Radiation Grafting of AMPSA and DMAEM onto Membranes of Acrylonitrile Copolymer and Polyamide for Immobilization of Glucose Oxidase

T. GODJEVARGOVA

University "Prof. Dr. A. Zlatarov," 8010 Bourgas, Bulgaria

SYNOPSIS

Membranes of acrylonitrile copolymer and polyamide were modified by radiation grafting of 2-acrylamido-2-methylpropanesulphonic acid and 2-dimethylaminoethyl methacrylate. The effect of irradiation dose (from an electron beam accelerator) and method of introduction of ferrous ions on the grafting degree was studied. The sulpho- and quaternary amino groups introduced into the membranes were determined quantitatively by potentiometric titration. The grafting degree and hydrophilicity of the modified membranes were studied. Glucose oxidase was immobilized onto modified membranes, and its basic characteristics were determined: bound protein, relative activity, pH_{opt}, and T_{opt}, pH, and thermal stability, as well as storage of activity. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

Polymer membranes are widely used for immobilization of enzymes. Additional modification of polymer membranes increases their suitability as enzyme carriers. Graft copolymerization is a well-known method for modification.[1]

The chemical graft copolymerization of 2-acrylamido-2-methylpropane sulphonic acid (AMPSA) and 2-dimethylaminoethyl methacrylate (DMAEM) onto cellulose and nylon has been reported earlier.[2-6] In our previous work,[7] we discussed the modification of membranes of acrylonitrile copolymer by chemical grafting of AMPSA and DMAEM and their use for immobilization of glucose oxidase. The radiation grafting is a better method for grafting monomers onto polymer membranes since the physico-mechanical and structural characteristics of the membranes are preserved.[8]

The aim of the present work is to study the modification of membranes of acrylonitrile copolymer and polyamide by radiation grafting and their use for immobilization of glucose oxidase. The effect of the method of introduction of ferrous ions on the grafting degree was studied.

Journal of Applied Polymer Science, Vol. 61, 343–349 (1996)
 CCC 0021-8995/96/020343-07

EXPERIMENTAL

Materials

Poly(acrylonitrile–methyl methacrylate–sodium vinylsulphonate) (PAN) membranes (molecular weight cut off 10,000) and polyamide (PA) membranes with a pore size of 0.2 μm, supplied by Spartak Co., Bulgaria, were used. The agents used for the modification were as follows: 2-acrylamido-2-methylpropanesulphonic acid, 2-dimethylaminoethyl methacrylate and benzyl chloride, supplied by Fluka Co., Switzerland; sodium hydroxide and nitric acid, from Chimsnable, Bulgaria; and ferrous ammonium sulphate, from Reachim, Russia. The glucose oxidase immobilized onto the modified membranes was of specific activity of 190 U/mg, a product of Bioprogress Co., Bulgaria.

Modification of PAN and PA Membranes by Radiation Grafting of AMPSA and DMAEM

The radiation grafting was carried out by the following two methods.

1. The membranes were partially hydrolyzed by immersion in 6 wt % aqueous solution of NaOH at 333 K for 1 h (PAN membranes) or in a 6 wt % aqueous solution of HCl at 313 K for 30 min (PA membranes). The hydrolyzed membranes were washed thoroughly with distilled water and immersed in a 0.5 wt % aqueous solution of ferrous ammonium sulphate (pH = 5) for 10 min at room temperature. Then they were washed again with distilled water to remove the nonsorbed Fe^{2+} ions. The membranes with sorbed Fe^{2+} ions were irradiated with 750 keV electron beam accelerator (UV-45-0.75-2000-1) with doses from 20 to 100 kGy at room temperature. The irradiated membranes were immersed in 5 wt % aqueous solution of AMPSA (pH = 4) or 5 wt % aqueous solution of DMAEM (pH = 2) at 333 K for 120 min. Nitrogen was continuously bubbled through the solution during the reaction.

