collected fractions by HPLC was analyzed using a Perkin Elmer 403 AAS .

Conductometer

Hanna HI 8820 conductometer for the measurement of conductivity was used.

Viscometer

The instrument was a Ubbelohde type glass viscometer. Figure 3.2. gives a picture of Ubbelohde type viscometer.

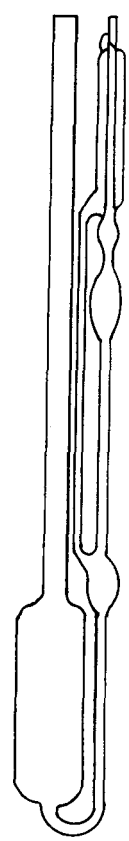


Figure 3.2. A Picture of Ubbelohde Type Viscometer

3.3. Experimental Procedure

3.3.1. Synthesis of Polymers

The synthesis of polyacrylamide with and without functional end groups are described below:

3.3.1.1. Synthesis of Polyacrylamide with Functional End Groups

Polymerization was carried out at 65 °C (water bath) in a two-necked round-bottom flask equipped with a stirrer. Methionine and monomer were dissolved in HNO_3. Then, the calculated amount of Ce (IV) salt solution was added dropwise with stirring in 5 min. After a chosen polymerization time of 1.5 hour, the reaction mixture was left to stand for 1 hour. The mixture was poured into an excess of acetone to precipitate the gross polymer. The polymer yield was determined by direct weighing of the polyacrylamide dried in air.

3.3.1.2. Synthesis of Polyacrylamide without Functional End Groups.

The synthesis of polyacrylamide without functional end group was achieved by reaction of 15 gr pure acrylamide dissolved in 150 ml of water under nitrogen for 70 min at 40 ±1 °C. Polymer was precipitated from aqueous solution twice by methanol addition and dried in vacuum at 35 °C.

3.3.2. Determination of Molecular Weight by Viscosity Measurement

The molecular weights of the polymer solutions were determined by using dilute solution viscosity method. The Ubbelohde type viscometer was mounted vertically in a water bath thermostated to 30°±0.02°C and left to equilibrate at that temperature. 20 ml of water was then drawn up the central capillary tube by suction using a hand aspirator until it was above the upper etched mark. The time for a constant volume of solvent to flow between the two etched marks was determined to high accuracy using a stopwatch. This value is indicated as t_o.

The viscometer was emptied, cleaned, dried and 20 ml of polymer solution containing approximately 0.5 gr polymer dissolved in 100 ml of water was drawn up the capillary without introducing air bubles. The flow time for the solution was determined in the same manner as for the water. The stock polymer solution was then progressively diluted by the addition of known amount of water to give at least 3 different concentrations, care being taken at each stage to mix thoroughly. This was achieved by repeated lowering and raising the solution up the capillary tube using the hand aspirator.

The flow times for each dilution were determined (t_c) and the molecular weights of polyacrylamide solutions (M_η) were calculated from the following equations [63, 30] :

$$\eta_r = t_c / t_o \tag{3.1}$$

$$\eta_{sp} = \eta_r - 1 \tag{3.2}$$

$$\eta_{sp} / c = [\eta] + b*c \tag{3.3}$$

$$[\eta] = 6.80 \times 10^{-4} M_\eta^{0.66} \tag{3.4}$$

where η_r, η_{sp}, and $[\eta]$ were previously defined in Section 2.6.1.

3.3.3. Quantitative Determination of Carboxyl End Groups

The procedure used for the quantitative determination of carboxyl groups by conductometric titrations consisted of dissolving polymer samples in 20 ml of distilled water and titrating with 0.01 N titrant. The conductometric titrations were carried out in a glass cell kept at constant temperature of 25°C. For the titration experiment, the cell was filled with 20 ml distilled water in with 20 ml distilled water in which a known amount of solid polymer was dispersed by magnetic stirring. The polymer completely dissolved within a few hours. The polymer solution was then titrated with 0.1 N NaOH added from a microburet.

In this work, the conductometric titration method was used for quantitative determination of the carboxyl end groups [32, 33].

$$\text{Equivalent of Carboxyl end groups / } 10^6 \text{ g} = \frac{V * n * 10^3}{m} \qquad (3.5)$$

where V, N, are the volume, and normality of titrant and m is the weight of polymer respectively.

3.3.4. Fractional Precipitation

The procedure of fractional precipitation of polyacrylamide with non-solvent (methanol) addition method has been published in detail by B. Baysal [63]. The polymer sample was dissolved in a distilled water to produce a 0.5 M solution and put in a 500 ml. three necked round bottom flask. The flask was placed in a constant temperature water bath regulated at 30°C. The solution was stirred

vigorously with a stirrer. The apparatus is shown in Figure 3.3. An appropriate amount of methanol was added slowly to the solution using a separatory funnel. After a certain amount of methanol has been added, the addition of one or more drop of methanol caused a turbidity which was not easily removed by agitation. The solution was then kept overnight to settle the precipitate. The precipitated phase was separated from the supernatant phase by siphoning of the latter using a pipette. The precipitated phase which contained the fraction consisting of the higher molecular weight species, was collected by dissolving it with a small amount of solvent. Then the fraction was transferred into a 100 ml beaker where it was dried at ambient temperature to constant weight. Once the weight of the fraction was determined its viscosity was measured as explained in Section 3.3.2.

The supernatant liquid was treated with a further volume of methanol, using the same procedure described above, to obtain the next fraction. Since the aim of the present work is to obtain the fraction consisting of 100,000 MW range the experiments were carried out until this fraction was collected.

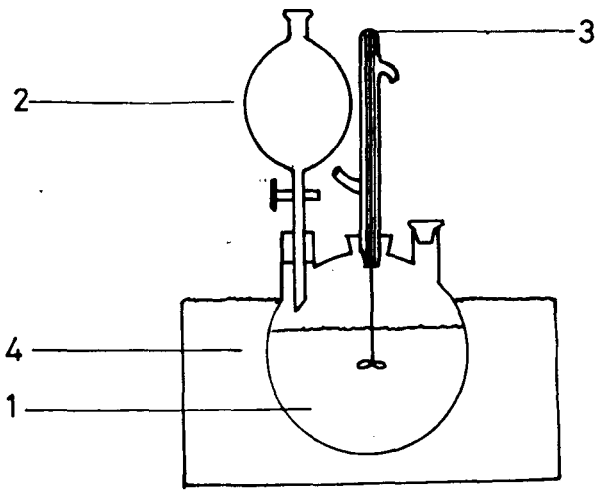


Figure 3.3. Fractional Precipitation Apparatus: 1. Tree Necked Round Bottom Flask, 2. Separatory Flask, 3. Stirrer, 4. Water Bath

3.3.5. Polymer-Metal Complexes

To produce a polymer-metal complex, 0.05 g polymer was first dissolved in 100 ml distilled water. Its pH was adjusted to 7 by adding a few drops of 1 N NaOH. Then water soluble polymer-metal complexes were obtained by simple mixing of polymer solution with %1 $CuSO_4.5H_2O$ solution and this was carried out by adding variable concentrations of metal to aqueous solutions of polymer at a constant concentration of polymer until the system lost its homogeneity, in other words, turbidity developed. In each metal addition, the pH of the mixture was adjusted to 7 by using the same procedure described above. Finally, the complex formation and the effect of irradiation on these complexes were investigated using HPLC and spectrophotometric methods.

3.3.6. Polymer-Metal-Protein Complexes

For the synthesis of ternary complexes of polymer-metal-protein, protein (Bovine Serum Albumin, BSA, MW=70,000) was dissolved in distilled water at pH 7 and its variable concentrations were added to the polymer-metal complex described above at constant concentrations of polymer and metal. The pH was then raised to 7. HPLC and spectrophotometric methods were employed to investigate the formation of the water soluble triple complexes of polymer with BSA in the presence of divalent copper ions (Cu^{2+}) and to analyze the effect of irradiation on these complexes.

3.3.7. Polymer-Protein Complexes

As mentioned in Section 2.8.2, transient metal ions play the role of fasteners between protein globules and PE chains by promoting the formation of water

soluble ternary complexes. To elucidate the role of the metal ions, various concentrations of protein solutions were added to the polymer dissolved in distilled water and Its pH was adjusted to 7 by adding a few drops of 1 N NaOH. The resultant differences between these polymer-protein complexes and polymer-metal-protein complexes, and also the effect of irradiation on these complexes were investigated using HPLC and spectrophometry.

3.3.8. Irradiation Conditions

. The samples were irradiated in wet condition using a Co^{60} source with a total dose of 1.2 kGy. The source was calibrated using a Fricke's dosimeter. The Fricke solution was prepared using the following procedure:

0.28 g of ferrous sulphate ($FeSO_47H_20$), 0.06 g of sodium chloride (NaCl), and 22 ml concentrated (95-98 %) sulphuric acid (H_2SO_4) were dissolved in enough distilled water to make 1 litre of solution.

In order to determine the adsorbed dose and the dose rate, a sample of the dosimeter solution is irradiated in a glass container, the irradiation exposure time being accurately measured was 4 hours. The determination of the ferric ion produced was made by spectrophotometric analysis which is a convenient and rapid method. In this method, the optical density of the irradiated solution was measured at 304nm, using the unirradiated solution as a reference. The dose rate was calculated from the following equation [45] :

$$\text{Dose Rate (rad)} = 28.516 \times 10^3 \times \text{OD} \qquad (3.6)$$

where OD is the optical density of the irradiated solution was measured at 304nm.

In this work, the dose rate was 1460.3 rad/hour where OD was 0.205. Since the samples were irradiated for 82 hours, the total irradiation dose was 120,000 rad (1.2 kGy).

IV. RESULTS and DISCUSSION

In the first sections of this chapter, the experimental results on the synthesis of polyacrylamide with and without functional end groups are presented. The dependence of molecular weight and yield on the reaction conditions are summarized. Next, the results on fractional precipitation and the determination of carboxyl end groups by conductometric titration and infrared measurements are given. Finally, based on the HPLC results, the proposed mechanisms of polymer-metal and polymer-metal-protein complexes, and gamma radiolysis of these complexes are discussed.

4.1. Synthesis of Polyacrylamide Containing Methionine End Group

Polymerization of acrylamide (AAm) was carried out with Cerium Ammonium Nitrate (CAN)-Methionine redox initiator system. The effect of Cerium (IV), polymerization time, and temperature on the molecular weight and polymerization yield were studied. The results are summarized in Table 4-1. The molecular weights of the polymers were determined by viscosimetric measurements. The polymer yield was determined by direct weighing of the polyacrylamide produced.

Table 4.1. Effect of Reaction Conditions (Ce(IV), Polymerization Time, and Temperature) on Molecular Weight and Yield

[Methionin], M	[AAm],M	[CAN],M	Polymerization Temperature (°C)	Polymerization Time (hour)	Molecular Weight	Yield (%)
0.0335	0.8	$1.25*10^{-3}$	60	2.00	141360	68
		$5*10^{-3}$			60944	75
		$10*10^{-3}$			41607	81
		$20*10^{-3}$			30155	88
0.0335	0.8	$1.25*10^{-3}$	60	0.50	132169	41
				1.00	53465	53
				2.00	141360	68
0.0335	0.8	$1.25*10^{-3}$	60	2.00	141360	68
			65		169727	74
			70		186785	87

4.1.1. Effect of Cerium (IV)

Polymerization was carried out with different concentrations of Ce (IV). Figure 4.1. and 4.2. shows the effect of Ce (IV) on molecular weight and polymerization yield, respectively. The results clearly show that as the concentration of Ce(IV) increases, the molecular weight decreases whereas the yield increases.

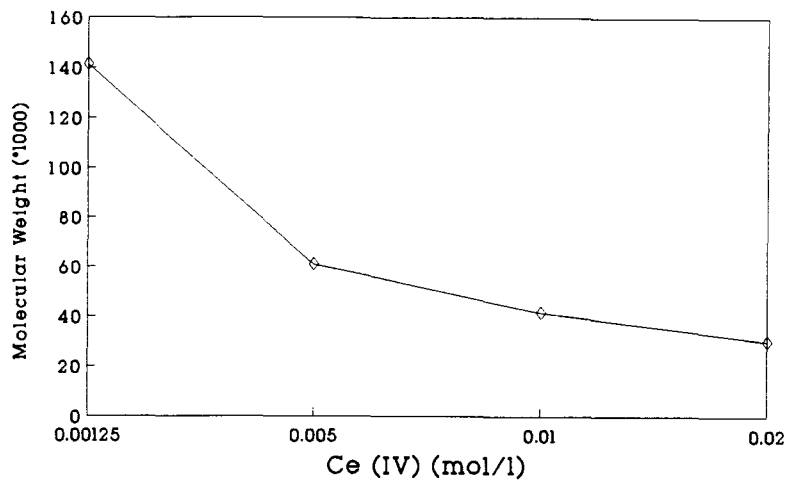


Figure 4.1. Effect of Ce (IV) Concentration on Molecular Weight (MW) of Polyacrylamide with Methionine End Group

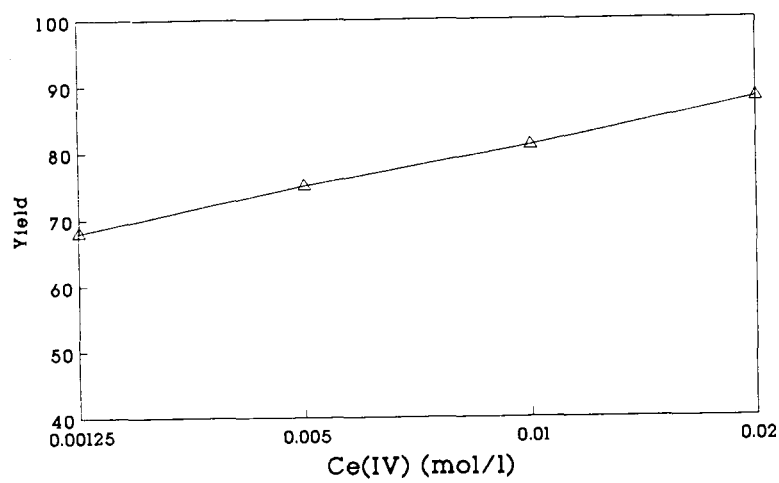


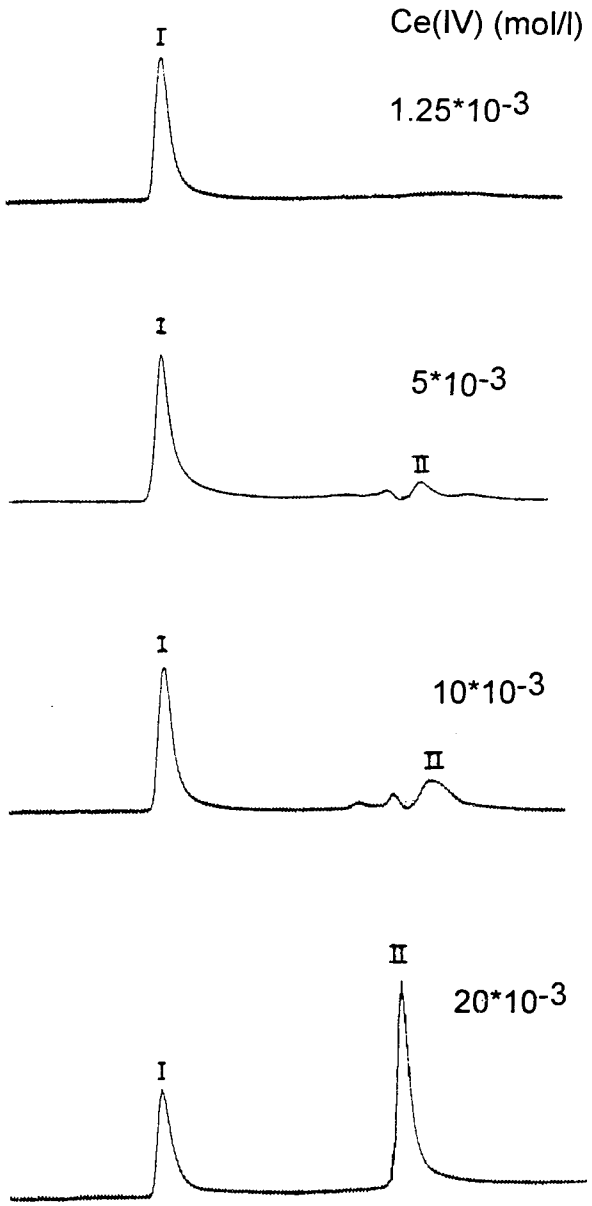
Figure 4.2. Effect of Ce (IV) Concentration on Yield of Polyacrylamide with Methionine End Group

Similar results on the radical polymerization of acrylamide initiated by ceric sulfate in the presence of amino acids were previously reported by C. Erbil et al. [64]. This probably indicates that at low catalyst [Ce(IV)] concentration, termination is due to the usual bimolecular collision between two chains. This finding eliminates the linear termination by Ce(IV) and emphasizes the mutual termination as a major process.

In fact, HPLC results support this assumption. As can be seen in Figure 4-3, a bimodal molecular distribution is observed at high Ce(IV) concentrations. According to size exclusion theory, the peak eluted first in the chromatogram (Peak 1) corresponds to the molecules of large size and similarly, the peak eluted last corresponds to those of small size (peak 2). As the concentration of Ce (IV) decreases the area of the peak 2 diminishes and ultimately, the peak 2 disappears and only peak 1 corresponding to higher molecular weight is seen in the chromatogram. These results play a significant role in understanding the polymerization mechanism of these polymers since the polyacrylamides initiated by Ce(IV)-reducing agent redox initiator system have not previously been investigated using by the method of HPLC.

4.1.2. Effect of Polymerization Time

The effect of polymerization time on molecular weight and yield was also examined at three different reaction times. The results are illustrated in Figure 4.4 and 4.5. The molecular weight intially shows a sharp decrease at low reaction times, attains a minimum at 1 hour and then rises at higher reaction times. The yield increases proportionally with reaction times. A series of HPLC chromatograms of the polymers obtained at different polymerization times is shown in Figure 4.6.



