0273-2289/01/90/0023/$13.25

Spectroscopic and Electrochemical Investigation of Ternary Complexes of D- or L-Aspartic Acid Containing Polyacrylamides-Cu^{2+}-Bovine Serum Albumin and Their Radiostability

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Received July 1, 1999; Revised February 1, 2000; Accepted June 1, 2000

Abstract

D- or L-aspartic acid containing polyacrylamides were synthesized. Binary and ternary complex formation between these polymers with copper and bovine serum albumin was studied by spectroscopic and electrochemical measurement. Depending on the ratio of the polymer/copper and protein/polymer, the mixture exhibited water-soluble and insoluble character. A hypothetical structural scheme for the formation of ternary complexes is proposed. The effect of radiation on these complexes was also investigated.

Index Entries: Ternary complexes; radiostability; biopolymer; electrochemistry of ternary complexes; polyacrylamide.

Introduction

Some synthetic polymers and polyelectrolytes have been found to increase the immunoresponse to the immunizing antigen and thus produce an adjuvant effect *(1,2)*. It is necessary to modify the carrier polymer, which can give rise to changes in its effect on biologic systems. In such systems, transition metal ions promote the complex formation without causing any appreciable change in chemical structure. Evidence has recently been presented for the existence of a ternary complex among proteins, Cu^{2+} ions, and amino acids *(3,4)*.

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Soluble and insoluble complexes of bovine serum albumin (BSA) have been studied previously *(5,6)*. Water-soluble polyelectrolytes and their various complexes have possible potential for radioprotective activity *(7)*.

Polymers used in the present study were water-soluble polyacrylamides (PAAm) that contain the amino acid end group. Polymerization and characterization of different end groups containing polyacrylamides have been studied in detail previously *(8–12)*. Such polymers have the potential for construction of high-molecular-weight polymers containing weakly bounded structure that can be destroyed in physiologic conditions. The rate of the interactions of the water-soluble glycine end group containing polyacrylamides with BSA in the presence of divalent copper ions was studied at different conditions *(5)*. On the basis of results represented in this study, hypothetical structural schemes for the formation of the soluble and insoluble ternary polycomplexes were proposed.

Because the amino acid end group containing polyacrylamides may represent biopolymer-like behavior, binary and ternary complexes of these polymers can be applicable for biosystems.

Recently, there has been a steady increase in the number of publications of immunologically active synthetic high-molecular-weight polyelectrolyte compounds. An investigation of the mechanism and the factors affecting complexation using well-characterized synthetic polyelectrolytes is important because these systems serve as models for complexes such as biopolymers *(12–16)*. Polyanions form ion coordinate complexes with proteins by crosslinks via metal ions. It has been shown that during metabolic processes in the organism, metals form more or less stable complexes with biologic ligands. The increasing interest in investigations of polymer–metal complexes (PMC) is primarily owing to the crucial role of metal ions in biologic processes *(17–19)*.

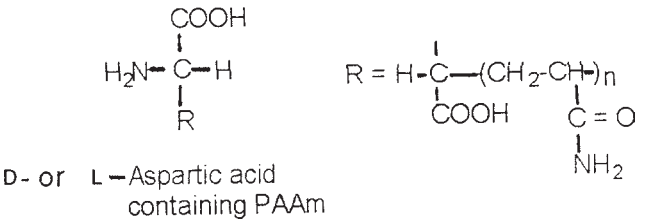
In the present study, water-soluble ternary complex formation between aspartic acid containing polyacrylamides and BSA in the presence of divalent copper ion was studied, and γ-radiolysis of these complexes was also investigated at various irradiation conditions before evaluating their possible use as a radioprotector.