   Modified membranes were thoroughly washed with distilled water to remove soluble homopolymer.
2. PAN and PA membranes were irradiated by accelerated electron beam with doses from 20 to 100 kGy at room temperature. Then, the membranes were immersed in water solution containing 5 wt % monomer (AMPSA or DMAEM) and 0.2 wt % ferrous ammonium sulphate for 120 min at 333 K. Nitrogen was continuously bubbled through the solution during the reaction.

Determination of the Grafting Degree, Hydrophilicity, and Concentration of Ionogenic Groups of Modified Membranes

The degree of grafting (X, %) was determined by the difference between membrane masses before ($G1$) and after ($G2$),[4] by the formula

$$X(\%) = \frac{G_2 - G_1}{G_1} \times 100$$

Membrane hydrophilicity was calculated as the weight difference between the water swollen membrane and dry membrane (water content) per unit membrane weight.[9] The contents of sulpho and quaternary ammonium groups were proved by potentiometric titration.[10] A Radelkis pH meter (Hungary) was used for these measurements.

Immobilization of Glucose Oxidase

Modified PAN and PA membranes were washed with 0.1 M phosphate buffer (pH = 5.5) and immersed in 0.1 wt % aqueous solution of glucose oxidase (GOD) with pH = 5.5 at 277 K for 16 h. The membranes were then washed with aq.0.1 M phosphate buffer, with pH = 5.5.

Free and immobilized glucose oxidase activities were measured spectrophotometrically (Specol 11, Carl Zeiss Jena) at 460 nm,[11] and bound protein was measured by the method of Lowry et al.[12]

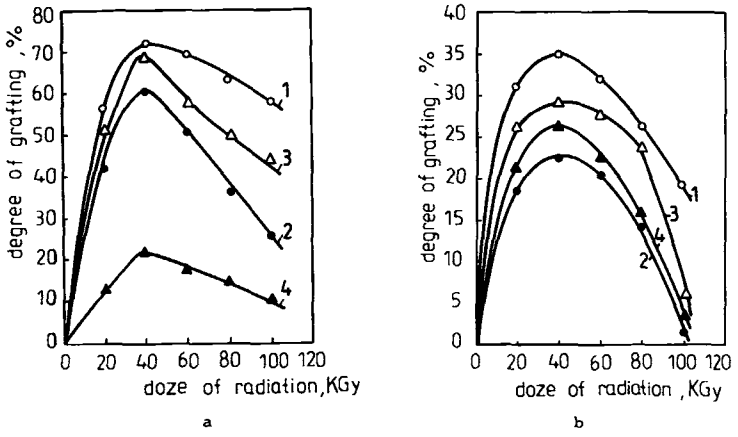

Figure 1 Effect of irradiation dose on the grafting degree of AMPSA (a) and DMAEM (b) on the following: PAN (1) and PA (3) membranes with sorbed Fe^{2+} ions before irradiation; PAN (2) and PA (4) membranes treated with monomer solution containing Fe^{2+} ions after irradiation. Monomer concentration, 5 wt %; temperature, 333 K; time, 120 min.

RESULTS AND DISCUSSION

Modified membranes of acrylonitrile copolymer and polyamide were used as carriers for the immobilization. The modification was carried out by radiation grafting of 2-acrylamido-2-methylpropanesulphonic acid and dimethylaminoethyl methacrylate in two stages: irradiation of polymer membranes by accelerated electron beam in air (irradiation dose from 20 to 100 kGy), and treatment of the irradiated membranes in a preliminarily inertized monomer solution of suitable concentration at a certain pH and temperature.[5,7] The main disadvantage of this method is that a high amount of homopolymer is obtained due to the presence of $^{\bullet}OH$ radicals. Therefore, an inhibitor of the homopolymerization (Fe^{2+} ions) was introduced in two ways.

1. By treatment of the polymer membranes with a solution of Fe^{2+} prior to irradiation and treatment with the monomer solution. Fe^{2+} ions are sorbed on membrane surface owing to the carboxylic groups formed during the preliminary hydrolysis of the membranes.[7] On the figures, the membranes treated in this way were denoted by the numbers 1 and 3.
2. By treatment of the irradiated membranes with a monomer solution containing Fe^{2+} ions.[7] On the figures, these membranes are denoted by 2 and 4.