ID	RT	Area	Area %
I	4.40	178624	100
I	4.43	187965	80.20
II	11.56	46350	19.80
I	4.45	188548	77.85
II	11.56	53620	22.15
I	4.45	143892	39.73
II	10.57	218319	60.27

Figure 4.3. A Series of Original HPLC Chromatograms of PAM-Methionine End Group, Synthesized at Four Different Ce(IV) Concentration (mol/l); Temperature = 60°C, Reaction Time =2 hour

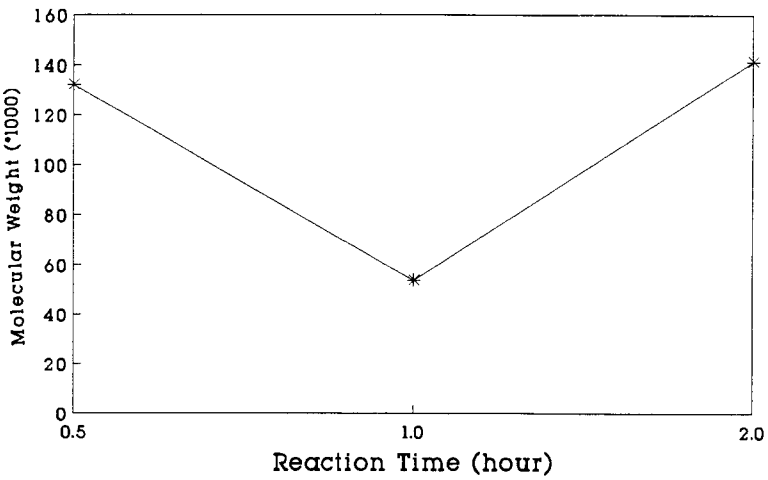


Figure 4.4. Effect of Reaction Time on Molecular Weight (MW) of Polyacrylamide with Methionine End Group

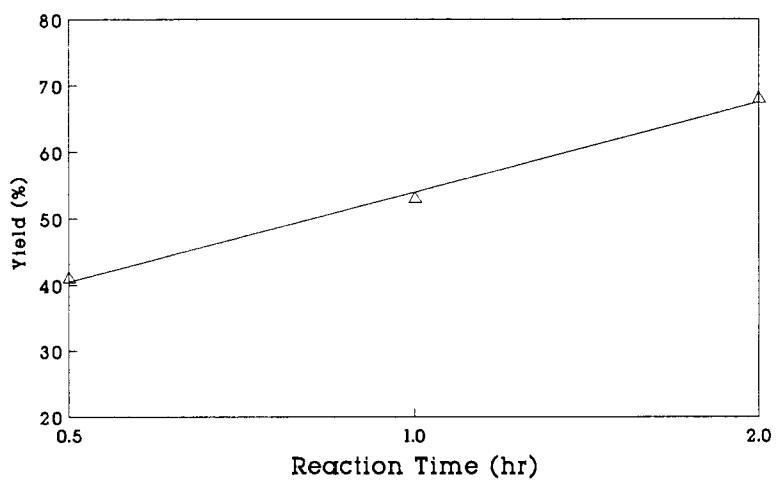
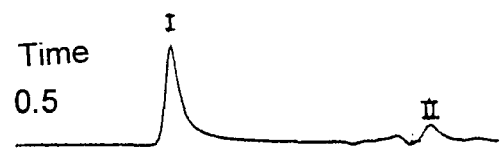


Figure 4.5. Effect of Reaction Time on Yield of Polyacrylamide with Methionine End Group



ID	RT	Area	Area %
I	4.46	152462	98.60
II	11.81	2183	1.40

I	4.49	70367	100

I	4.45	129006	100

Figure 4.6. A Series of Original HPLC Chromatograms of PAM-Methionine End Group, Synthesized at Three Different Reaction Times (Hour): Ce(IV)=$20*10^{-3}$; Temperature = 60°C

4.1.3. Effect of Temperature

By considering the effect of temperature on the molecular weight and yield, the polymerization was carried out at three different temperatures. The results are shown in Figure 4.7. and 4.8. The molecular weight and yield are found to increase in proportion with temperature.

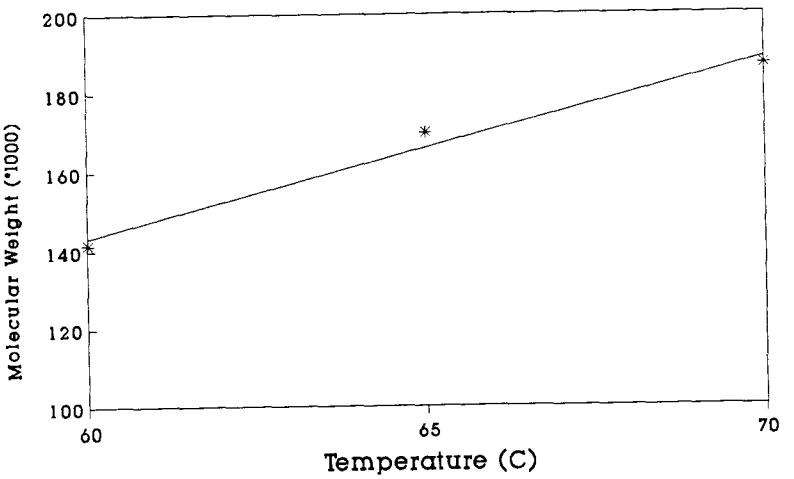


Figure 4.7. Effect of Temperature on Molecular Weight (MW) of Polyacrylamide with Methionine End Group

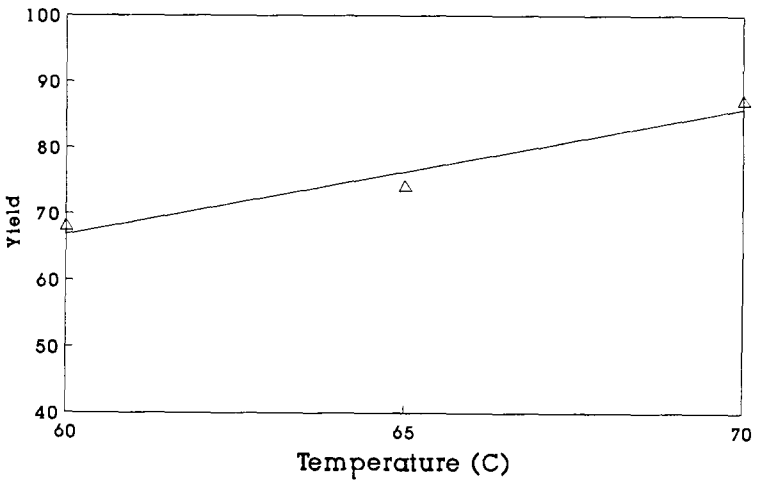


Figure 4.8. Effect of Temperature on Yield of Polyacrylamide with Methionine End Group

A series of HPLC chromatograms of the polymers synthesized at various temperatures is illustrated in Figure 4.9. As it follows from this figure, with increase in temperature, the area of peak 1 corresponding to the molecules of large size decreases whereas that of peak 2 corresponding to the molecules of small size increases.

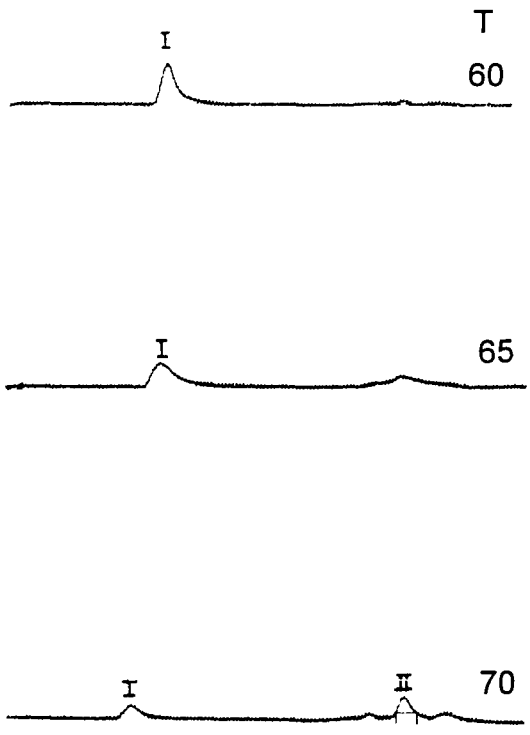
4.1.4. Determination of Carboxyl End Groups by Conductivity

Carboxyl end groups of the polyacrylamide with methionine end groups were determined by conductometric titration in water at 25 °C in order to confirm that the polymer showing only one peak in the chromatogram contains methionin end group.

The conductometric titration curve for the polymer are illustrated in Figure 4.10. The equivalence point corresponds to 2 ml. The number of gram equivalents of end groups per gram of polymer is therefore $46.3*10^{-6}$ -COOH. In this work, on the hypothesis that salt formation was responsible for the second breaks, the -COOH ends were titrated by addition of sodium hydroxide solution. After completing this reaction, further addition of base suppressed ionization of the $-NH_3^+$ ion, and this reaction was completed at 5 ml.

4.1.5. FT-IR Results

From FT-IR spectrum of the polyacrylamide with methionine end group showing only one peak in the chromatogram (Figure 4.9.), the appearance of characteristic peak attributed to sulfur group was evident at 646 cm^{-1} (Figure 4.11.). The other absorption bands corresponding to methionine are listed in Table 4.2.



ID	RT	Area	Area %
I	4.45	15317	100

I	4.41	25528	100

I	4.39	129006	91
II	11.81	12700	9

Figure 4.9. A Series of HPLC Chromatograms of PAM-Methionine End Group, Synthesized at Three Different Temperatures (°C); Ce(IV)=$1.25*10^{-3}$ mol/l, Reaction Time= 2 hour

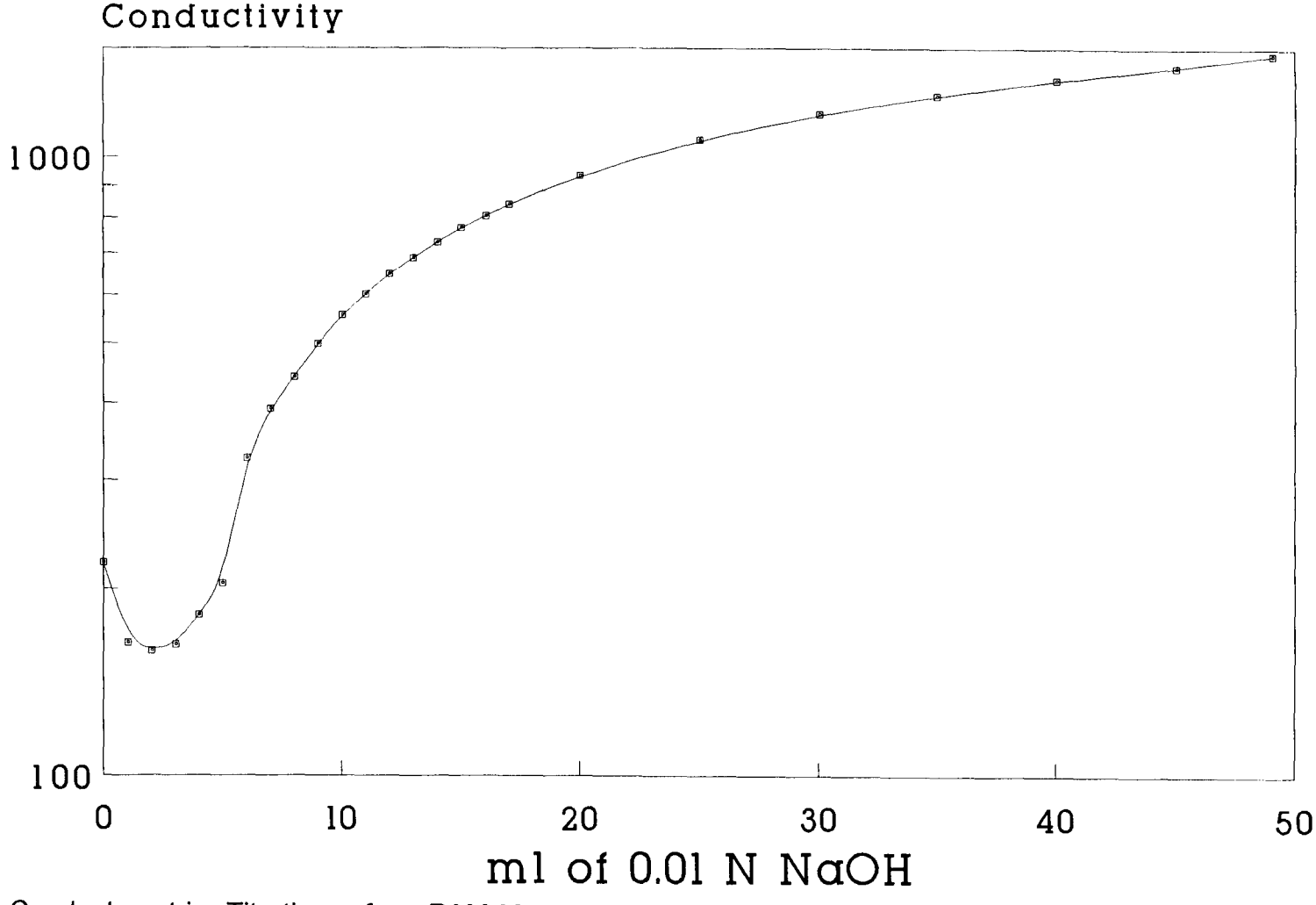


Figure 4.10. Conductometric Titration of a PAM-Methionine End Group Having a Narrow Molecular Weight Distribution in Water at 25 °C

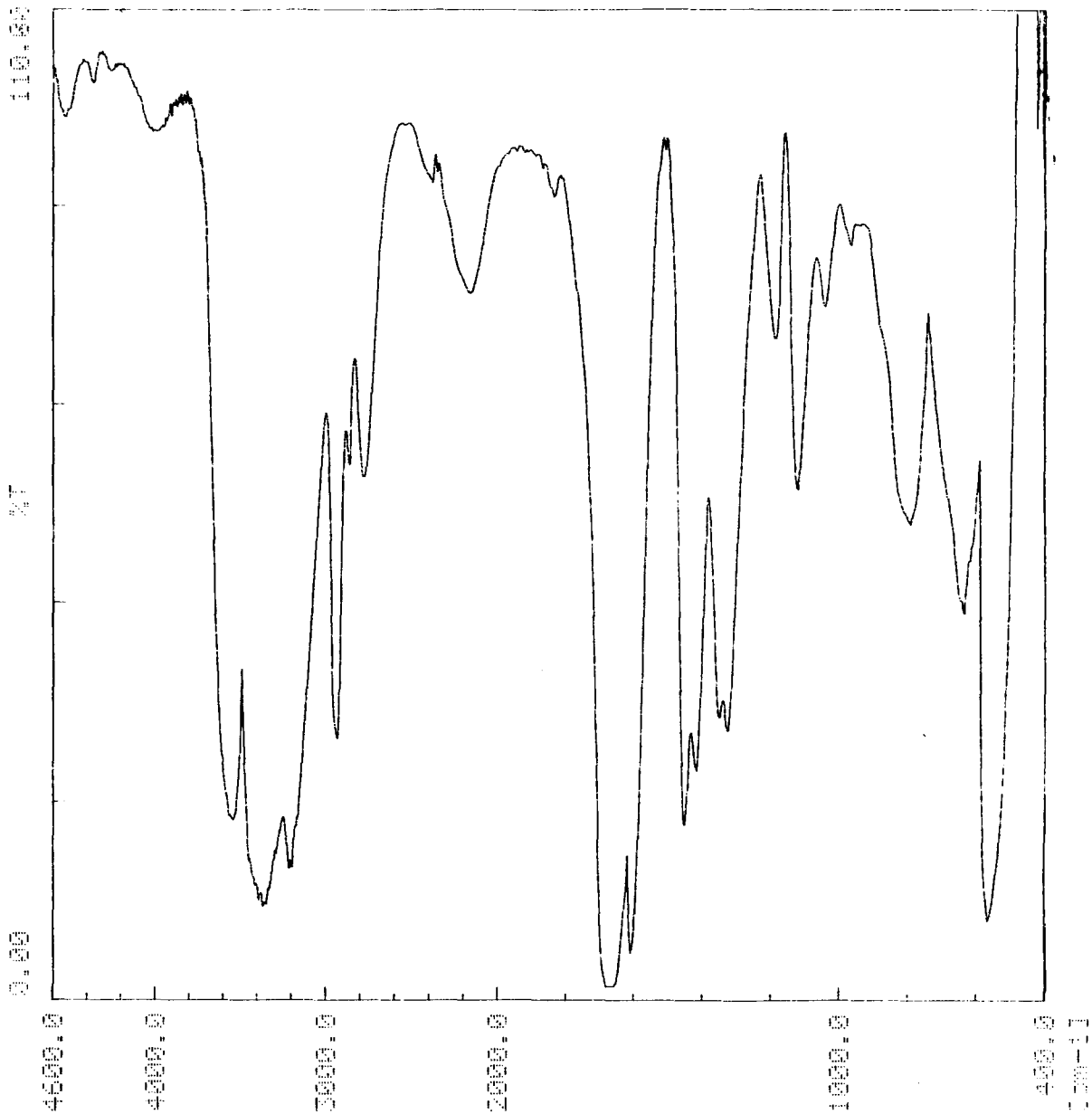


Figure 4.11. FT-IR Spectrum of a PAM-Methionine End Group Having a Narrow Molecular Weight Distribution

Table 4.2. IR Spectra of Polyacrylamide with Methionine End Group

Type of Absorption Bands	Methionine
O-H Streching	2942
Carboxylic Acid	3193 3363
N-H Bending	1613
Primary Amide and Amine	1664
C-N Bending	1418
Primary Amides	1452
C-N	1124
Alifatic Amine	1187
C-O Streching	1354
Sulfur Groups $-(CH_2-S-CH_2)-$	646

Figure 4.12. shows a comparison of FT-IR spectrum of polyacrylamide containing methionine end group with that of polyacrylamide containing no end group. From this figure, it is clearly seen that the polyacrylamide containing no end group does not show the characteristic bands of methionine at 646 cm^{-1} (sulfur group); 3193, 3363, and 2942 cm^{-1} (carboxylic acid, and OH streching groups).

4.2. Polymerization of Acrylamide Without End Groups

In the present work, the synthesis of polyacrylamide containing no functional end group was achieved by exposing aqueous solutions of acrylamide monomer to heat under nitrogen atmosphere.

4.2.1. Fractional Precipitation

Figure 4.13. shows the HPLC chromatograms of polyacrylamide containing no end group. From this figure it is seen that the molecular weight distribution of the polymer is very broad.

To obtain a polymer having a narrow molecular weight distribution (monodisperse), the fractional precipitation of polyacrylamide with nonsolvent (methanol) was carried out. Obtaining a fraction showing only one polymer peak in the chromatogram is an essential prerequisite of studying the complex formation of polymers. Furthermore, to collect the fraction consisting of 50,000-150,000 molecular weight range gives an excellent opportunity to compare the complex formation of polyacrylamide containing methionine end groups to that of polyacrylamide containing no end groups. For these reasons, the

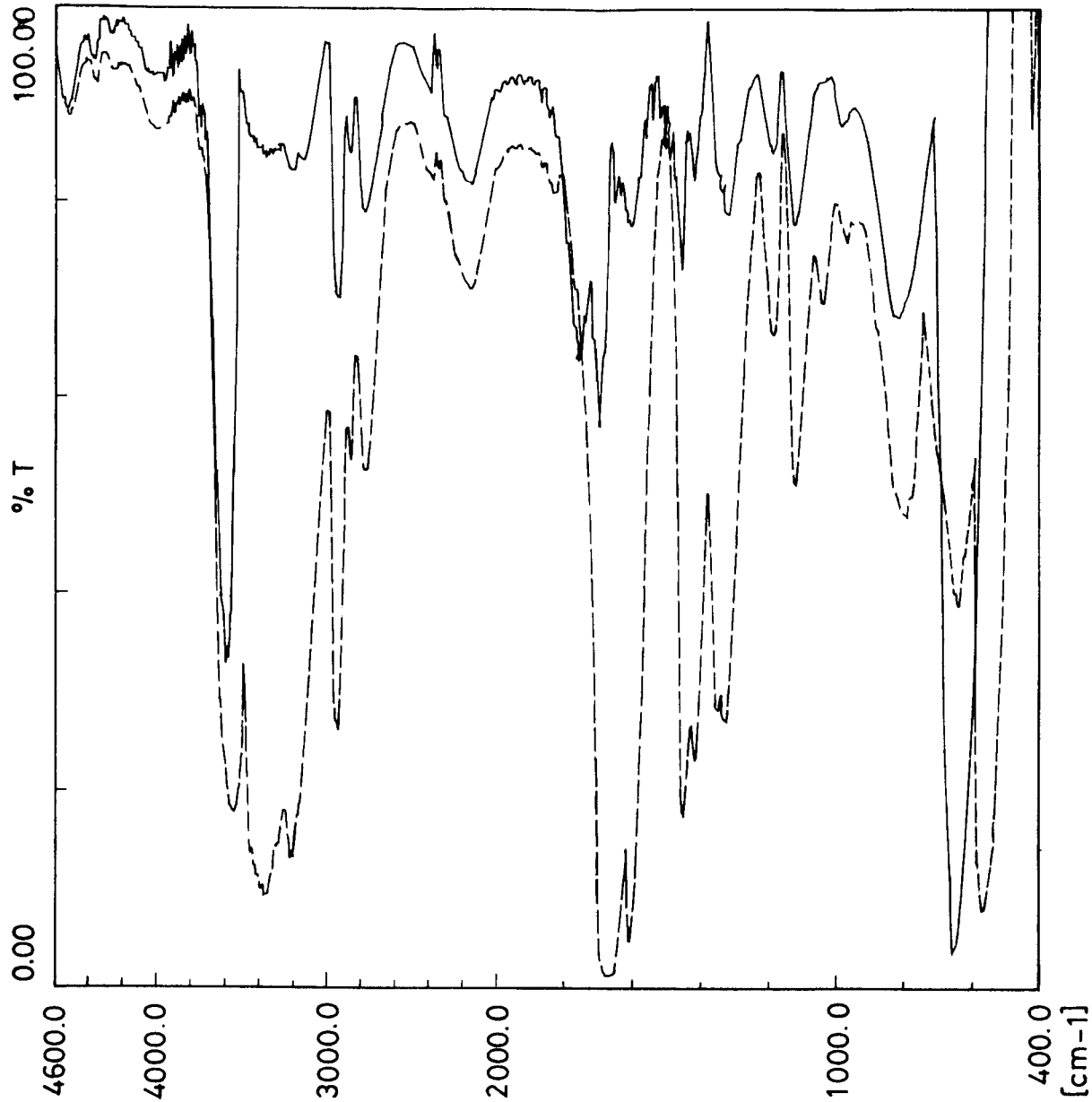


Figure 4.12. FT-IR Spectra of PAM-Methionine End Group (- - -) and PAM-No End Group