Materials and Methods

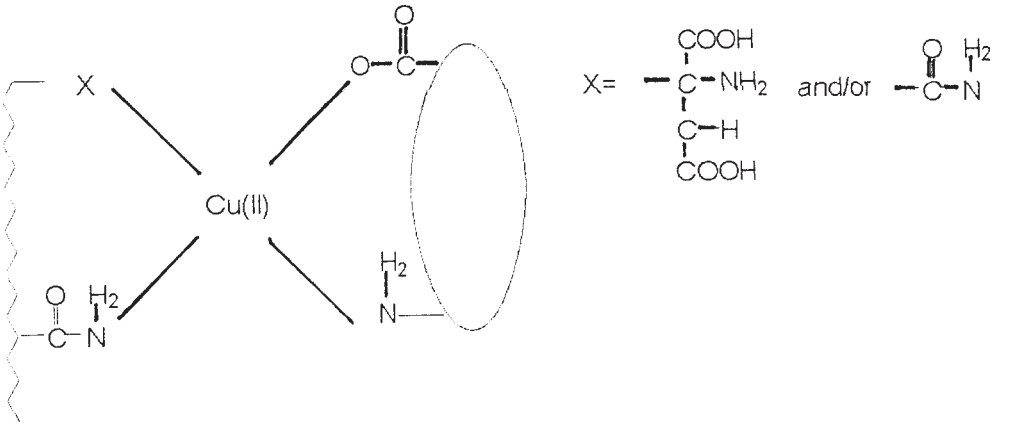
Materials

Ammonium cerium (IV) nitrate (Ce[IV]) (Merck), acrylamide (AAm), L-aspartic acid, D-aspartic acid BSA (Fluka AG), and $CuSO_4 \cdot 5H_2O$ (Merck) were used without further treatment.

The polymerization procedure was similar to those reported earlier *(8–12)*. In each experiment, the concentrations of AAm, Ce(IV), and amino acids were kept at 0.7034, 0.02, and 0.0335 mol/L, respectively. The polymerization of the AAm monomer is initiated by an aspartic acid radical, which is formed by the reactions of Ce(IV)–Ce(III). The polyacrylamides synthesized, using this procedure, have the chemical structures given in Scheme 1.



Scheme 1. Structures of polyacrylamides used.



Scheme 2. Possible structure of ternary complex.

The molecular weights of polyacrylamides were determined by viscosity measurements in water at 30°C in an Ubbelohde viscometer, using the relationship derived by Collinson et al. *(20)*.

Preparation of Complexes

D-Aspartic acid (mol wt = 19,100) or L-aspartic acid (mol wt = 20,650) containing PAAm was dissolved in a bidistilled water and the pH was adjusted to 7.0 by the addition of 1 *M* NaOH. For the preparation of binary complex, the molar ratio of Cu^{2+} to AAm was chosen as $n_{Cu^{2+}}/n_{PAAm} = 0.15$ and $[Cu^{2+}] = 2.1 \times 10^{-3}$ *M*. Ternary complex was prepared by adding BSA to binary complex to obtain $n_{PAAm}/n_{BSA} = 1.0$. Details of the preparation of polymer–metal and polymer–metal–protein complexes are given in a previous study *(21)*. The chemical structure of ternary complex may be given in Scheme 2.

Measurements

Electrochemical measurements were made in a standard three-electrode cell with a Wenking POS 73 Model potentiostat and Kipp and Zonen X-Y recorder. A platinum wire electrode as a working electrode and as a counterelectrode and Ag/AgCl as a reference electrode were chosen. All measurements were made in deoxygenated medium, by passing nitrogen gas through the solution for 20 min.

Spectrophotometric measurements were made using a Shimadzu UV-2401 PC UV-Vis Recording Spectrophotometer. Fourier transform infrared (FT-IR) measurements were made using a Mattson 1000 FT-IR Spectrophotometer in KBr pellets.

High-performance liquid chromatography (HPLC) measurements were made using a Waters Model 510 Pump, a Waters Lamda-Max Model 481 LC spectrophotometer, a Waters 746 data module integrator, and an Ultrahydrogel Linear Column (7.8×300 mm). The HPLC experiments were run by using a buffer containing 0.05 *M* Na_2HPO_4, 0.05 *M* NaH_2PO_4, 0.15 *M* NaCl, 0.01 *M* NaN_3 (pH 6.8). Elution was isocratic at a flow rate of 0.5 mL/min. The mobile phase and samples were filtered (0.45-μm filter pore size; Waters). Twenty-microliter samples were injected into the chromatograph. The samples were monitored at 280-nm wavelength, at which BSA has a maximum peak. The column was maintained at ambient room temperature.