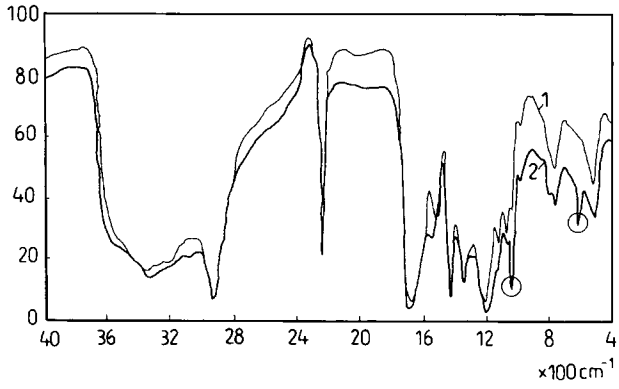

Figure 3 Infrared spectra of initial (1) and modified (2) PA membranes by radiation grafting with 5 wt % AMPSA. Experimental conditions are as in Figure 2.

In our system, the homopolymerization is initiated by hydroxyl radicals, which are formed from the decomposition of the peroxide during the irradiation. The thermal breakdown of hydroperoxide is

$$POOH \rightarrow PO^{\bullet} + OH^{\bullet} \qquad (1)$$

When Fe^{2+} is present, reaction (1) becomes

$$POOH + Fe^{2+} \rightarrow PO^{\bullet} + OH^{-} + Fe^{3+} \qquad (2)$$

where hydroxyl radicals initiating the homopolymerization do not form, thereby eliminating the ini-

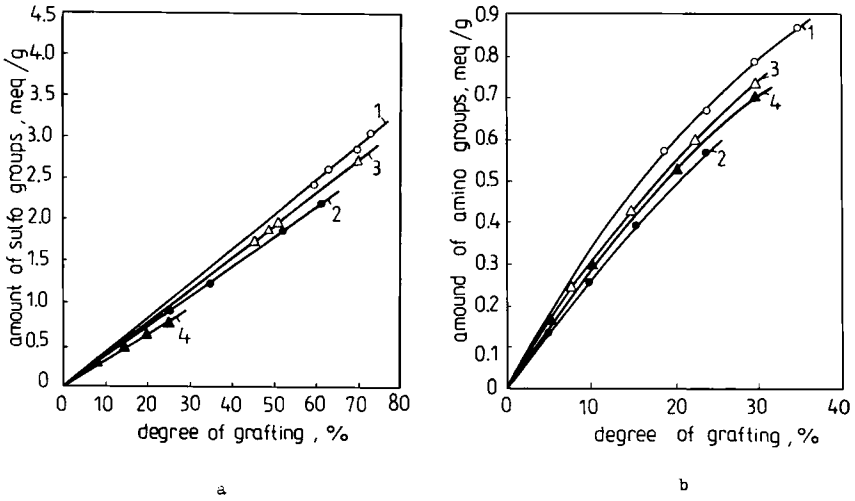

Figure 2 Dependence of the amount of sulpho groups (a) and quaternary amino groups (b) on the grafting degree of AMPSA (a) and DMAEM (b) onto the following: PAN (1) and PA (3) membranes with sorbed Fe^{2+} ions before irradiation; PAN (2) and PA (4) membranes treated with monomer solution containing Fe^{2+} ions after irradiation. Dose, 20–100 kGy; other experimental conditions are as in Figure 1.