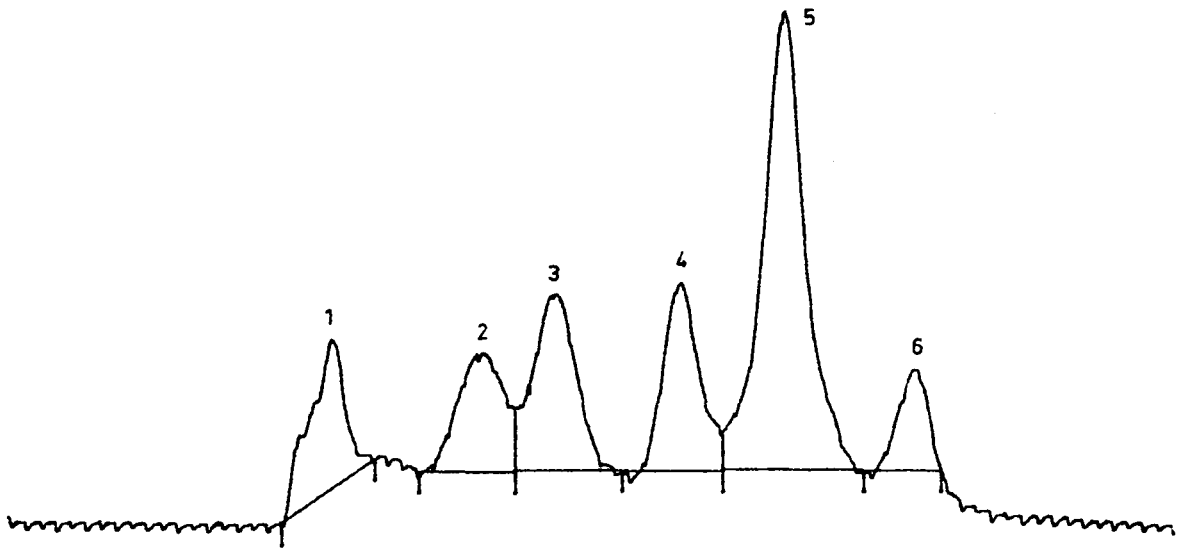


Figure 4.13. Original HPLC Chromatogram of Polyacrylamide With No End Group

Peak ID	RT	Area	Normalized Area %
1	4.33	109294	10.39
2	6.28	117105	11.71
3	7.24	155886	15.59
4	8.84	143440	14.35
5	10.21	415382	41.55
6	11.91	58487	5.85
TOTAL		999594	100

experiments were carried out until this fraction was collected. The MW of the fraction collected is 149,756. HPLC chromatograms of the polyacrylamide before and after fractionation are shown in Figure 4.14.

4.3. Polymer-Metal Complexes (PMC)

Water soluble polymer-metal complexes were prepared by simple mixing of polymer with metal in neutral aqueous media. The composition and structure of PMC were controlled by adding variable concentrations of metal to aqueous solutions of polymer at a constant concentration of polymer.

Addition of a metal ion to polymer starts to give homogeneous solution for certain value of n_{me}/n_p at pH 7, where n_{me} and n_p are the numbers of moles of metal and polymer, respectively. Although metal salt ($CuSO_4$) is not soluble at pH 7, its mixture with the polymer is soluble up to certain n_{me}/n_p ratio. This result confirms the idea of complex formation.

Figure 4.15. shows the plot of n_{me}/n_p versus absorbance. As can be seen from this figure, water soluble polyacrylamide with methionine end group-metal complexes were obtained until $n_{me}/n_p \leq 0.2$ and with further increase in metal concentrations, turbidity suddenly developed. This indicates that high concentrations of Cu^{2+} leads to the crosslinking of macromolecules, as a result the system loses its homogeneity. Similar trend was observed for polyacrylic acid (Figure 4.16.).

Figure 4.17., 4.18., and 4.19. illustrate the typical HPLC chromatograms of polyacrylic acid (PAA), polyacrylamide containing methionine end group (PAM-Met), and polyacrylamide containing no end group (PAM) and their mixture with copper ions, respectively, at different ratios of n_{me}/n_p.

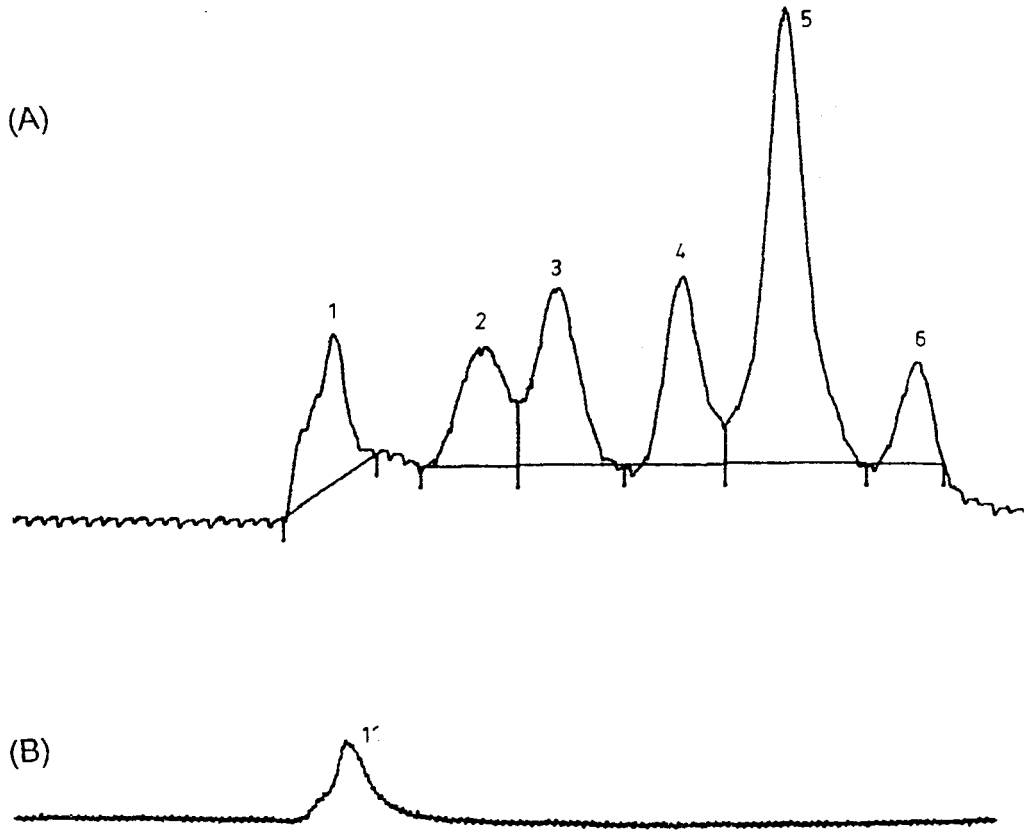


Figure 4.14. HPLC Chromatograms of Polyacrylamide with No End Group Before (A) and After Fractionation (B)

Peak ID	RT	Area	Normalized Area %
1	4.33	109294	10.39
2	6.28	117105	11.71
3	7.24	155886	15.59
4	8.84	143440	14.35
5	10.21	415382	41.55
6	11.91	58487	5.85
TOTAL		999594	100.00

Peak ID	RT	Area	Normalized Area %
1'	4.33	45780	100
TOTAL		45780	100

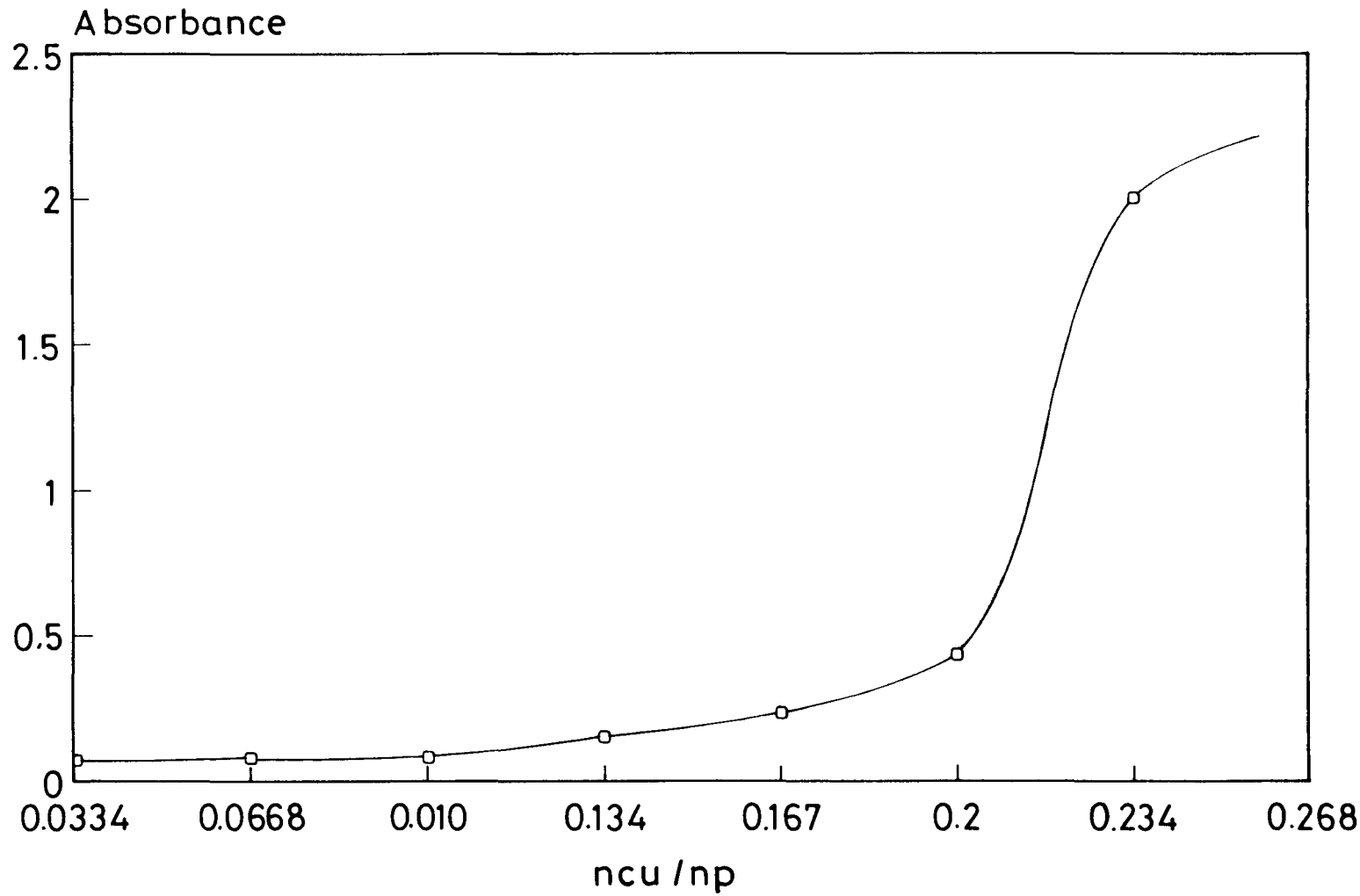


Figure 4.15. A plot of n_{me}/n_p versus Absorbance for Polyacrylamide with Methionine End Group

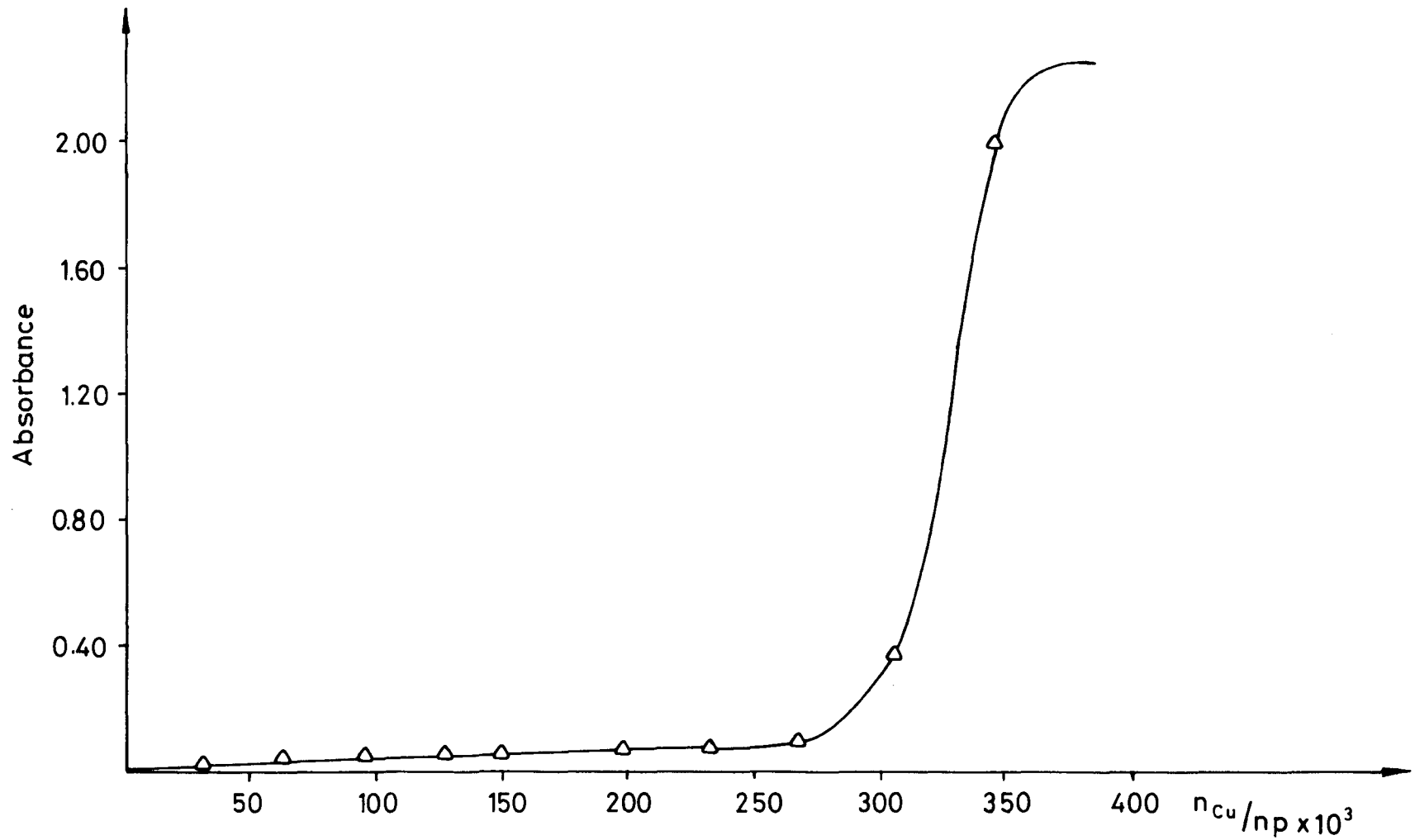
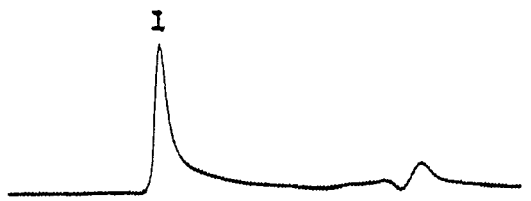
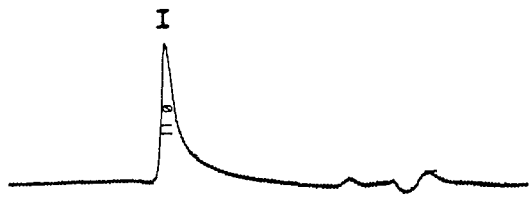


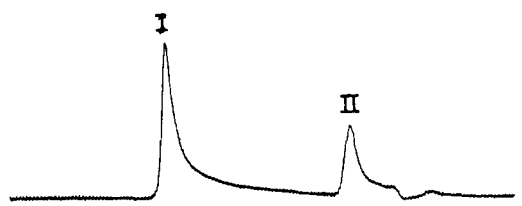
Figure 4.16. A plot of n_{me}/n_p versus Absorbance for PAA



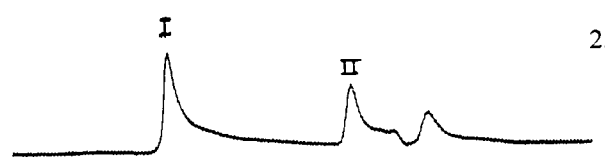
$[Cu^{2+}]$	ID	RT	Area	Area %
0 (PAA)	I	4.31	233626	100
	II	-	-	-



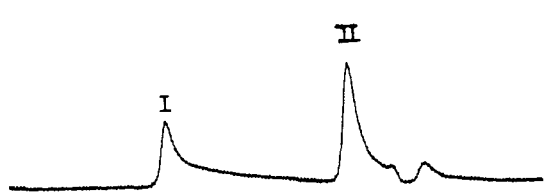
$0.7x10^{-3}$	I	4.31	233500	100
	II	-	-	-



$1.4x10^{-3}$	I	4.31	171609	70.131
	II	9.34	73421	29.869



$2.1x10^{-3}$	I	4.31	109104	54.93
	II	9.33	89531	45.07



$2.8x10^{-3}$	I	4.30	53685	25.96
	II	9.33	136099	64.64

Figure 4.17. Original HPLC Chromatograms of PAA-Cu^{2+} Complexes prepared at different $[Cu^{2+}]$ concentrations (ml/l)

$[Cu^{2+}]$

0 (PAM)

ID	RT	Area	Area %
I	4.32	152878	100

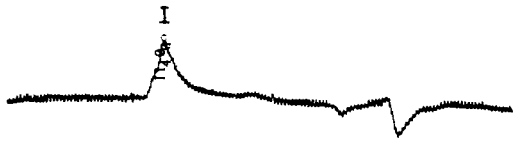
$1.4x10^{-3}$

I	4.32	76809	50.22
II	11.50	76151	49.78

$2.1x10^{-3}$

I	4.32	54694	35.37
II	11.50	99924	64.63

Figure 4.18 Original HPLC Chromatograms of PAM with Methionine End Group-Cu^{2+} Complexes prepared at different $[Cu^{2+}]$ concentrations (ml/l)



$[Cu^{2+}]$

0 (PAM)

ID	RT	Area	Area %
I	4.32	29893	100

$1.4x10^{-3}$

I	4.32	27971	33
II	11.50	65623	67

$2.1x10^{-3}$

I	4.32	17575	20.39
II	11.50	68620	79.61

Figure 4.19 Original HPLC Chromatograms of PAM with No End Group-Cu^{2+} Complexes prepared at different $[Cu^{2+}]$ concentrations (ml/l)

As can be observed from these figures, at relatively low concentrations of Cu^{2+} only one peak is seen in the chromatogram and further increase in n_{me}/n_p leads to a bimodal distribution of components and as well as a decrease in the area of the peak 1, and an increase in the area of the peak 2.