Irradiation was performed by using a ^{60}Co γ-source of 36 Ci. Dosimetry measurements were performed by using the Fricke dosimetry method *(22)*, and the dose rate was 44.77 Gy/h.

Results and Discussion

Electrochemical Measurements

The interaction of Cu^{2+} with the end group containing PAAm and BSA in the ternary mixture was investigated by cyclic voltammetry analysis at different pH values. To obtain some qualitative information before doing that, the interaction of the two components was checked. No peaks were observed for PAAm and BSA solution in the range of study (+500 to –800 mV vs Ag/AgCl). The cyclic voltammograms of 2×10^{-3} *M* Cu^{2+} ions and PAAm–Cu^{2+} mixture at pH 4.5 are given in Fig. 1, [a] and [b], respectively. A single cathodic peak was observed at about –300 mV for Cu^{2+}, and the reverse scan exhibited an anodic peak at –100 mV. The cathodic peak was attributed to the reduction in Cu^{2+} to Cu^0 and the reverse peak to its oxidation. In the presence of PAAm with aspartic acid functional end group, peak potentials shifted to a more cathodic region and peak currents decreased owing to difficulty of electron transfer in the presence of polymer (Fig. 1).

At a higher pH (10.5), for binary and ternary complexes, the cathodic peak shifted to a more cathodic region and the anodic peak disappeared (Fig. 2). PAAm and BSA contain both -COOH and -NH_2 groups, and at

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Fig. 1. *(opposite page)* Cyclic voltammograms of Cu(II) (a) and PAAm-Cu(II) binary complexes (b) at pH 4.5 in 0.1 *M* $NaClO_4$ containing water: [Cu(II)] = 2×10^{-3} *M*; [PAAm] = 4.84×10^{-6} *M*. Working and counterelectrodes, Pt; reference electrode, Ag/AgCl; scan rate, 50 mV/s[1].

Fig. 2. *(opposite page)* Cyclic voltammograms of PAAm-Cu(II) binary complexes (a) and PAAm-Cu(II)-BSA ternary complexes (b) at pH 10.5 in 0.1 *M* $NaClO_4$ containing water: [Cu(II)] = 2×10^{-3} *M*; [PAAm] = 4.84×10^{-6} *M*. Working and counterelectrodes, Pt; reference electrode, Ag/AgCl; scan rate, 50 mV/s[1] $n_{BSA}/n_{PAAm} = 1$.

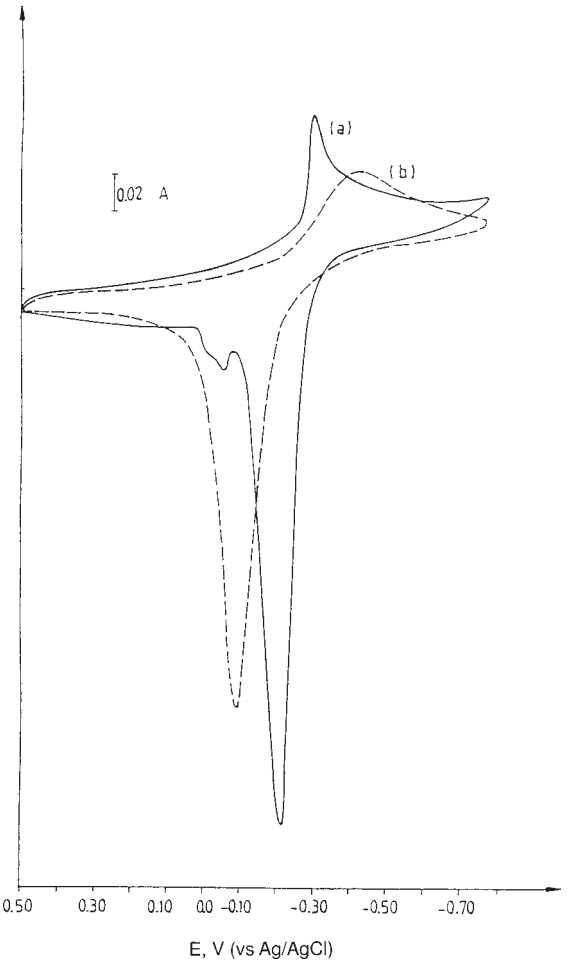
(a)
(b)
0.02 A
0.50
0.30
0.10
0.0
-0.10
-0.30
-0.50
-0.70
E, V (vs Ag/AgCl)