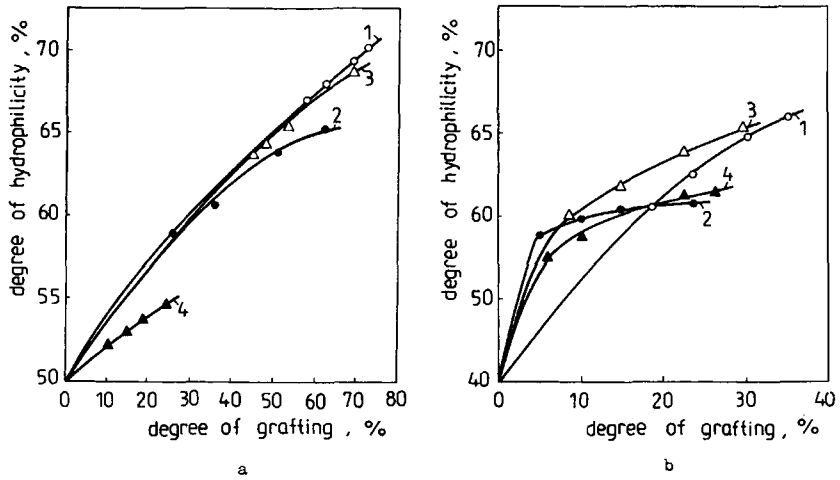

Figure 4 Dependence of the degree of hydrophilicity on the degree of radiation grafting of AMPSA (a) and DMAEM (b) onto the following: PAN (1) and PA (3) membranes with sorbed Fe^{2+} ions before irradiation; PAN (2) and PA (4) membranes treated with monomer solution containing Fe^{2+} ions after irradiation. Experimental conditions are as in Figure 2.

tiation of homopolymerization. Besides, the ferrous ions increase the initiation rate via the redox process, as well as the rate of chain termination.

The net result of the surplus of ferrous ammonium sulphate in the reaction medium is the reduction of the grafting degree by enhancing chain termination

$$P—CH_2^{\bullet} + Fe^{2+} + H_2O \rightarrow P—CH_3 + Fe^{3+} + OH^- \quad (3)$$

This reaction occurs not only with growing homopolymer chains but also with growing grafted branches, thus reducing the yields of both processes.

The effect of the Fe^{2+} ion concentration was studied in a number of papers.[5,13,14] In the present work, whether and how the way of the introduction of Fe^{2+} ions in the system affects the radiation grafting is investigated.

The effect of irradiation dose on the grafting degree was also studied. Figure 1 shows that the maximum grafting degree was obtained at an irradiation

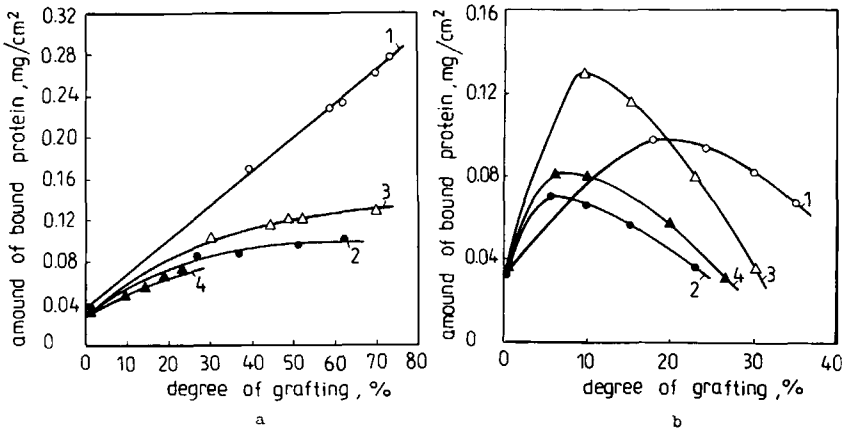

Figure 5 Dependence of the amount of bound protein of immobilized GOD on the degree of radiation grafting of AMPSA (a) and DMAEM (b) onto the following: PAN (1) and PA (3) membranes with sorbed Fe^{2+} ions before irradiation; PAN (2) and PA (4) membranes treated with monomer solution containing Fe^{2+} ions after irradiation.