One may assume that peak 1 corresponds to a free polymer and peak 2 corresponds to a complex polymer-Cu^{2+} . Based on this assumption, the most suitable metal concentration at which maximum complex formation takes place between PAA and Cu^{2+} ions is 2.8×10^{-3} mol/l. In the case of polyacrylamide with and without end group, the most suitable metal concentration is 2.1×10^{-3} mol/l. The existence of the free polymer in the system indicates a non-random distribution of the copper ions between polymer chains.

In the light of HPLC results mentioned above, it can be suggested that at relatively low concentrations of Cu^{2+}, these cations are randomly distributed between adsorbing polyions and on further increase in n_{me}/n_p the distribution changes considerably so that the systems consists of two fractions: part of polymer binds the maximal amount of Cu^{2+}, whereas the other part is in the free state. This mechanism is further confirmed by Atomic Absorption Spectrophotometer (AAS) analysis of the collected HPLC fractions.

4.4. Polymer-Metal-Protein Complexes

For the ternary complexes, soluble polymer-metal complexes were used and variable concentrations of protein were added to them. In a wide range of n_{BSA}/n_p, these triple polymer-metal-protein complexes remained soluble, where n_{BSA} and n_p are the numbers of moles of protein (bovine serum albumin) and polymer, respectively.

The formation of some water soluble triple complexes with bovine serum albumin in the presence of divalent copper ions (Cu^{2+}) was investigated by sedimentation analysis, turbidimetric titration, viscometry, and u.v. spectroscopy in neutral aqueous media according to the method developed by Mustafaev and Kabanov [27]. HPLC studies in homogeneous systems at different ratios of the components will give an excellent opportunity to elucidate some important features characterizing triple polymer-metal-protein complex formation. Hence, complex formation of some water soluble polymers [polyacrylic acid (PAA), polyacrylamide with methionine end group (PAM-Met), and polyacrylamide containing no end group (PAM)] were analyzed by HPLC method at the different ratio of components ($n_{BSA}/n_{p)}$ and concentrations of Cu^{2+}.

A series of HPLC chromatograms of the triple PAA-Cu^{2+}-BSA complexes at the different ratio of components ($n_{BSA}/n_{p)}$ and concentrations of Cu^{2+} are given in Appendix 1 and 2. As shown from the chromatograms, the distribution of the ternary mixtures revealed a multimodel character. The retention (RT) values of the peaks corresponding to mixture products are insignificantly different from those of individual BSA and PAA molecules. However, the area of peak 1, 2, and 3 are considerably different from those of individual PAA and BSA molecules. It should be pointed out that an increase in the n_{BSA}/n_{PAA} ratio (the weight concentration of PAA = 0.05 g/ml and n_{Cu}/n_{PAA} = 0.15 are kept constant) initially leads to an increase in the area of the peak 1, and also the area of peak 2 and 3 of the mixture are smaller than those of individual dimer and monomer of BSA. These results probably indicate that protein binding by PAA takes place and appears as peak 1 of the mixture and peak 2 and peak 3 of the mixture corresponds to free dimer and monomer of BSA. Based on these results, the following mechanism can be suggested:

$$_n\text{PAA-Cu}^{2+} + _m\text{BSA} \Leftrightarrow (\text{PAA-Cu}^{2+})_n\text{-BSA}_m \xrightarrow{x\ \text{BSA}} (\text{PAA-Cu}^{2+})_n\text{-BSA}_{m+x}$$

On further increase in n_{BSA}/n_{PAA} ratio ($n_{BSA}/n_{PAA}>2$), a considerable decrease in the area of peak 1 was observed. This result may indicate that the formation of ternary complexes is partially destroyed because of increasing

concentration of protein in the mixture. In the light of the HPLC results, the most suitable n_{BSA}/n_{PAA} ratio at which maximum complex formation takes place between polymer and protein molecules in the presence of metal ions is 1.48. The same mechanism was observed for polyacrylamide containing methionine end group (PAM-Met)-Cu^{2+}-BSA, and polyacrylamide containing no end group (PAM)-Cu^{2+}-BSA, as shown in Appendix 3, and 4. As shown from the chromatograms, the most suitable protein/polymer ratio at which maximum complex formation takes place is 1.00 for both polyacrylamide with and without end group.

A comparison of HPLC results of PAA-BSA complexes in the absence of metal with those of PAA-Cu^{2+}-BSA complexes are given in Appendix 5 . The area of peak 1 of the double PAA-BSA complexes was smaller than those of triple PAA-Cu^{2+}-BSA complexes, whereas the area of peak 2 and 3 were larger than those of triple complexes. These results of triple complexes can be interpreted that complex formation of PAA with BSA in the presence of metal ions is larger than those in the absence of metal ions.

In fact, Petrov et al. have previously reported that under conditions where both polymer and protein have same (negative) charges and are in capable of binding to another in the absence of a mediator (metal ions), the divalent Cu^{2+} act as "fasteners" between BSA globules and PAA chains and promote the formation of a soluble ternary complex which is stable under physiological conditions [11]. The following hypothetical scheme for the structure of the ternary PAA-Cu^{2+}-BSA complex is shown in Figure 4.20. In each molecule of ternary polymer-metal-protein complex, the protein globules interact with one another via copper ions crosslinked to a linear polymer. Part of Cu^{2+} ions form chelate complexes and thus promote the aggregation of ternary complex molecules, whereas others form intramolecular crosslinks in the free sites of polymer, thus stabilizing the overall structure. Fragments of polymer that are not directly involved in the complex formation form free loops accessible to H_2O [11].

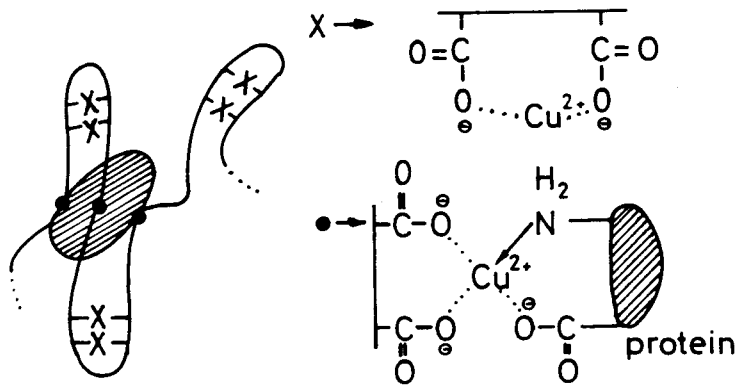


Figure 4.20. A Hypothetical Scheme for the Structure of the Ternary PAA-Cu^{2+}-BSA Complex

4.4.1. Effect of Metal Concentration on Triple PAA-Cu-BSA Complexes

The effect of n_{me}/n_p ratio on the complex formation of ternary PAA-Cu^{2+}-BSA was analysed by HPLC, where n_{me} and n_p are the numbers of moles of metal and polymer, respectively. A series of HPLC chromatograms obtained at three different n_{me}/n_p ratio are illustrated in Appendix 6. As expected, the area of peak 1 of the mixture which corresponds to ternary PAA-Cu^{2+}-BSA complexes, is found to increase in proportion with n_{me}/n_p ratio. From the chromatograms, it is evident that with the increasing concentration of metal ions in the mixture, complex formation increases since the metal ions between polymer chains and protein globules play their role as a crosslinking agent more effectively.

4.5. Radiation Chemistry

Aqueous solutions of polyacrylic acid (PAA), Bovine Serum Albumin (BSA), PAA-Cu^{2+}, PAA-Cu^{2+}-BSA, and PAA-BSA were irradiated in O_2 atmosphere

using a 6 curie Co-60 gamma source for a total dose of 1.2 kGy. Furthermore, HPLC and spectrophotometric methods were applied in order to investigate the effect of irradiation on them.

4.5.1. Radiolysis of Polymers

Aqueous solution of polyacrylic acid (PAA) were irradiated with Co-60 gamma rays with a dose of 1.2 kGy and the changes occurred were measured by spectrophotometric and HPLC methods.

Figure 4.21. shows UV-Visible spectrum of PAA in aqueous solution before and after irradiation. On irradiation, the absorbance of PAA increases

HPLC results of the irradiated and unirradiated PAA solution are illustrated in Figure 4.22. As can be observed by the chromatograms, irradiated PAA elutes later than unirradiated PAA, indicating the decrease in molecular weight of the polymer. Since according to size exclusion theory, the polymers elute in reverse order of their molecular weight, this result can be interpreted that the changes occurred after irradiation are due to degradation of PAA chains. Indeed, Alexander and Charlesby made a similar study on the effect of ionizing radiations (x- and γ-rays) on aqueous solutions of the following vinyl polymers: PAA, polyacrylamide and polyvinyl pyrollidone, and polyvinyl alcohol. They found that in dilute solutions (<0.3 % for the polymer used) all the polymers are degraded by main fracture and higher concentration, crosslinking becomes the dominant action of all the polymers studied with the exception of polymethacrylic acid [58]. Since 0.05 % PAA was used in this work, the experimental results obtained by HPLC is in agreement with the literature mentioned above.

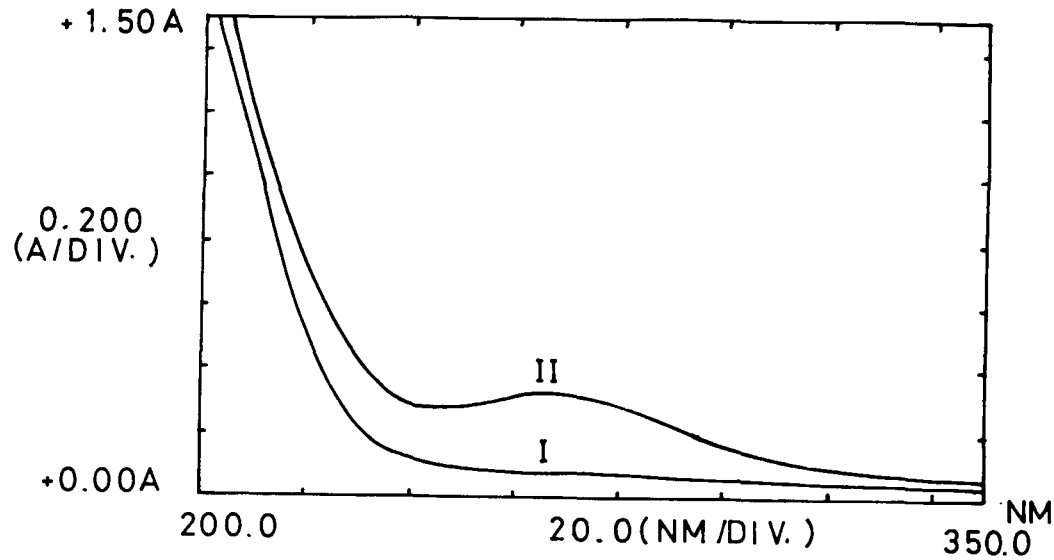


Figure 4.21. UV-Visible spectrum of PAA in aqueous solution before (I) and after irradiation (II)

4.5.2. Radiolysis of Proteins

Aqueous solution of the protein (BSA) irradiated in O_2 atmosphere at 1.2 kGy, when subjected to HPLC analysis, yielded the chromatograms shown in Appendix 7. These chromatograms clearly provide evidence of the formation of a new form of the protein. This has been interpreted to indicate a change of the protein from the native form to the denatured form.

Spectrophotometric measurements confirmed the chromatographic picture. As illustrated in Figure 4.23., a significant increase in absorbance of the UV spectrum was observed after irradiation, indicating aggregation process. The reason for this appears to be tied in with the free radicals produced in water bringing about a change in the shape of the protein molecule (e.g. unfolding) and thereby facilitating subsequent aggregation processes. Similar results including denaturation and aggregation have been obtained with the irradiation of proteins including human and bovine serum albumin, egg albumin and casein [65].

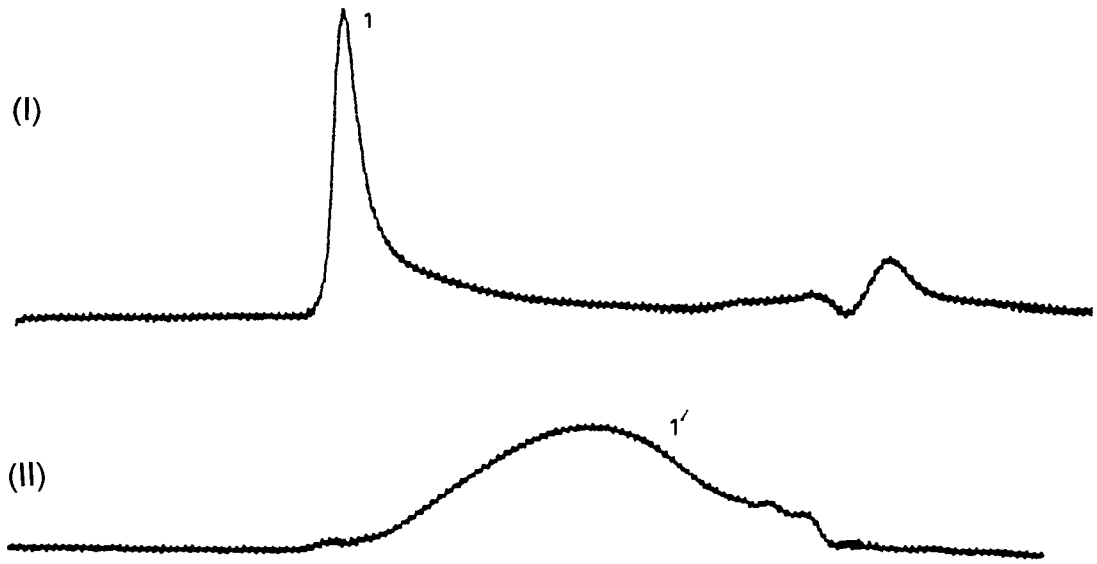


Figure 4.22. HPLC Chromatograms of PAA in aqueous solution before (I) and after irradiation (II)

Peak ID	RT	Area	Normalized Area %
1	4.31	233626	100
1'	7.69	529974	100

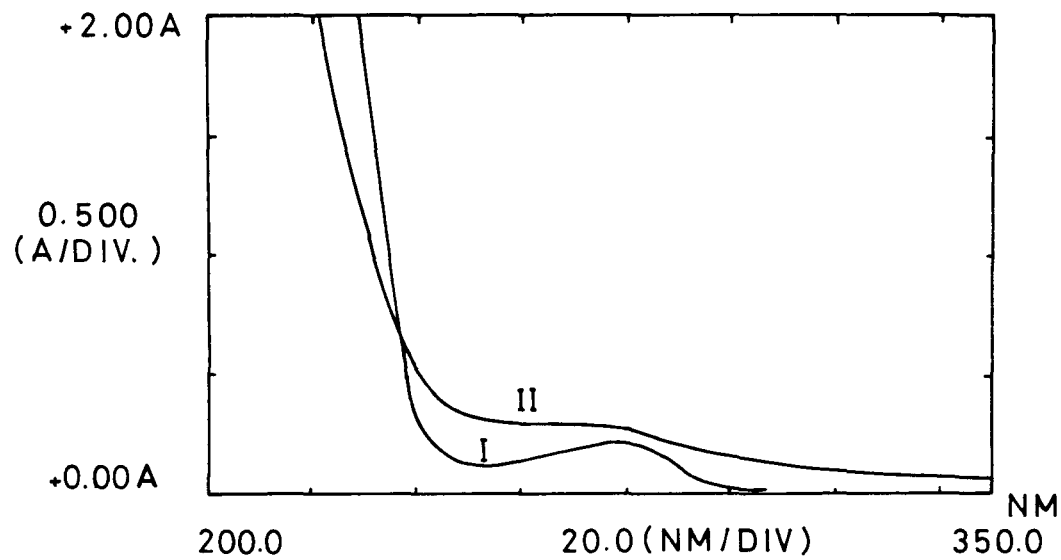


Figure 4.23. UV-Visible spectrum of BSA in aqueous solution before (I) and after irradiation (II)

4.5.3. Radiolysis of Polymer-Metal Complexes

Spectrophotometric and HPLC results of the irradiated and unirradiated PAA-Cu^{2+} solution in O_2 atmosphere at 1.2 kGy were shown in Appendix 8, and 9.

Spectrophotometric results clearly shows that irradiation leads to a decrease in the absorbance of polymer PAA-Cu^{2+}. In addition, from the examination of HPLC chromatograms, it can be seen that the bimodal character of molecular weight distribution of polymer-metal complexes does not change after irradiation. However, it was observed that the extent of the decomposition in peak 1 corresponding to free polymer is very high while decomposition process does not take place in peak 2 which corresponds polymer-metal

complex. This suggest a partial stabilizing effect of Cu^{2+} added to PAA solution.

4.5.4. Radiolysis of Polymer-Metal-Protein Complexes

The spectrophotometric and HPLC results obtained for the irradiated solutions of ternary PAA-Cu^{2+}-BSA complexes in a wide range of n_{BSA}/n_P and at two different concentrations of Cu^{2+} are given in Appendix 10, and 11.

As shown from the UV spectrum of ternary complexes, a decrease in absorbance was observed after irradiation. Furthermore, HPLC results clearly shows that as the concentration of protein (BSA) in the mixture increases, the extent of decomposition in PAA-Cu^{2+}-BSA complexes decreases. At high concentration of n_{BSA}/n_P ratio, the chromatograms of irradiated and unirradiated PAA are remarkably alike in appearance. This probably indicate the stabilizing effect of Cu^{2+} added to polymer-protein mixture.

Moreover, PAA-BSA complexes in the absence of metal ions were investigated using HPLC. As expected, the extent of decomposition in PAA-BSA complexes is significantly higher than PAA-Cu^{2+}-BSA complexes (Appendix 12). This result is agreement with the assumption mentioned above, that is, the addition of metal ions to polymer-protein mixture creates a stabilizing effect on both polymer chains and protein globules.

4.5.4.1. Effect of Irradiation Dose Rate

To examine the effect of irradiation dose rate on aqueous solutions of PAA, BSA, PAA-Cu^{2+}, and ternary PAA-Cu^{2+}-BSA complexes they were irradiated at two different dose rate of 24.36, and 102857.14 rad/min,

respectively, while keeping total irradiation dose same (1.2 kGy). As is obvious from HPLC chromatograms, shown in Appendix 13, 14, 15, and 16, the shapes of the above mentioned samples exposed to two different dose rate of irradiation were similar to each other. This clearly demonstrate that extent of decomposition of these samples does not depend on the dose rate providing that the same total irradiation dose is exposed to them.

V. CONCLUSIONS AND RECOMMENDATIONS

The conclusions which can be drawn from the experimental studies are summarized below, together with the recommendations for future work.

5.1. Conclusions

The polymerization of acrylamide, initiated by the Cerium Ammonium Nitrate (Ce(IV)) -Methionine redox initiator system, was carried out in aqueous solution at different reaction conditions. The dependence of molecular weight and yield of polymer on the concentration of Cerium (IV), polymerization time, and temperature was determined.

The experimental results show that:

- As the concentration of Ce(IV) increases, the molecular weight decreases whereas the yield increases.

- By considering the effect of reaction time, it is found that the yield increases proportionally with reaction times, and also noticed that at low reaction times, the molecular weight gives a sharp decrease, attains a minimum at 1 hour and then rises at higher reaction times.

- As temperature increases, the molecular weight and yield also increase.

Taking the HPLC results mentioned in Section 4.1.1 into account, a decrease in the concentration of Ce(IV) results in a decrease in the area of peak 2 corresponding to the molecules of small size, but an increase in the area of peak 1 corresponding to the molecules of large size. In view of these results, the mutual termination seems to be a major process at low catalyst [Ce(IV)] concentration. Hence, these findings play a crucial role in understanding the polymerization mechanism of these polymers. Moreover, optimum reaction conditions (concentration of Ce(IV), polymerization time, and temperature) which provide an opportunity to obtain a polymer having a narrow molecular weight distribution are determined. After obtaining such a polymer, one question is arised in mind, that is, whether this polymer actually contains methionine end group or not. For this purpose, both conductometric titration and infrared measurements were carried out. Based on the results mentioned in Sections 4.1.4, and 4.1.5, it can be concluded that the polyacrylamide contains methionine end group.