Fig. 1

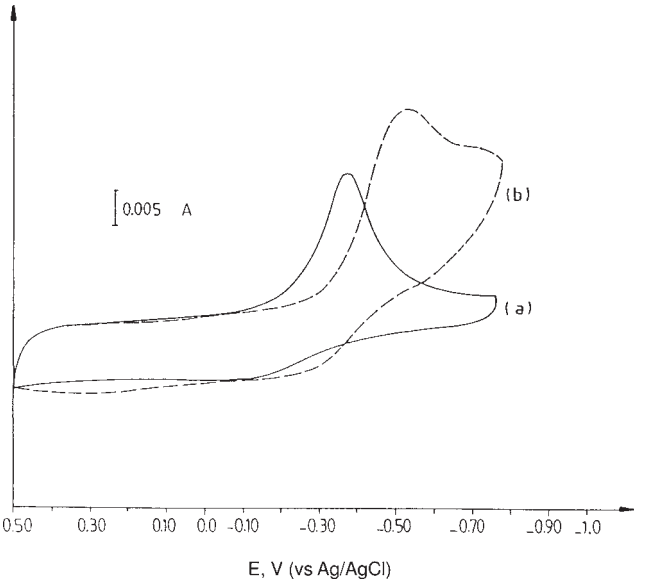
0.005 A
(b)
(a)
0.50
0.30
0.10
0.0
-0.10
-0.30
-0.50
-0.70
-0.90
-1.0
E, V (vs Ag/AgCl)

Fig. 2

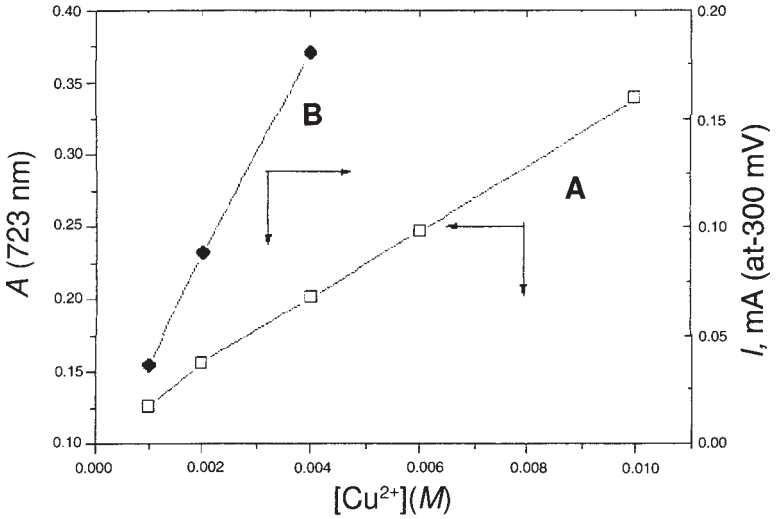

Fig. 3. Changes in current and absorbance values with copper concentration. [PAAm] = 0.1%; t = 25°C.

acidic pH, interaction owing to basic group occurs and at basic pH vice versa. An increase in pH increases the amount of copper ion incorporated into the complex structure, and irreversible charge transfer occurs owing to difficulties resulting in more inclusion of macromolecules.

One can suggest that at basic pH, hydrolysis of Cu^{2+} ions can occur. Although this is true for Cu^{2+} solution alone, there is no precipitation in the presence of polymer. In the presence of monomer at pH > 6.0, hydrolysis of Cu^{2+} also occurred, whereas in the presence of polymer solution, no precipitation was observed up to pH 11.0 (Fig. 2).