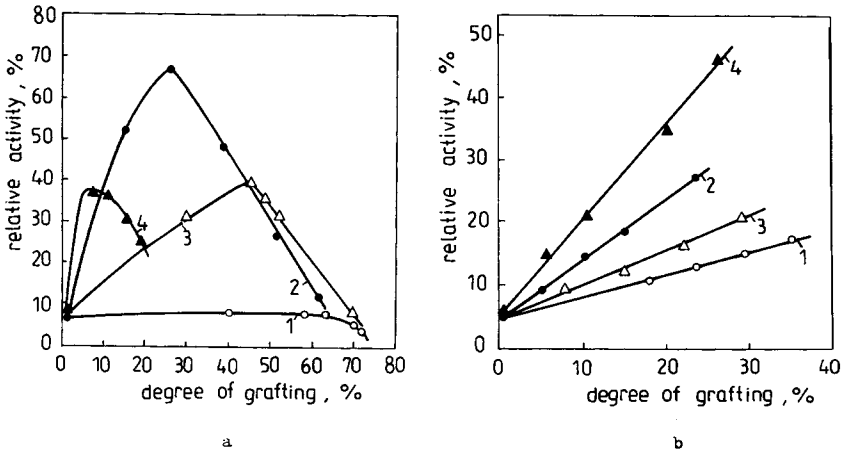

Figure 6 Dependence of the relative activity of immobilized GOD on the grafting degree of AMPSA (a) and DMAEM (b) onto the following: PAN (1) and PA (3) membranes with sorbed Fe^{2+} ions before irradiation; PAN (2) and PA (4) membranes treated with monomer solution containing Fe^{2+} ions after irradiation.

dose of 40 kGy. The grafting degree of AMPSA [Fig. 1(a)] and DMAEM [Fig. 1(b)] for membranes with sorbed Fe^{2+} before irradiation (membranes 1 and 3) is higher than that of membranes 2 and 4 treated with monomer solution containing Fe^{2+} ions after irradiation. It means in the first two cases, that homopolymerization did not practically occur, and that high grafting degree has been achieved. This is probably due to the immediate interaction between the Fe^{2+} ions sorbed on the membrane surface and the hydroperoxides. Thus, the formation of $OH^{\bullet}$ [eq. (2)], which initiated the homopolymerization, is eliminated. PAN membranes in both cases show higher grafting degree compared to PA membranes. It could be suggested that this is due to the different nature of the polymer membranes.[15]

The comparison of the results from Figures 1(a) and 1(b) shows that AMPSA was grafted to higher degrees, especially on membranes with Fe^{2+} ions sorbed before irradiation (60–70%). Besides, the grafting of AMPSA revealed most clearly the difference between both ways of introduction of Fe^{2+} ions in the system: Membranes with Fe^{2+} sorbed before irradiation show a grafting degree of 40% higher than that of membranes treated with monomer solution containing Fe^{2+} after irradiation.

New groups were introduced in the membranes during the modification—sulpho groups by the grafting of AMPSA, and tertiary amino groups by the grafting of DMAEM. The tertiary amino groups were quaternized with benzyl chloride. The sulpho and quaternized amino groups were determined

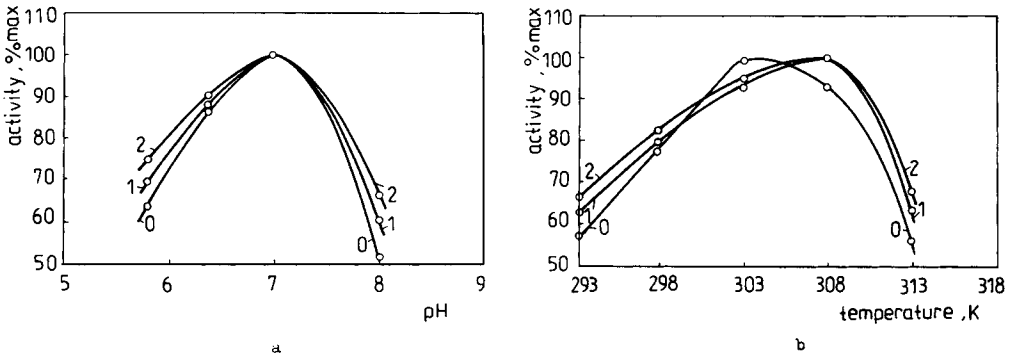

Figure 7 pH and temperature dependences of native (0) and immobilized GOD onto membranes modified with AMPSA (1) and DMAEM (2): (a) 0.1*M* phosphate buffer, 15 min, 303 K; (b) 0.1*M* phosphate buffer, pH = 7, 15 min.