In addition, the synthesis of polyacrylamide containing no functional end group was achieved by exposing aqueous solutions of acrylamide monomer to heat under nitrogen atmosphere. HPLC results show that the molecular weight distribution of the polymer is very broad. It should be pointed out that obtaining a fraction showing only one polymer peak in the chromatogram is an essential prerequisite of studying the complex formation of polymers. Therefore, fractional precipitation was applied to polyacrylamide having a broad molecular weight distribution in order to obtain a fraction having a narrow molecular weight distribution.

From the examination of both spectrophotometric and HPLC results, it is concluded that the chemical structure and composition of PMC depend on both metal concentration in solution and the chemical nature of PE. At relatively low concentrations of Cu^{2+} these cations coordinate mostly intramolecularly both the adjacent and distant fragments of the polymeric chain (intramolecular

crosslinks). With an increase in metal concentration the distribution changes considerably. The systems consist of two fractions: part of PE bind the maximal amount of metal whereas the other part is in free state. This mechanism is further confirmed by Atomic Absorption Spectrophotometer (AAS) analysis of the collected HPLC fractions. This complex formation occurs as a result of non-random distribution of Cu^{2+} within polymeric molecule. With a further increase in metal concentration the whole bulk of the free polymer is transformed into PMC as the same mechanism before. At relatively high concentrations of Cu^{2+}, an intermolecular crosslinking of polymer also takes place. This process culminates in the aggregation of macromolecules so that the system loses its homogeneity and the formation of a spatial network, in which the role of the crosslinking agent is also played by metal ions.

It is well known that transient metal ions as well as other biphilic low molecular weight compounds (e.g., surfactants) possess the ability to bind to neutral or charged water soluble polymers, and they confer on them adhesive properties and the capacity to form complexes with complementary surfaces (protein, etc.). So, both components of the reaction system bearing the like (positive or negative) charge and being incapable of binding to each other in the absence of mediator, metal ions play the role of "crosslinking agent" between the protein globules and the polymer chains.

In this work, the complex formation of some water soluble polymers (polyacrylic acid, polyacrylamide containing methionine end group, polyacrylamide containing no end group) with protein (bovine serum albumin) in the presence of copper ions was examined using the method of High Performance Liquid Chromatography (HPLC). Based on the HPLC results mentioned in Section 4.4, the most suitable metal concentration and protein/polymer ratio at which maximum complex formation takes place between polymer and protein molecules in the presence of metal ions was determined. The increasing concentration of the metal ions is found to promote complex formation as the metal ions play their fastener roles between polymer chains and protein globules more effectively.

HPLC and spectrophotometric methods were employed to investigate the effect of irradiation on aqueous solutions of polyacrylic acid (PAA), Bovine Serum Albumin (BSA), PAA-Cu^{2+}, PAA-Cu^{2+}-BSA, and PAA-BSA in O_2 atmosphere. For this purpose, they were irradiated with 6 curie Co-60 gamma source for a total dose of 1.2 kGy.

On the basis of spectrophotometric and HPLC results obtained, it is concluded that upon irradiation PAA undergoes degradation, and also considering the effect of irradiation on protein (BSA), irradiation causes a change of the protein from the native form to the denatured form. The reason for this seems to be tied in with the free radicals produced in water, which in turn bring about a change in the shapes of the polymer and the protein molecules. Furthermore, it is noticed that upon irradiation, the rate of decomposition in PAA-BSA complexes is considerably higher than PAA-Cu^{2+} and PAA-Cu^{2+}-BSA complexes. This may be due to the stabilizing effect of copper ions added to these complexes.

In order to investigate the effect of irradiation dose rate on aqueous solutions of PAA, BSA, PAA-Cu^{2+}, and ternary PAA-Cu^{2+}-BSA complexes they were irradiated at two different dose rate of 24.36, and 102857.14 rad/min, respectively, while keeping total irradiation dose same (1.2 kGy). The chromatographic pictures of the above mentioned samples exposed to two different dose rate of irradiation are found to be similar to each other. Based on these results, it can be concluded that extent of decomposition of the irradiated samples don't depend on the dose rate provided they are exposed to the same total irradiation dose.

5.2. Recommendations

Further investigations in the same field, namely, the intraperitonal injection of the above mentioned polymers and their various complexes to mice, exposure of these mice to a lethal dose of 500 rem gamma-rays by Co-60 and finally, chromosomal aberration of the exposed mice should be carried out in order to observe radioprotective effect of the polymer and their various complexes.

For future study, polyacrylamide or polyacrylic acid, initiated by cerium ammonium nitrate-cysteamine redox initiator system, may be synthesized since it was well known that cysteamine (or β-mercaptoethylamine, or MEA) was an excellent radioprotective compound [6,10] and the observations about the radioprotective effect of cysteamine have been confirmed many times and on a wide range of living organism [7-9]

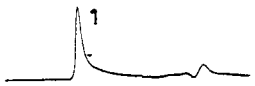
Instead of using copper ions as crosslinking agent between polymer chains and protein globules; zinc, iron or other transient metal ions may be used and in the presence of these metal ions, the complex formation mechanism of polymer with proteins may be studied using HPLC.

Although complex formation mechanisms of polyacrylic acid and polyacrylamide with protein in the presence of copper ions were studied, other water soluble polymers, such as polymethacrylic acid, polyvinylsulfonic acid, etc. may be examined in such systems.

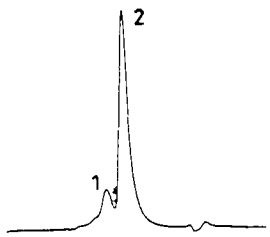
In the present work, bovine serum albumin was used as a model protein. Similarly, human serum albumin or hemoglobin may be used in order to investigate the mechanism of protein binding by synthetic polymer

Considering fractional precipitation procedure, instead of waiting for the solution to settle overnight in order to separate precipitate from supernatant phase, the solution may be divided into small portions and each portion may be centrifuged. Hence, this procedure would be less time consuming.

APPENDICES

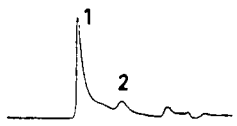


	ID	RT	Area	Area %
PAA	1	4.31	199216	100

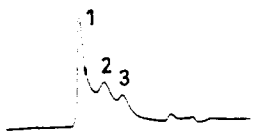


BSA	2	5.66	71057	8.24
	3	6.65	791619	91.76

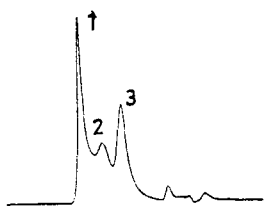
n_{BSA}/n_p



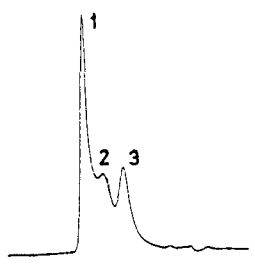
0.13	1	4.31	233568	77.94
	2	6.61	66108	22.06



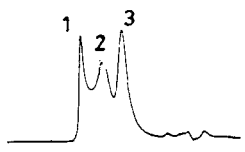
0.51	1	4.31	277743	58.2
	2	5.60	112384	23.64
	3	6.66	86795	18.16



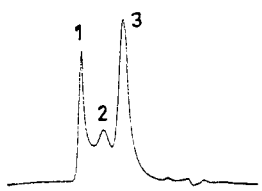
1.00	1	4.31	367384	53.24
	2	5.61	121306	17.58
	3	6.66	201322	29.18



1.48	1	4.33	467895	58.70
	2	5.73	155965	19.56
	3	6.67	173294	21.74

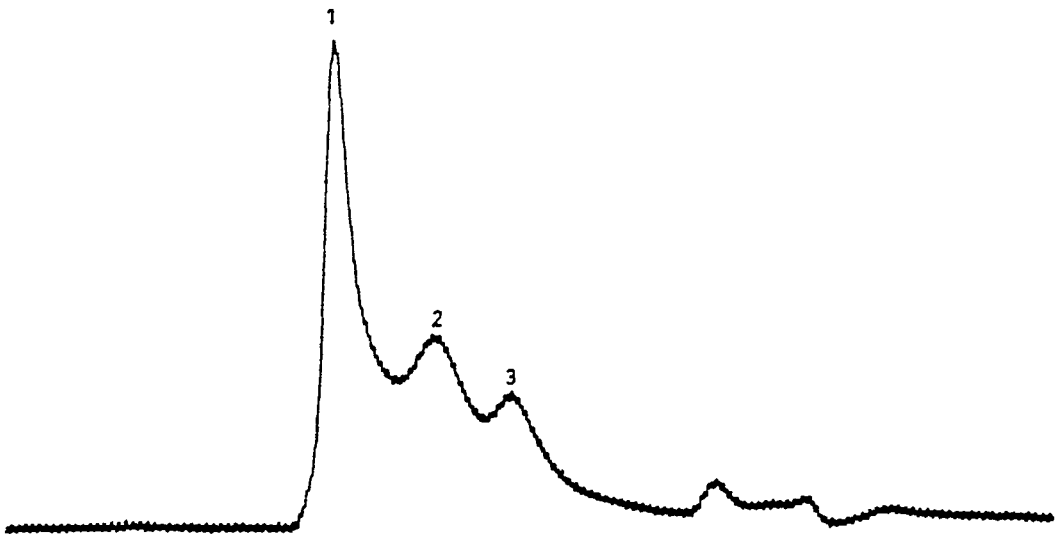


1.93	1	4.28	207593	34.64
	2	5.55	162897	27.18
	3	6.65	228749	34.18



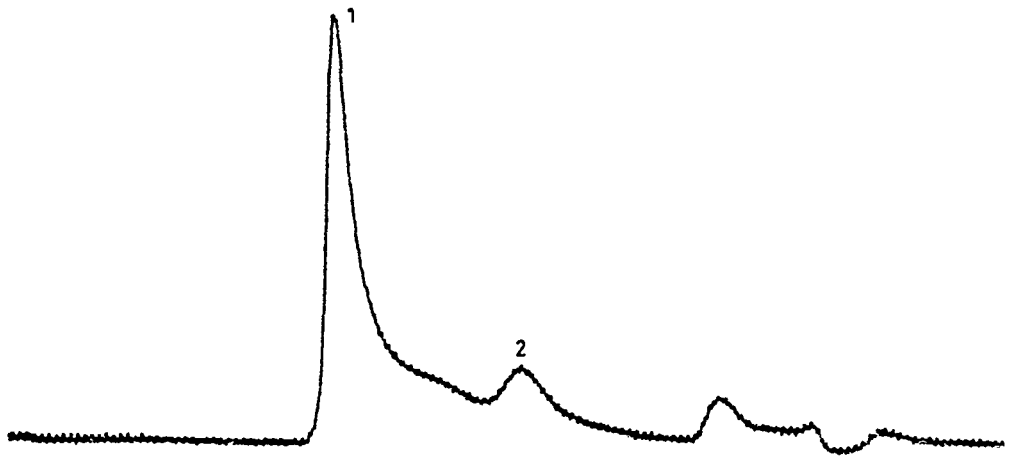
2.96	1	4.28	259942	37.5
	2	5.55	103997	15
	3	6.69	329959	47.5

Appendix 1.1. Original HPLC Chromatograms of PAA-Cu^{2+}-BSA Complexes at different n_{BSA}/n_p ratio, $[Cu^{2+}]=2.08x10^{-3}$ mol/l



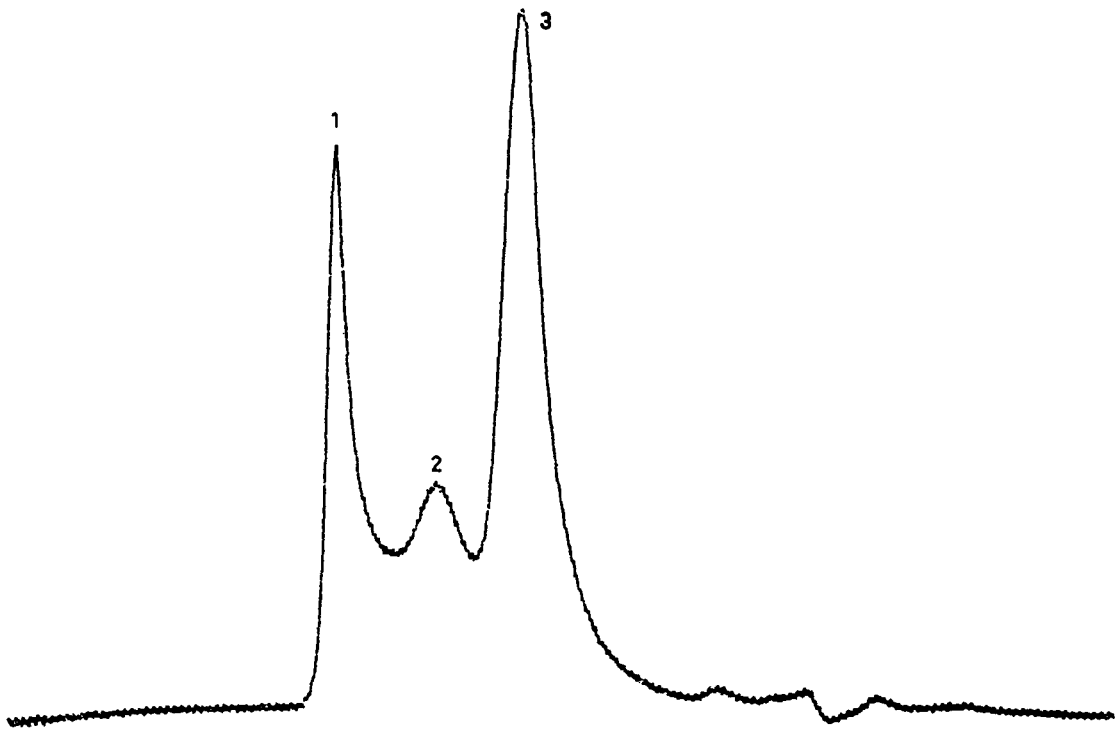
Appendix 1.3. Original HPLC Chromatogram of PAA-Cu^{2+}-BSA Complex at n_{BSA}/n_{AA}=0.51; $[Cu^{2+}]$ = 2.08x10^{-3} mol/l

Peak ID	RT	Area	Normalized Area %
1	4.31	277743	58.20
2	5.60	112834	23.64
3	6.66	86795	18.16
TOTAL		477372	100



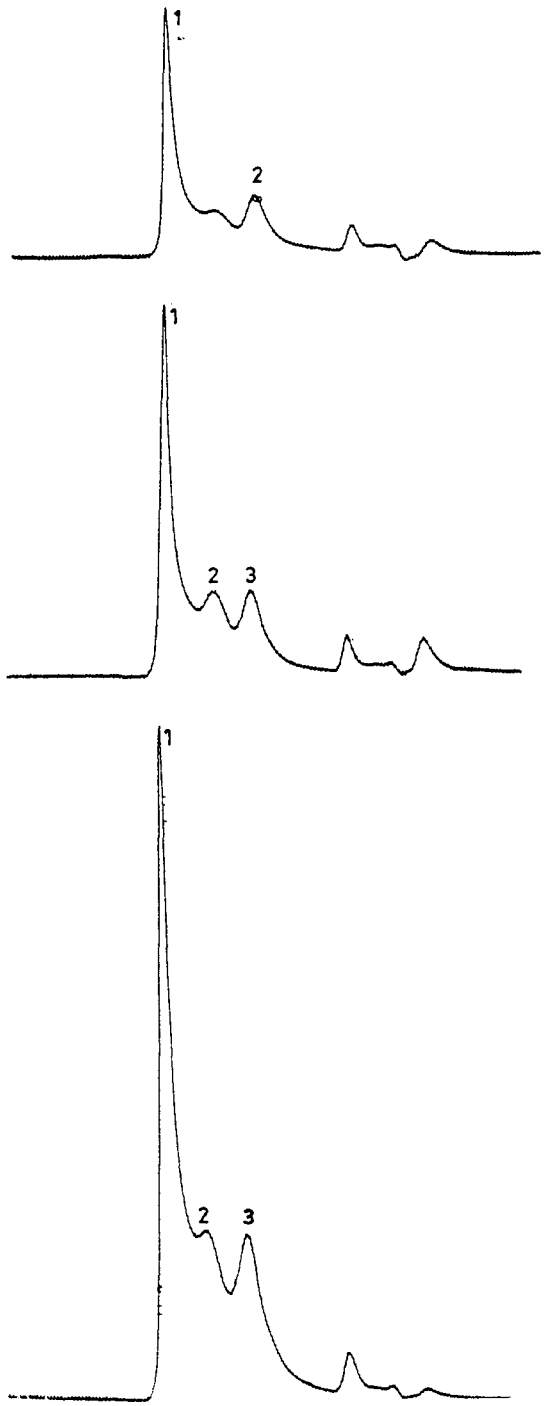
Appendix 1.2. Original HPLC Chromatogram of PAA-Cu^{2+}-BSA Complex at n_{BSA}/n_{AA}=0.13; $[Cu^{2+}]$ = 2.08x10^{-3} mol/l

Peak ID	RT	Area	Normalized Area %
1	4.31	233568	77.94
2	6.61	66108	22.06
TOTAL		299676	100



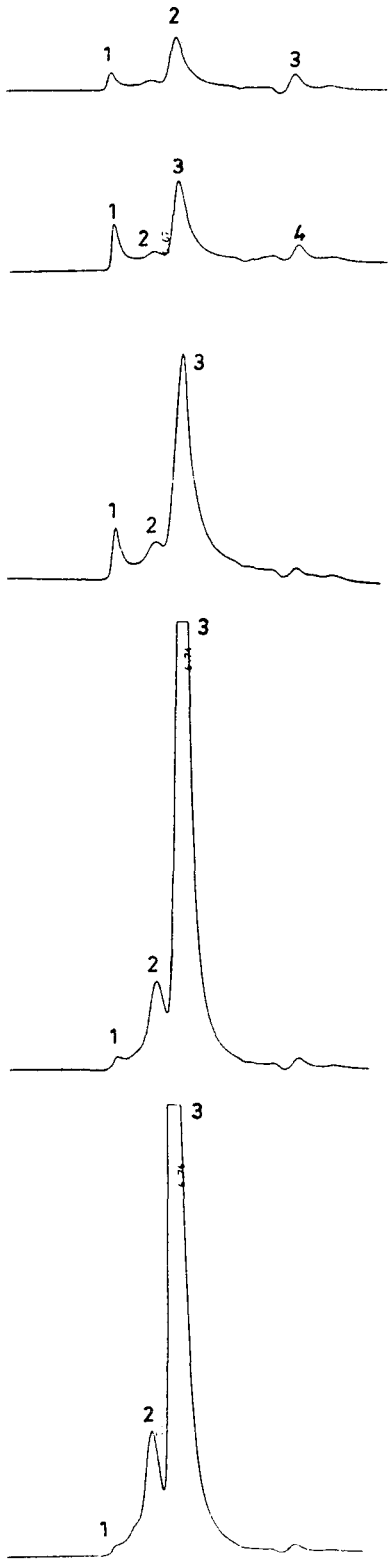
Appendix 1.4. Original HPLC Chromatogram of PAA-Cu^{2+}-BSA Complex at n_{BSA}/n_{AA}=2.96; $[Cu^{2+}]$ = 2.08x10^{-3} mol/l

Peak ID	RT	Area	Normalized Area %
1	4.28	259942	37.50
2	5.55	103997	15.00
3	6.69	329259	47.50
TOPLAM		599239	100



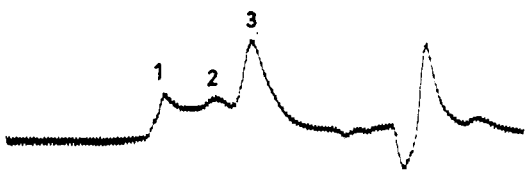
n_{BSA}/n_p	ID	RT	Area	Area %
0.13	1	4.31	249701	86.03
	2	6.54	40556	13.97
1.00	1	4.31	351359	82.79
	2	5.61	16522	3.90
	3	6.65	56522	13.31
1.93	1	4.31	1332461	87.88
	2	5.66	30040	1.98
	3	6.55	88238	5.82