On the other hand, peak currents, corresponding to a reduction in Cu^{2+} (at E_a = –300 mV) increase with an increase in copper concentrations (Fig. 3, [a]). This result shows parallelism with the UV-visible spectrophotometric measurements (Fig. 3, [b]), suggesting the formation of complex.

Spectrophotometric Measurements

UV-Visible Measurements

The UV-visible absorption spectra of PAAm, PAAm-Cu^{2+} binary, and PAAm-Cu^{2+}-BSA ternary complexes were measured before and after exposure to irradiation, and the results are given in Figs. 4, 5, 6, and 7, respectively. The UV-visible absorption spectrum of D-aspartic acid containing PAAm has two broad maximums at about 260 and 280 nm (Fig. 4, curve 1).

When D-aspartic acid containing PAAm (Fig. 4, curve 1) was exposed to 300 (Fig. 4, curve 2) and 1200 Gy (Fig. 4, curve 3), the spectral shape remained almost the same as before irradiation (Fig. 4, curve 1). The absor-

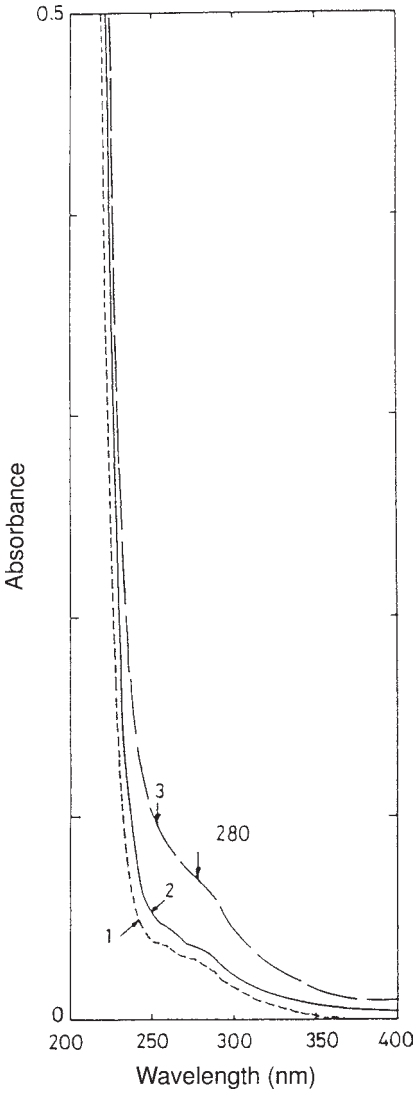

Fig. 4. UV-visible spectra of D-aspartic acid end group containing PAAm; curve 1, nonirradiated; curve 2, after exposition to 300 Gy; curve 3, 1200 Gy irradiation.

bance values regarding the nonirradiated samples increased with increasing radiation dose (Fig. 4, curves 2 and 3), and this may be owing to some changes in conformation.

In the case of binary complex, a maximum was observed at $\lambda = 254$ nm, and only a minor change was observed after irradiation (Fig. 5, curves 1–3).

The ternary complexes showed a maximum at $\lambda = 265.2$ nm. These red shifts in accordance with D-PAAm alone and binary complexes also indicate the formation of complexes. The absorbance values increased with increasing radiation dose (Fig. 6, curves 2 and 3). In addition to this peak,

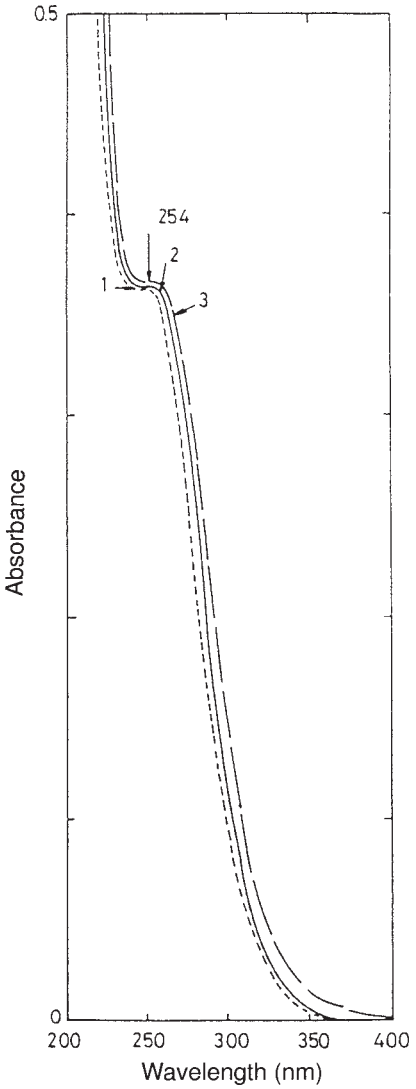

Fig. 5. UV-visible spectra of binary complexes of D-aspartic acid end group containing PAAm with Cu(II); curve 1, nonirradiated; curve 2, after exposition to 300 Gy; curve 3, 1200 Gy irradiation.

in the case of binary and ternary complexes, a new peak, which was not observed in the case of PAAm homopolymer (curve 1) and BSA (curve 4) alone, also appeared at λ = 723 nm (Fig. 7, curves 2 and 3). Changes in optical density (OD) at 280 and 723 nm were calculated according to the following equation, taking into account that the OD before and after irradiation, that lowest OD% indicates more stability of compound against the irradiation:

$$OD\% = (\Delta OD/OD) \times 100$$

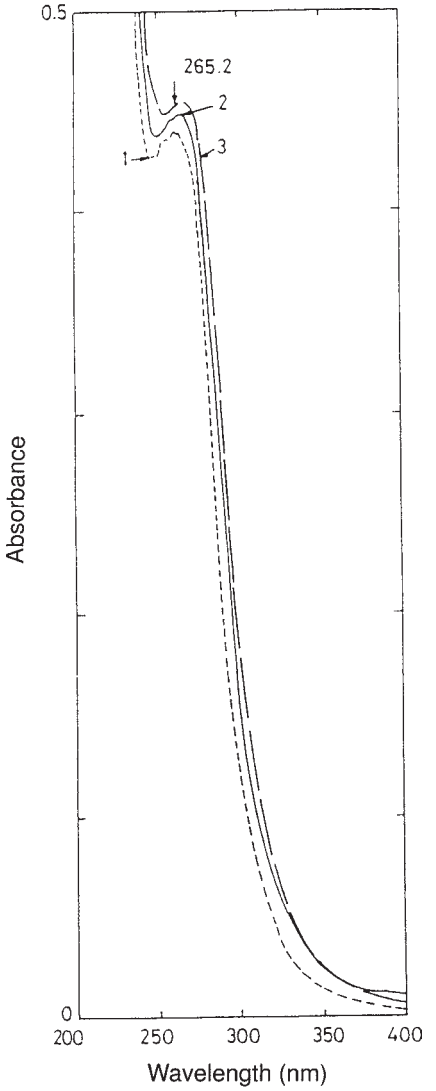

Fig. 6. UV-visible spectra of ternary complexes of D-aspartic acid end group containing PAAm with Cu(II) and BSA; curve 1, nonirradiated; curve 2, after exposition to 300 Gy; curve 3, 1200 Gy irradiation. [PAAm] = 0.1 g/L; $n_{BSA}/n_{PAAm} = 1$; $n_{Cu(II)}/n_{PAAm} = 0.15$.

The Cu^{2+}-L-aspartic acid end group containing PAAm binary and corresponding ternary complexes was more stable than the D-aspartic acid end group containing binary and ternary complexes, which is reflected in the OD changes after 300 and 1200 Gy irradiation (Table 1).

FT-IR Measurements

To study the effect of radiation on the binary and ternary complexes, these complexes were also investigated by FT-IR measurements. Although