Appendix 2. Original HPLC Chromatograms of PAA-Cu^{2+}-BSA Complexes at different n_{BSA}/n_p ratio, $[Cu^{2+}]=2.8x10^{-3}$ mol/l



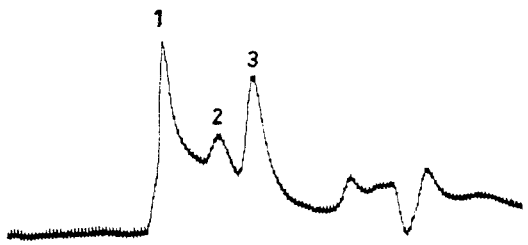
n_{BSA}/n_p	ID	RT	Area	Area %
0.13	1	4.32	41750	9.94
	2	6.63	300954	71.62
	3	11.32	77531	18.44
0.51	1	4.32	132460	20.14
	2	5.67	23983	3.65
	3	6.69	451269	68.61
	4	11.37	50048	7.60
1.00	1	4.32	163627	11.97
	2	5.67	47966	3.51
	3	6.69	1155141	84.52
2.00	1	4.32	86350	3.44
	2	5.71	115118	4.59
	3	6.74	2310282	91.97
4.00	1	4.32	55777	1.15
	2	5.77	167444	3.46
	3	6.76	4620564	95.40

Appendix 3. Original HPLC Chromatograms of PAM (Polyacrylamide with Methionine End Group)-Cu^{2+}-BSA Complexes at different n_{BSA}/n_p ratio, $[Cu^{2+}]=1.4x10^{-3}$ mol/l



n_{BSA}/n_p	ID	RT	Area	Area %
0.13	1	4.35	22705	15.60
	2	6.68	57713	39.64
	3	11.43	65156	44.76

0.51	1	4.35	40544	23.9
	2	6.73	68619	40.21
	3	11.46	60836	35.88

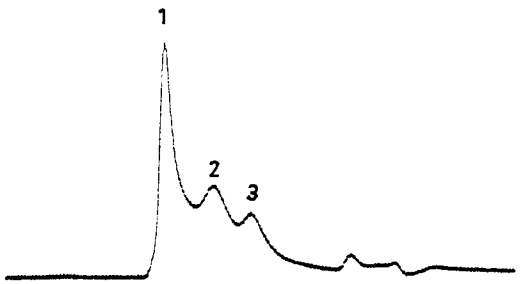


1.00	1	4.31	622142	11.45
	2	5.79	46242	8.51
	3	6.74	416142	80.04

Appendix 4. Original HPLC Chromatograms of PAM (with no end group)-Cu^{2+}-BSA Complexes at different n_{BSA}/n_p ratio, $[Cu^{2+}]=1.4x10^{-3}$ mol/l

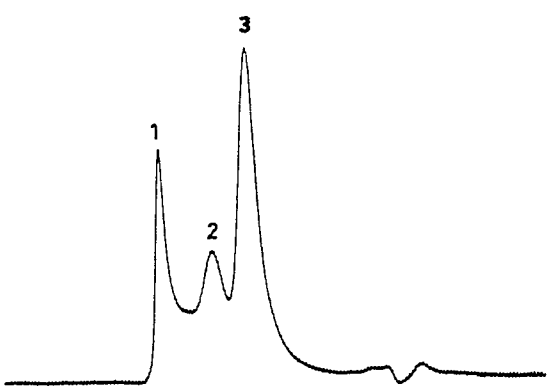
ID	RT	Area	Area %
1	4.31	162777	51.58
2	5.65	26459	8.38
3	6.69	126340	40.04

PAA-BSA (n_{BSA}/n_p=0.51)



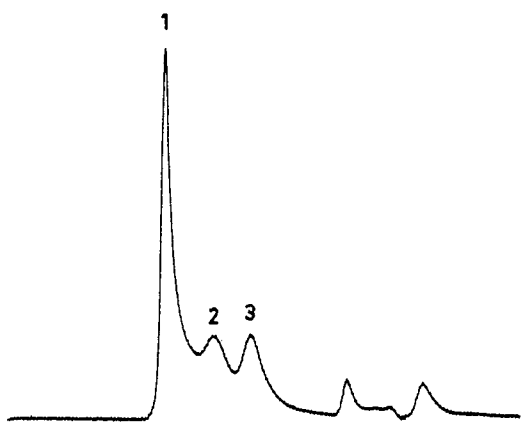
ID	RT	Area	Area %
1	4.31	277743	58.20
2	5.60	112834	23.64
3	6.66	86795	18.16

PAA-Cu^{2+}-BSA (n_{BSA}/n_p=0.51)



ID	RT	Area	Area %
1	4.31	209780	25.75
2	5.65	102796	12.62
3	6.69	502006	61.63

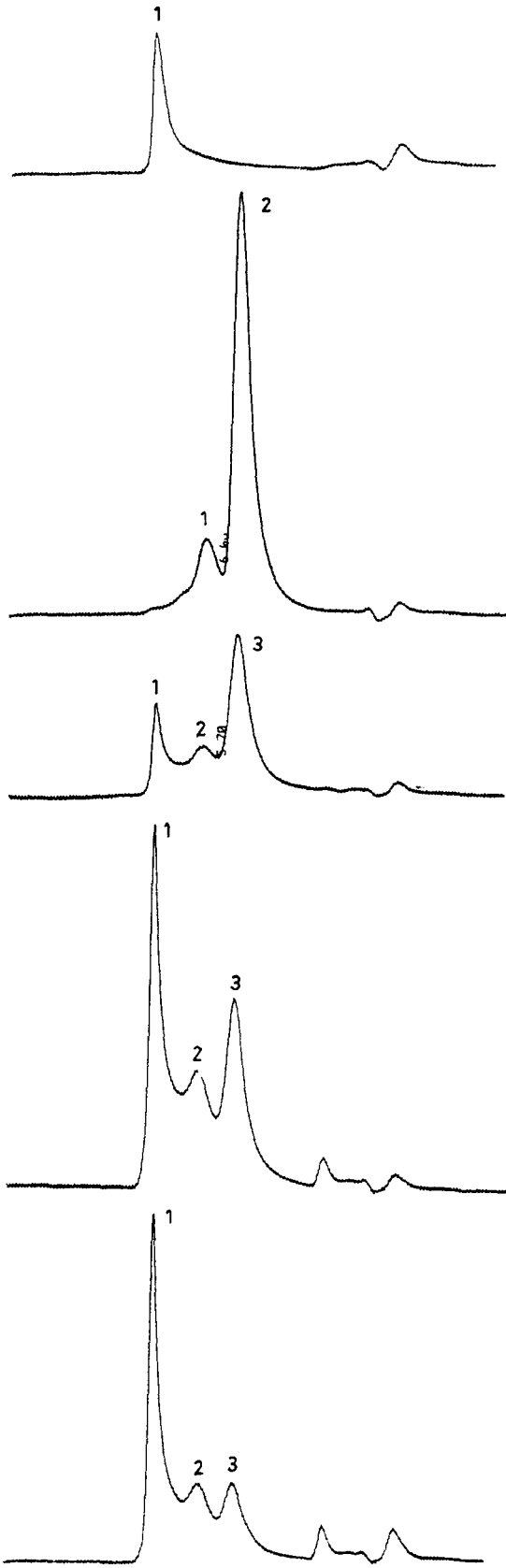
PAA-BSA (n_{BSA}/n_p=1.93)



ID	RT	Area	Area %
1	4.31	351359	82.79
2	5.61	16522	3.90
3	6.65	56522	13.31

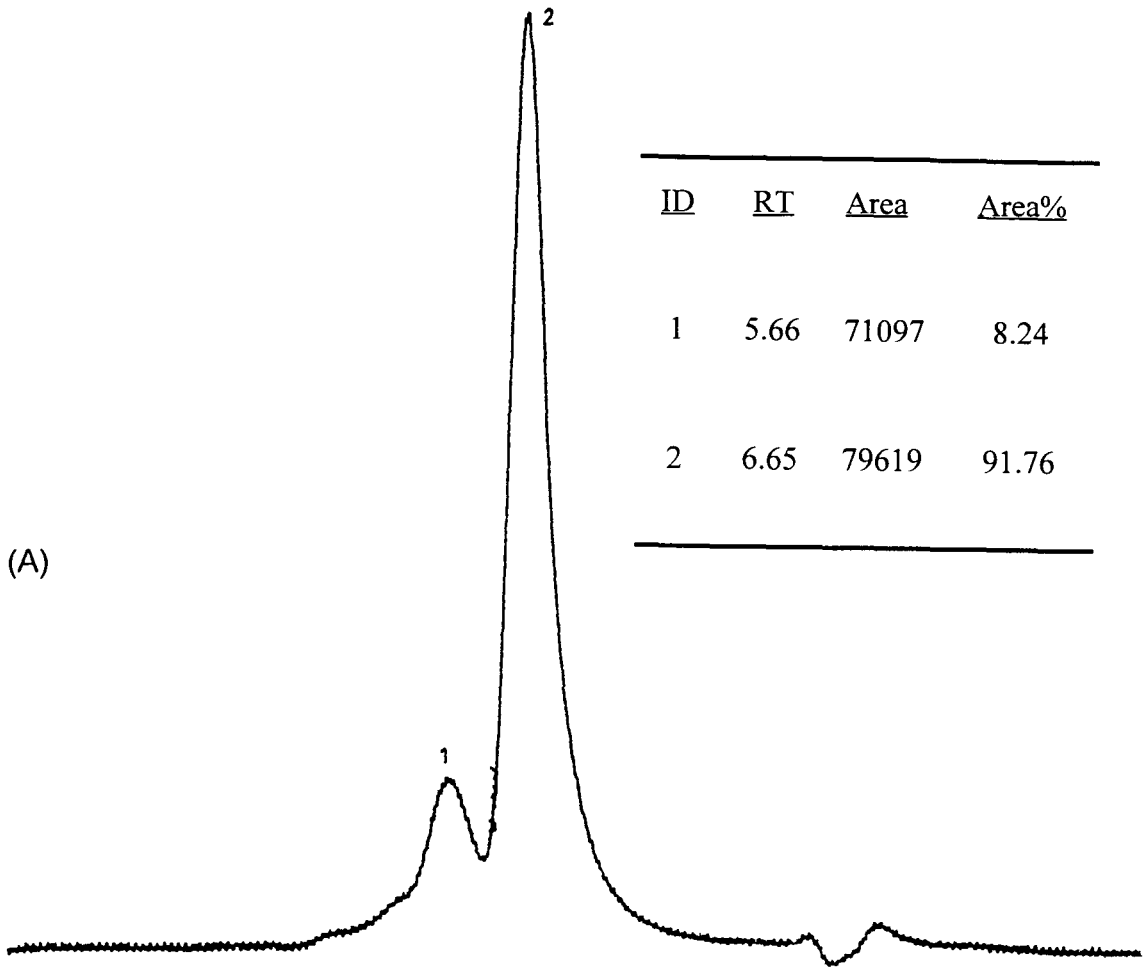
PAA-Cu^{2+}-BSA (n_{BSA}/n_p=1.93)

Appendix 5. Original HPLC Chromatograms of PAA-BSA and PAA-Cu^{2+}-BSA Complexes at the same n_{BSA}/n_p ratio



$[Cu^{2+}]$	ID	RT	Area	Area %
PAA	1	4.31	199216	100
BSA	1	5.66	71097	8.24
	2	6.65	79619	91.76
$1.4x10^{-3}$	1	4.31	93647	30.52
	2	5.61	56609	18.45
	3	6.66	156584	51.03
$2.1x10^{-3}$	1	4.31	367384	53.24
	2	5.61	121306	17.58
	3	6.66	201322	29.18
$2.8x10^{-3}$	1	4.31	351359	82.79
	2	5.61	16522	3.89
	3	6.65	56522	13.32

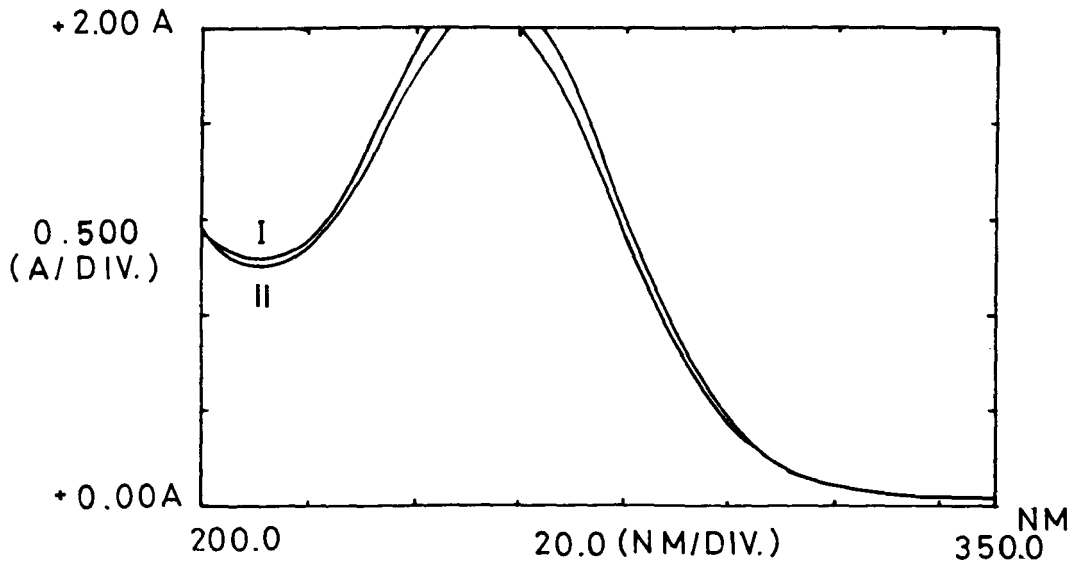
Appendix 6. Original HPLC Chromatograms of PAA-Cu^{2+}-BSA Complex at different $[Cu^{2+}]$ concentrations (mol/l)



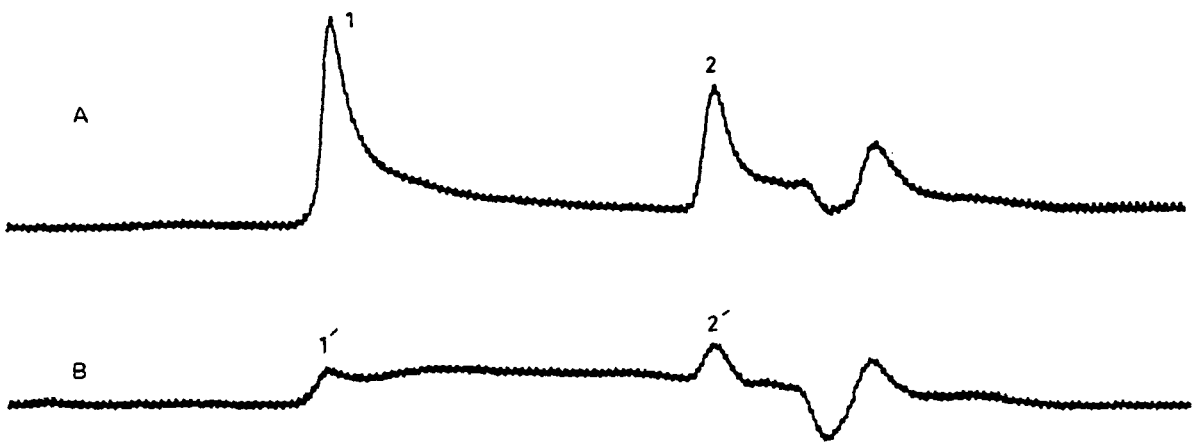
ID	RT	Area	Area%
1	5.66	71097	8.24
2	6.65	79619	91.76

Peak ID	RT	Area	Normalized Area %
1'	6.53	689867	100

Appendix 7. Original HPLC Chromatograms of BSA before (A) and after irradiation (B)

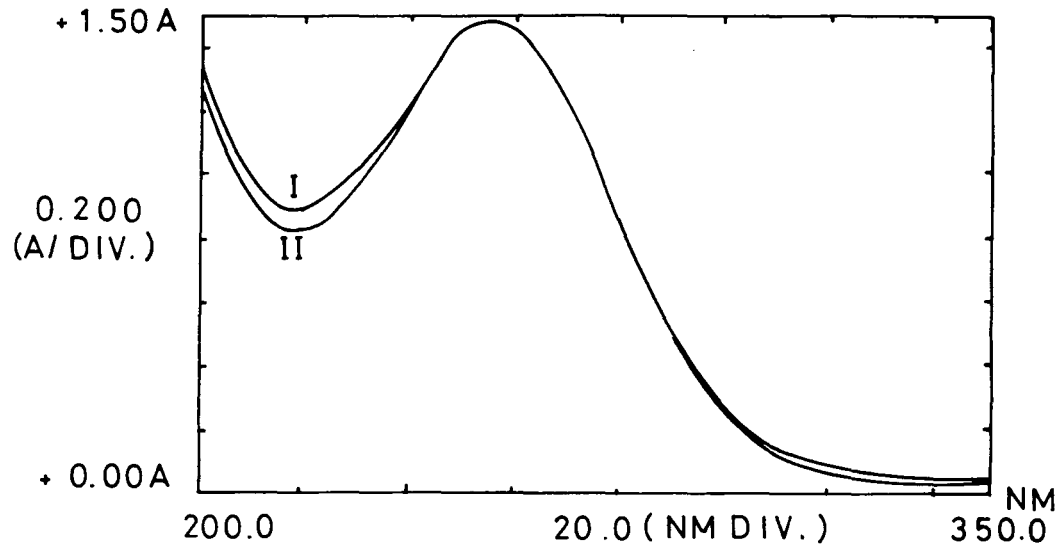


Appendix 8. UV-Visible spectrum of PAA-Cu^{2+}in aqueous solution before (I) and after irradiation (II)

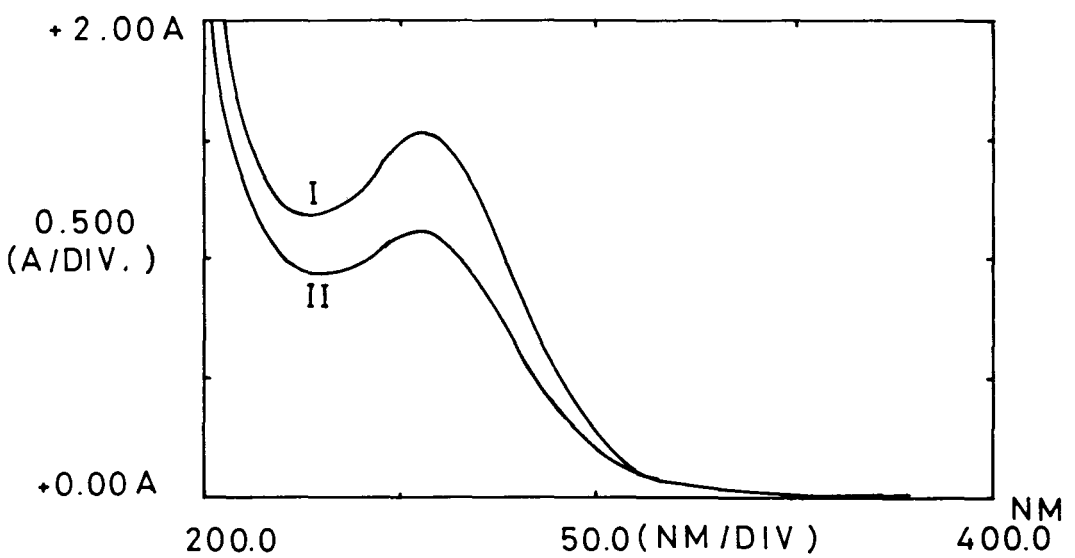


Appendix 9. HPLC Chromatograms of PAA-Cu^{2+} in aqueous solution before (A) and after irradiation (B)

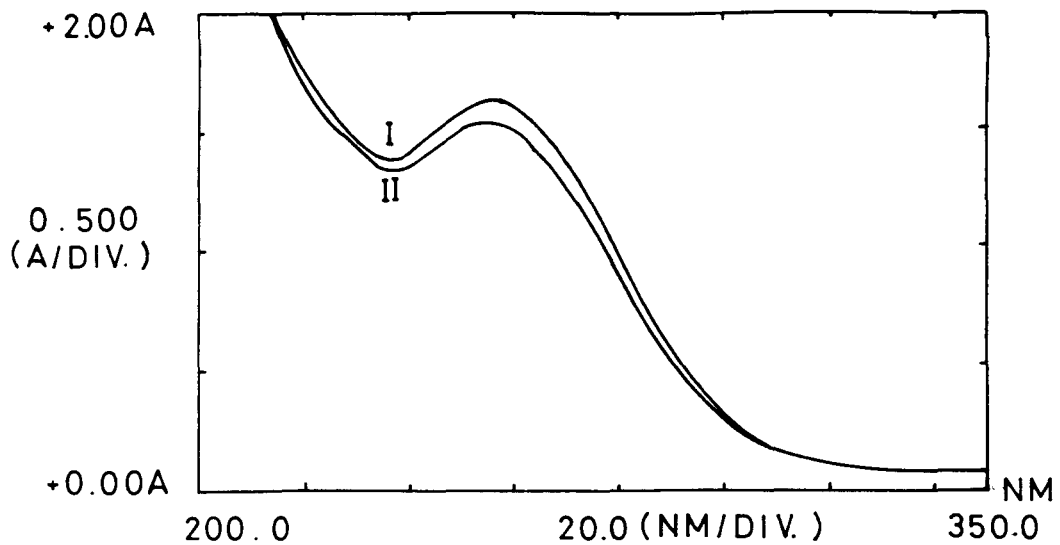
Peak ID	RT	Area	Normalized Area %
1	4.31	109104	54.93
2	9.33	89531	45.07
1'	4.48	35276	56.65
2'	9.33	26691	43.44



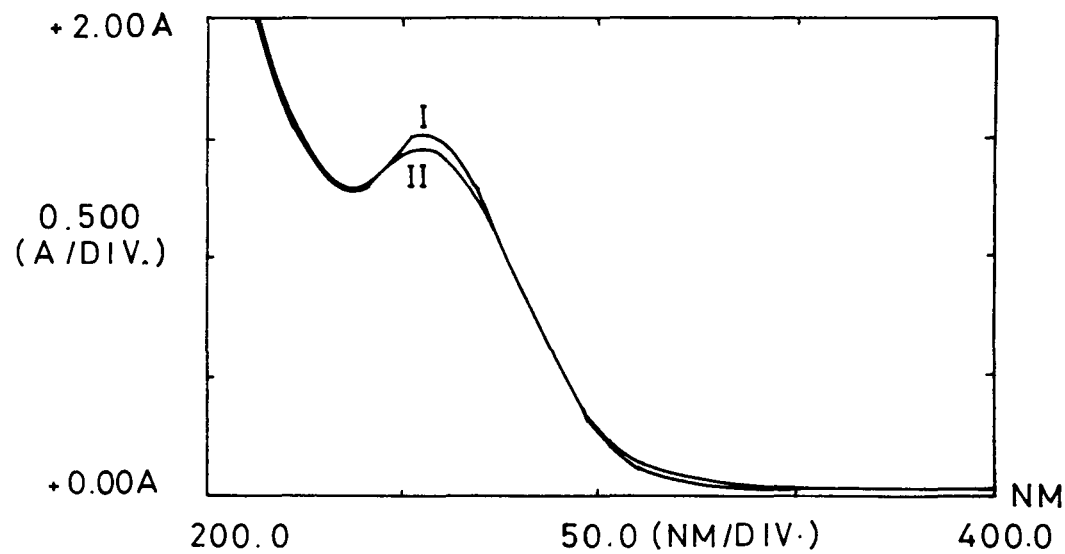
Appendix 10.1. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}=0.13; [Cu^{2+}]=1.4x10^{-3} mol/l before (I) and after irradiation (II)



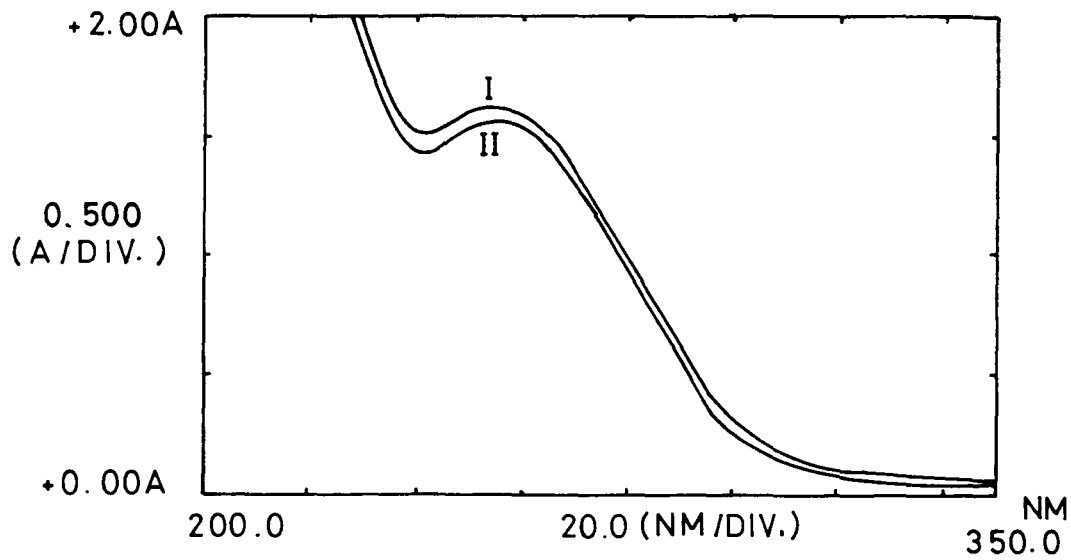
Appendix 10.2. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}=0.51; [Cu^{2+}]=1.4x10^{-3} mol/l before (I) and after irradiation (II)



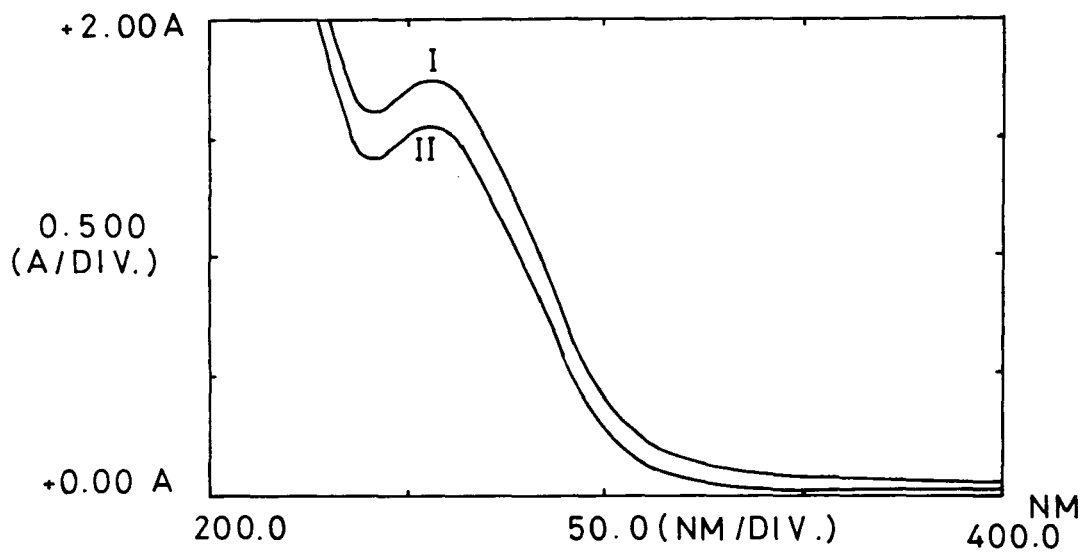
Appendix 10.3. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}= 1.00; $[Cu^{2+}]=1.4x10^{-3}$ mol/l before (I) and after irradiation (II)



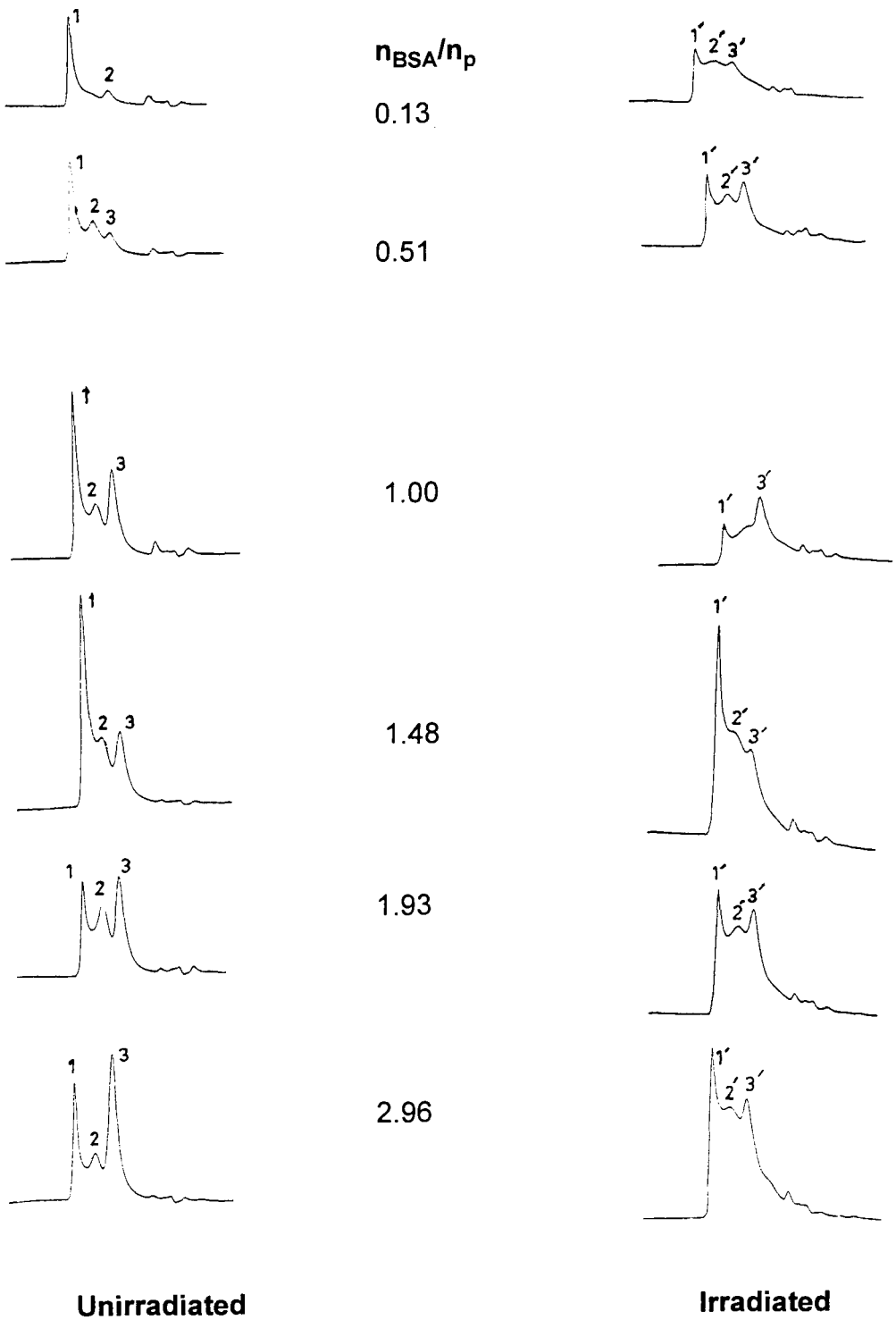
Appendix 10.4. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}= 1.48; $[Cu^{2+}]=1.4x10^{-3}$ mol/l before (I) and after irradiation (II)



Appendix 10.5. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}= 1.93; $[Cu^{2+}]$= $1.4x10^{-3}$ mol/l before (I) and after irradiation (II)



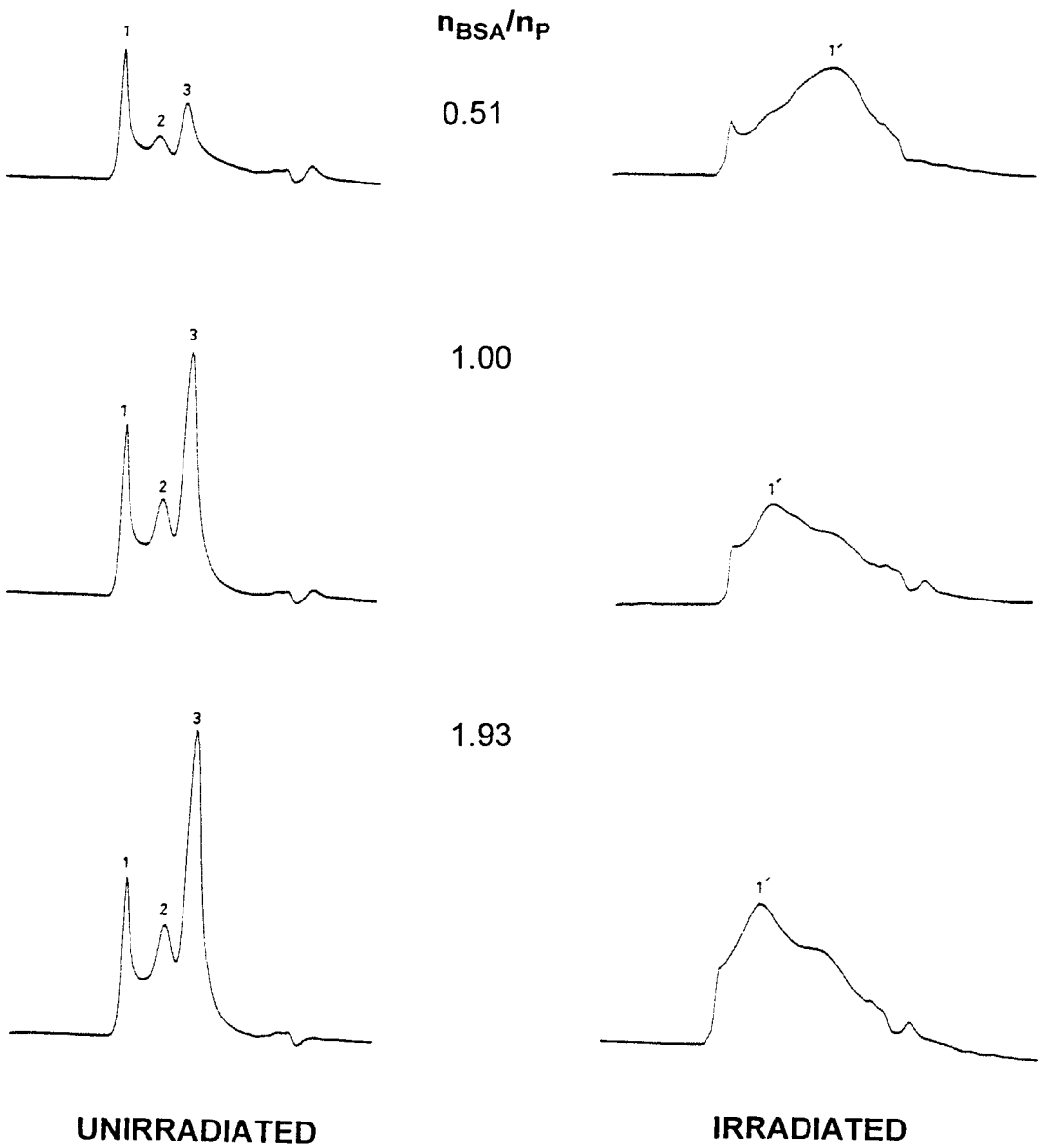
Appendix 10.6. UV-Visible spectrum of ternary PAA-Cu^{2+}-BSA complexes at n_{BSA}/n_{AA}= 2.96; $[Cu^{2+}]$= $1.4x10^{-3}$ mol/l before (I) and after irradiation (II)



Appendix 11.1. Original HPLC Chromatograms of unirradiated and irradiated PAA-Cu^{2+}-BSA Complex at different n_{BSA}/n_p ratio, $[Cu^{2+}]=2.8x10^{-3}$ mol/l

Appendix 11.2 Unirradiated and irradiated PAA-Cu^{2+}-BSA Complexes at different n_{BSA}/n_p ratio, $[Cu^{2+}] = 2.08 \times 10^{-3}$ mol/l

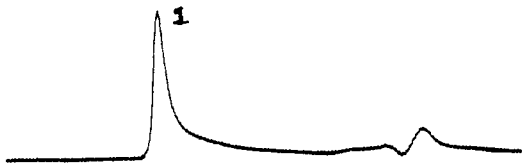
	UNIRRADIATED				IRRADIATED			
n_{BSA}/n_P	ID	RT	Area	Area %	ID	RT	Area	Area %
0.13	1	4.31	233568	77.94	1'	4.37	17340	18.46
	2	6.61	66108	22.06	2'	6.67	76596	81.54
0.51	1	4.31	277743	58.20	1'	4.33	233645	31.60
	2	5.60	112834	23.64	2'	5.63	117840	15.94
	3	6.66	86795	18.16	3'	6.69	387784	52.46
1.00	1	4.31	367384	53.24	1'	4.33	134995	50.45
	2	5.61	121306	17.58	2'	-	-	-
	3	6.66	201322	29.18	3'	6.67	132570	49.55
1.48	1	4.33	467895	58.70	1'	4.33	467875	51.43
	2	5.73	155965	19.56	2'	5.74	233948	25.71
	3	6.67	173294	21.74	3'	6.67	207953	22.86
1.93	1	4.28	207953	34.64	1'	4.37	281733	39.00
	2	5.55	162897	27.18	2'	5.64	195476	27.10
	3	6.59	228749	34.18	3'	6.67	245088	33.90
2.96	1	4.28	259942	37.50	1'	4.37	363919	41.38
	2	5.55	103997	15.00	2'	5.62	238356	27.10
	3	6.69	329259	47.50	3'	6.76	277221	31.52



Appendix 12.1. Unirradiated and irradiated PAA-BSA Complexes at different n_{BSA}/n_p ratio

Appendix 12.2 Unirradiated and irradiated PAA-BSA Complexes at different n_{BSA}/n_D ratio

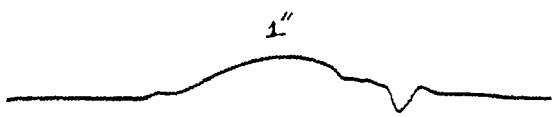
	UNIRRADIATED				IRRADIATED			
n_{BSA}/n_P	ID	RT	Area	Area %	ID	RT	Area	Area %
0.51	1	4.31	162777	58.20	1'	4.35	94552	14.35
	2	5.65	26549	23.64	2'	8.04	564288	85.65
	3	6.69	126340	18.16	3'	-	-	-
1.00	1	4.32	209780	25.75	1'	4.32	96760	27.73
	2	5.74	102796	12.62	2'	5.03	251193	72.27
	3	6.70	502006	61.63	3'	-	-	-
1.93	1	4.29	187462	18.75	1'	5.36	217740	100
	2	5.81	134886	13.50	2'	-	-	-
	3	6.76	677158	67.75	3'	-	-	-