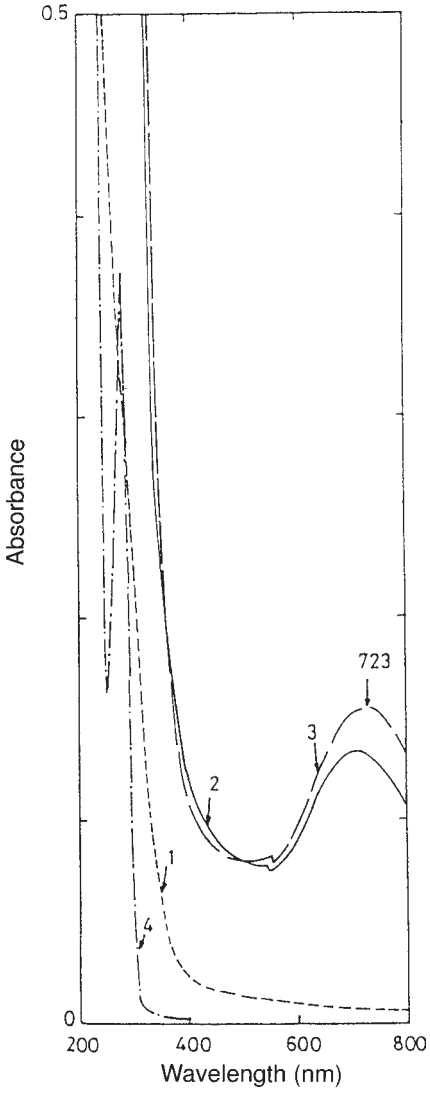

Fig. 7. UV-visible spectra of D-aspartic acid end group containing the folllowing: curve 1, PAAm; curve 2, binary complexes with Cu(II); curve 3, ternary complexes with PAAm-Cu(II)-BSA; curve 4, BSA. [PAAm] = 1.0 g/L; $n_{BSA}/n_{PAAm} = 1$; $n_{Cu(II)}/n_{PAAm} = 0.15$.

Table 1
Percentage Changes in OD Values by γ-Radiolysis (medium: aerated)

	OD% = (ΔOD/OD) × 100					
Irradiation dose (Gy)	D-PAAm[a]	L-PAAm[a]	D-PAAm+ Cu^{2+}[b]	L-PAAm+ Cu^{2+}[b]	D-PAAm-Cu^{2+}-BSA[b]	L-PAAm-Cu^{2+}-BSA[b]
300	19.59	6.28	34.61	2.08	19.11	6.6
1200	5.78	17.71	35.89	3.12	21.32	6.6

[a]OD% values: λ = 280 nm. ΔOD = difference in ODs of sample between irradiated and nonirradiated conditions.

[b]OD% values: λ_{max} = 723 nm.

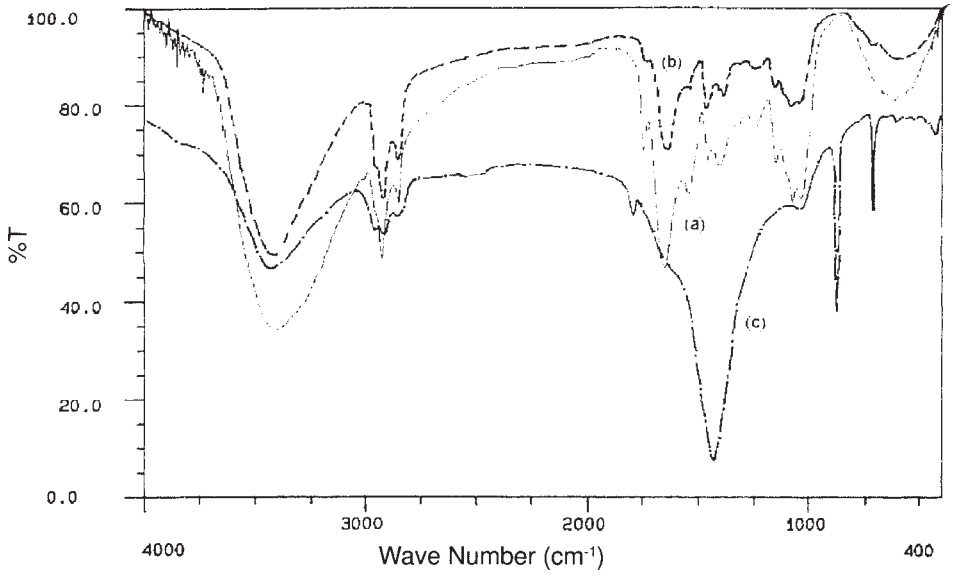

Fig. 8. FT-IR spectra of binary complex L-aspartic acid end group containing PAAm with Cu(II); (a) nonirradiated; (b) after exposition to 300 Gy; (c) after exposition to 300 Gy ternary complexes with Cu(II) and BSA.

the binary complex remained stable before and after 300 Gy of γ-irradiation (Fig. 8, [a] and [b]), the ternary complex seemed to decompose when it was exposed to 300 Gy of radiation (Fig. 8, [c]), because the characteristic band owing to the -C=O group at 1676 cm^{-1} shifted to lower wave numbers (ν = 1446 cm^{-1}).

HPLC Measurements

Figure 9 shows a series of HPLC chromatograms of D-aspartic acid containing PAAm. Although D-aspartic acid containing PAAm has very small peak areas, before irradiation (Fig. 9A, [a]), the peak area increased with increasing radiation dose (Fig. 9A, [b] and [c]). At the same polymer concentration, in the presence of Cu^{2+}, a new peak at higher retention time (RT) suggests the formation of binary complex (Fig. 9C). In the presence of BSA, formation of ternary complex (PAAm-Cu[II]-BSA) can also be followed by the presence of a new peak (Fig. 9D). The peak areas at the same retention values increase, probably owing to electronic interaction of the functional group of polymer with metal and protein and the difference in conformation of polymeric chain in the complex structure. In the UV-visible spectrum of polymer alone, the absorbance values increased with increasing radiation dose (Fig. 4), and the parallel behavior was also obtained from HPLC results. After irradiation, separation of ternary complex by HPLC was not possible, probably owing to the interaction of compound with column material of HPLC.

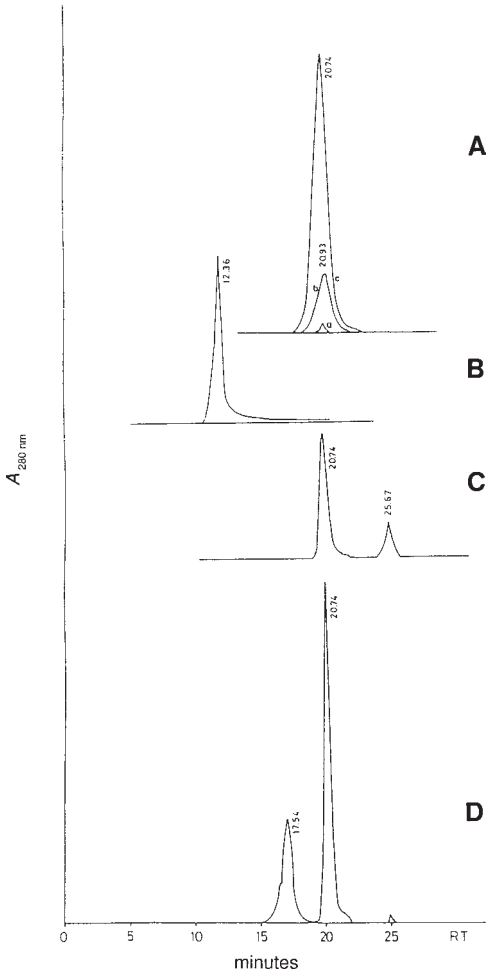

Fig. 9. A series of HPLC chromatograms of D-aspartic acid end group containing **(A)** PAAms (a) nonirradiated, (b) after exposition to 300 Gy, and (c) 1200 Gy irradiation; **(B)** BSA; **(C)** binary complexes with Cu; and **(D)** ternary complexes with Cu(II) and BSA. (RT is retention time.)

Conclusion

Physicochemical characterization of ternary complex indicates that the L-aspartic acid end group containing PAAm and its complexes seems more stable than the D-aspartic acid end group containing PAAm to irradiation. In addition, binary complexes of these L-aspartic acid end group containing polymers become more stable against irradiation in accordance with the homopolymer alone.

Acknowledgment

We thank the staff of the Industrial Application Department of ÇNAEM who kindly performed our gamma irradiation measurements and Dr. M. I. Mustafaev for helpful discussion.

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