ID	RT	Area	Area %
1	4.31	233626	100

24.36 rad/min

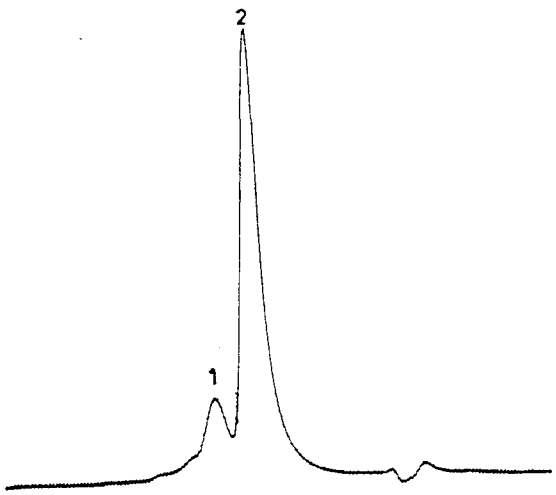
ID	RT	Area	Area %
1'	7.69	529974	100



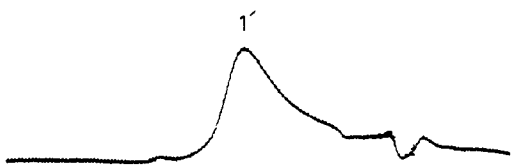
102857 rad/min

ID	RT	Area	Area %
1"	7.68	331235	100

Appendix 13. HPLC Chromatograms of unirradiated PAA (1) and irradiated PAA solutions (1', 1") at two different dose rate



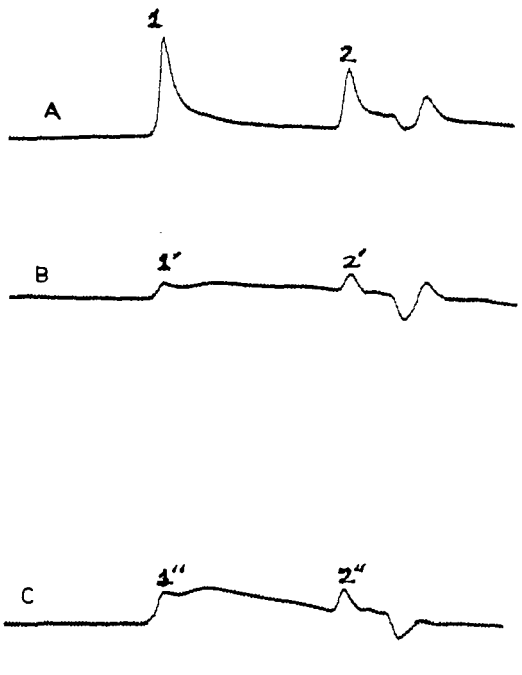
ID	RT	Area	Area %
1	5.66	71097	8.24
2	6.65	79619	91.76



	ID	RT	Area	Area %
24.36 rad/min	1'	6.53	689867	100

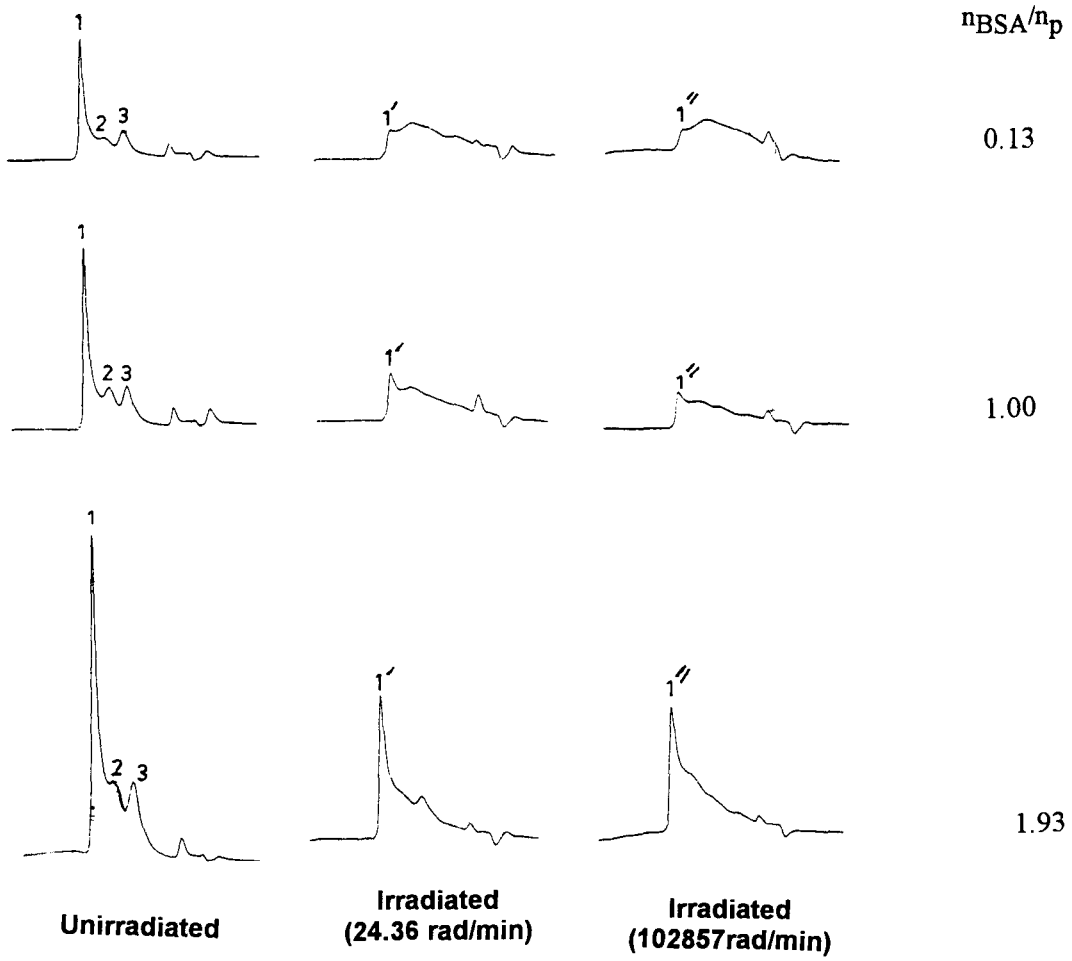
	ID	RT	Area	Area %
102857 rad/min	1''	6.55	687960	100

Appendix 14. HPLC Chromatograms of unirradiated (1) and irradiated BSA solution at two different dose rate (1', 1'')



	ID	RT	Area	Area %
	1	4.31	109104	54.93
	2	9.33	89531	45.07
24.36 rad/min	1'	4.48	35276	56.65
	2'	9.33	26691	43.44
102857 rad/min	1"	4.45	50550	57.16
	2"	9.35	37890	42.84

Appendix 15. HPLC Chromatograms of unirradiated (A) and irradiated PAA-Cu^{2+} solutions (B, C) at two different dose rate



Appendix 16. Original HPLC Chromatograms of unirradiated and irradiated PAA-Cu^{2+}-BSA Complexes at two different dose rate, $[Cu^{2+}]=2.8x10^{-3}$ mol/l

n_{BSA}/n_P	ID	RT	Area	A. %	ID	RT	Area	A. %	ID	RT	Area	A. %
0.13	1	4.31	249701	86.03	1'	4.48	124534	100	1"	4.47	123990	100
	2	6.54	40556	13.97	2'	-	-	-	2"	-	-	-
					3'	-	-	-	3"	-	-	-
1.00	1	4.31	351359	82.79	1'	4.27	86998	87.73	1"	4.25	85590	87.61
	2	5.61	16522	3.90	2'	9.27	12169	12.27	2"	9.29	12100	12.39
	3	6.65	56522	13.31								
1.93	1	4.31	1332461	87.88	1'	4.25	666230	45.45	1"	4.28	646780	100
	2	5.66	30040	1.98	2'	-	-	-	2"	-	-	-
	3	6.55	88238	5.82	3'	6.50	799477	54.45	3"	-	-	-

REFERENCES

1. Gordy, W., "Electron Spin Resonance of Free Radicals in Irradiated Biochemicals", *Radiation Research Suppl.* Vol. 1, pp. 491-510, 1959.

2. Gordy, W. and I. Miyogawa, "Electron Spin Resonance Studies of Mechanisms for Chemical Protection from Ionizing Radiation", *Radiation Research*, Vol. 12, pp. 211-229, 1960.

3. Eidus, K., M.B. Kalamkarova, and G. K. Otarova, "The Migration Mechanism of Radiation Protection", *Biophysics*, Vol. 2, pp.564-566, 1957.

4. Saldick, J., "Thermal Initiation of Vinyl Polymerization by Ceric Solutions", *Journal of Polymer Science*, Vol.19, pp. 73-75, 1956.

5. Mino, G., S. Kaizerman, and B. Rasmussen, "Polymerization of Acrylamide Initiated by Ceric Nitrate-3-Chloro-1-Propanol Redox Systems", *Journal of Polymer Science,* Vol.38, pp. 393-401, 1959.

6. Subramanian, S.V., and M. Santappa, " Vinyl Polymerization Initiated by Ceric Ion Reducing Agent Systems in Sulfuric Acid Medium", *Journal of Polymer Science*, Part A-1, Vol.6, pp. 493-504, 1968.

7. Saraç, A.S., H. Başak, A.B. Soydan, and A. Akar, "Polymerization of Acrylamide by Electrolytically Generated Ce(IV)-Organic Acid Redox Systems", *Die Angewandte Macromolekulare Chemie,* Vol. 198, pp. 191-198, 1992.

8. Hanna, S. B., and A.S. Saraç, "Metal-Ion Oxidative Decarboxylations. 9. Reaction of Benzilic Acid and Cerium (IV) in Acidic Perchlorate and Sulfate Media", *Journal of Organic Chemistry*, Vol. 11, 2063-2068, 1977.

9. Erbil, C., C. Cin, A.B. Soydan, and A.S. Saraç, "Polyaminocarboxylic Acids- Ce(IV) Redox Systems as an İnitiator in Acrylamide Polymerization", *Journal of Applied Polymer Science*, Vol. 47, pp. 1643-1648, 1993.

10. Saraç, A.S., C. Erbil, and A.B. Soydan, "Polymerization of Acrylamide Initiated with Electrogenerated Cerium (IV) in the presence of EDTA", *Journal of Applied Polymer Science*, Vol. 44, pp. 877-881, 1992.

11. Petrov, R.V., M.I. Mustafaev and A. Norimov, "Physico-Chemical Criteria for the construction of Artificial Modulators and Immunogens on the Basis of Polyelectrolyte Complexes", *Soviet Medical Rewievsl* Section D, Vol. 4, part 3, pp. 1-113, 1992.

12. Patt, H. M., E. B. Tyree, R. L. Straube, and D. E. Smith, "Cysteine Protection Against X-Irradiation", *Science*, Vol. 10, pp. 213-214, 1949.

13. Bacq, Z. M., and A. Herve, "Protection of a Mice Against a Lethal Dose of X-Rays by Cyanide, Azide and Malonitrile", *British Journal of Radiology*, Vol. 24, pp. 617-621, 1949.

14. Shapiro, B., and E. A. Dickens, "The Mechanism of Action of AET. I. The Radiation Chemistry of 2-Mercaptoethylguanidine and Bis(2-guanidoethyl) disulfide in Aqueous Buffered Solutions", *Radiation Research*, Vol. 13, pp. 857-870, 1960.

15. Pihl, A., and L. Eldjarn, *In Advances in Radiobiology*, Oliver & Boyd, Edinburg, 1957.

16. Pizzarello, D. J., and L.G. Colombetti, *Radiation Biology*, CRC Press, Inc., Florida, 1960.

17. Bacq, Z.M., and P. Alexander, *Fundamentals of Radiobiology*, Pergamon Press, New York, 1961.

18. Brown, P.E., "Mechanism of Action of Aminothiol Radioprotectors", *Nature*, Vol. 213, pp. 363-364, 1967.

19. Eldjarn, L., and A. Phil, *Mechanisms in Radiobiology*, Academic Press, New York, 1960.

20. Doherty, D. G., *Radiation Protection and Recovery*, Pergamon Press, London, 1961.

21. Bacq, Z.M., *Chemical Protection Against Ionizing Radiation*, Charles C. Thomas Publisher, New York, 1965.

22. Breccia, A., and E. Gattavecchia, "Radiation Chemistry Studies for the Development of New Drugs", *La Chimica E L'Industria*, Vol. 65, No:5, pp. 354-358, 1983.

23. Patt, H.M., "Protective Mechanism in Ionizing Radiation Injury", *Physiol. Revision*, Vol. 33, pp. 35-76, 1953.

24. Kabanov, V.A., A.B. Zezin, M.I. Mustafaev, and V.A. Kasaikin, "Soluble nterpolymer Complexes of Polyamines and Polyammonium Salts", *Polymeric Amines and Ammonium Salts,* Pergamon Press, New York, pp. 173-192., 1980.

25. Mustafaev, M.I. and A.S. Norimov, "Immunogeneicity and Protective Activity of Protein Antigenic Water-Soluble Polymer-Metal Complexes", *Biomedical Science*, Vol. 1, pp. 274-278, 1990.

26. Hara, M., *Polyelectrolytes Science and Technology*, Marcel Dekker, Inc., 1993.

27. Mustafaev, M.I., and V.A. Kabanov, "A Water-Soluble Ternary Poly-4-vinylpyridine - Bovine Serum Albumin - Cu^{2+} Complex", *Vysokomolekulyarniye Soyedineniya High Molecular Weight Compounds*, Vol, 23 A, pp. 275-2271, 1979.

28. Immergut, E.H., "Cellulose, Graft Copolymers", *Encylopedia of Polymer Science and Technology*, Vol. 3, pp. 242-283, 1965.

29. Mino, G., and S. Kaizerman, "A New Method for the Preparation of Graft Copolymers", *Journal of Polymer Science,* Vol.31, pp. 242-243, 1958.

30. Fanta, C.F., and E.B. Bagley, "Starch, Graft Copolymers", *Encylopedia of Polymer Science Technology, Suppl.* Vol. 2 , pp. 665-699, 1977.

31. Akar, A., O.Galioğlu, A. Göçmen, and A. S. Saraç, "Copolymer of Ketonic Resin - Polyacrylonitrile", *Journal of Applied Polymer Science*, Vol. 39, pp. 1657-1663, 1990.

32. Waltz, J.E., and G.B. Taylor, "Determination of the Molecular Weight of Nylon", *Analytical Chemistry*, Vol. 19, pp 448-450, 1947.

33. Garmon, R.G., and M.G. Gibson, "New Specific, Sensitive Methods for Determining Amine and Carboxyl End Groups in Nylon 60, *Analytical Chemistry*, Vol. 37, pp. 1309-1312, 1965.

34. Flory, P.J., *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York, 1953.

35. Rodriguez, F., Principles of Polymer Chemistry, McGraw-Hill Book Co., New York, 1970.

36. Campbell, D., and J.K. White, *Polymer Characterization Physical Techniques,* Chapman and Hall, New York, 1963.

37. Huggins, M.L., "The Viscosity of Dilute solutions of Long-Chain Molecules, IV. IV. Dependence on Concentration", *Journal of American Chemical Society*, Vol. 64, pp. 2716-2718, 1942.

38. Kraemer, E.O., "Molecular Weights of Cellulose and Cellulose Derivatives", *Industrial Engineering Chemistry*, Vol. 30, pp. 1200-1203, 1938.

39. Simpson, C.F., *Practical High Performance Liquid Chromatography*, Heyden and Son Limited, London, 1978.

40. Ottenbrite, R. M. and W. Regelson, "Water Soluble Polymers", Encylopedia of Polymer Science and Technology, pp. 111-128, 1977.

41. Harland, R.S., and R. K. Prud'homme, *Polyelectrolyte Gels Properties, Preparation and Applications*, American Chemical Society (ACS) Symposium 480 Series, Washington, 1992.

42. Michaels, A.S., "Polyelectrolyte Complexes", *Enyclopedia of Polymer Science and Technology*, Vol.10, pp. 765-780, 1969.

43. Zezin, A.B., and Kabanov, V.A., "A New Class of Complex Water-Soluble Polyelectrolytes", *Advances in Chemistry*, Vol. 11, pp. 1447-1451, 1983.

44. Frickie, H., and S. Morse, "The action of X-rays on Ferrous Sulphate Solutions", *Phil. Mag.*, Vol. 7, pp.129-141, 1929.

45. Draganic, I.G., and Z. D. Draganic, *The Radiation Chemistry of Water*, Academic Press, New York, 1971.

46. Allen, A.O., "Radiation Chemistry of Aqueous Solutions", *Journal of Physics and Colloid Chemistry*, Vol. 52, pp. 479-490, 1948.

47. Weiss, J., "Radiochemistry of Aqueous Solutions", *Nature*, Vol. 153, pp. 748-756, 1944.

48. Allen, A. O., "Mechanism of Decomposition of Water by Ionizing Radiations", Discussions Faraday Society, Vol. 12, pp. 79-87, 1952.

49. Swallow, A. J., *Radiation Chemistry An Introduction*, John Wiley & Sons, Great Britain, 1973.

50. Hart, E.J., and Anbar, M., *The Hydrated Electron*, Wiley Interscience, New York, 1970.

51. Stein, G., "Some Aspect of the Radiation Chemistry of Organic Solutes" *Discussions Faraday Society*, Vol. 12, pp. 227-234, 1952.

52. Platzman, R.L., "Superexcited States of Molecules, and the Primary Action of Ionizing Radiation", *The Vortex*, Vol. 28, pp. 372-385, 1962.

53. Hart, E.J., and J.W. Boag, "Absorption Spectrum of the Hydrated Electron in Water and in Aqueous Solutions", *Journal of the American Chemical Society*, Vol. 84, pp. 4090-4095, 1962.

54. Rabani, J., and G. Stein, "Yield and Reactivity of Electrons and H Atoms in Irradiated Aqueous Solutions", *Journal of Chemical Physics*, Vol. 37, pp. 1865-1873, 1962.

55. Frickie, H., and S. Morse, "The Chemical Action of Roentgen rays on Dilute Ferrosulphate Solutions as a Measure of Dose", *American Journal of Roentgenology and Radium Therapy*, Vol. 18, pp. 430-432 , 1927.

56. Scharf, K., and R.M. Lee, " Investigation of the Spectrophotometric Method of Measuring the Ferric Ion Yield in the Ferrous Sulfate Dosimeter", *Radiation Research*, Vol. 16, pp. 115-124, 1962.

57. Chapiro, A., *Radiation Chemistry of Polymeric Systems*, John Wiley & Sons, New York, 1962.

58. Alexander, P., and A. Charlesby, "Effect of X-rays and γ-Rays on Synthetic Polymers in Aqueous Solution", *Journal of Polymer Science*, Vol. 23, pp. 355-375, 1957.

59. Charlesby, " Cross-linking of Polyethylene by Pile Radiation", *Proc. Roy. Soc.* (London), Vol A125, pp. 187-214, 1952.

60. Henglein, A., "Crosslinking of Polymers in Solution under the Influence of γ-Radiation", *Journal of Physical Chemistry*, Vol. 63, pp. 1852-1858, 1959.

61. Broams, R., "Rate Constants of Hydrated Electron Reactions with Peptides and Proteins", *Radiation Research*, Vol. 31, pp. 8-26, 1967.

62. Billmeyer, F. W., Textbook of Polymer Science, John Wiley & Science, New York, 1984.

63. Baysal, B., G. Adler, D. Ballantine, and A. Glines, "Polymer Fractionation Studies in the Solid State Polymerization of Acrylamide", *Polymer Letters*, Vol.1, pp. 257-262, 1963.

64. Erbil, C., A. B. Soydan, A.Z. Aroğuz, A.S. Saraç, "The Free Radical Polymerization of Acrylamide Initiated with Ceric Sulfate in the Presence of a Amino Acids", *Die Angewandte Makromolekulare Chemie*, 1994 (In Press).

65. Alias, P. S., and A. J. Cohen, *Radiation Chemistry of Major Components*, Elsevier Scientific Publishing Company, Amsterdam, 1977.

REFERENCE NOT CITED

1. Lindsay, S. *High Performance Liquid Chromatography*, John Wiley & Sons, Chichester, 1992.

2. Meyer, V. R., *Practical High-Performance Liquid Chromatography*, John Wiley & Sons, Chichester, 1988.

3. Synder, L. R., and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, John Wiley & Sons, New York, 1979.

4. Engelhardt, H., *Practice of High Performance Liquid Chromatography*, Springer-Verlag, Berlin, 1986.