Table I Storage of Activity of Immobilized GOD after 50 Days

Membrane No.	Modified[a] PAN Membrane		Modified[a] PA Membrane	
	with AMPSA	with DMAEM	with AMPSA	with DMAEM
1	99.7	99.9	99.4	99.8
2	99.5	99.8	99.6	99.7
3	99.5	99.7	99.7	99.8
4	99.3	99.8	99.7	99.8

GOD-bound membranes were stored in distilled water at 277 K for 50 days. Values reflect percentages.
[a] Modified membranes providing high relative activity of immobilized GOD were used.

quantitatively by residual potentiometric titration. Figure 2 shows the effect of the grafting degree on the amount of ionogenic groups in the membranes. Obviously, the amount of ionogenic groups increases with the grafting degree. Membranes modified with AMPSA and possessing a grafting degree of 60–70% contain the highest amount of sulpho groups (2.5–3 meq/g). Membranes with sorbed Fe^{2+} have a higher concentration of ionogenic groups than the membranes treated with monomer solution containing Fe^{2+}, which fully corresponds to their grafting degree. The new sulpho groups introduced during the modification with AMPSA were proved qualitatively by comparison of the infrared spectra of initial and modified membrane. Figure 3 illustrates this comparison for PA membranes. The new absorption bands at 630 and 1050 nm, corresponding to the valent oscillation of S—O bonds and valent symmetric oscillation of SO_2 groups, prove the modification of the initial membranes with AMPSA.

The degree of hydrophilicity of the modified membranes was found to be higher than that of initial membranes (Fig. 4). Membranes 1 and 3, modified with AMPSA and having the highest grafting degree, showed up to 70% hydrophilicity [Fig. 4(a)].

Modified membranes were used as carriers for immobilization of GOD. The immobilization is based on electrostatic interaction between the sulpho and quaternized amino groups of the membrane and the amino and carboxylic groups of the enzyme, respectively.

The amount of bound protein was determined (Fig. 5). Membranes modified with AMPSA (especially 1 and 3) and containing the highest amount of sulpho groups adsorbed twice as much protein as other membranes.

Figure 5(a) shows that the amount of bound GOD increases with the grafting degree of AMPSA. This is not the same with membranes modified with DMAEM [Fig. 5(b)], where the curve passes through a maximum increase.

The relative activity of the immobilized glucose oxidase was determined as the ratio of immobilized enzyme activity to total activity of the free enzyme used for the immobilization (Fig. 6). Membrane 1, modified with AMPSA and containing the highest amount of bound protein, showed the lowest relative activity [Fig. 6(a)]. This is probably due to the high concentration of bound protein leading to local aggregations and, respectively, difficult diffusion of the substrate to the active centers of the immobilized GOD. Membrane 2 containing ca. 0.07–0.09 mg/cm^2 bound protein provides higher relative activity (ca. 70%) of the immobilized GOD [Fig. 6(a)].

The dependence of the relative activity of GOD immobilized onto membranes modified with DMAEM on the grafting degree of DMAEM is presented in Figure 6(b). The relative activity of the immobilized GOD increases with the grafting degree. As in previous cases, the membranes with the highest amount of bound protein (membranes 1 and 3) showed the lowest relative activity of the immobilized GOD. Membranes 2 and 4 (modified with DMAEM), containing 0.04–0.08 mg/cm^2 bound protein, provide comparatively better relative activity of immobilized GOD (40 ÷ 50%).

Temperature and pH optima of immobilized GOD were determined. In both cases, these optima were found to be the same as those of the native GOD (303 K and pH = 7).

Temperature and pH stability are two of the most important characteristics of the immobilized GOD. Figure 7(a) shows that the immobilized GOD is stable in a wider pH interval than the native GOD. The temperature stability of immobilized GOD is similar to that of native GOD [Fig. 7(b)].

The storage of activity of the immobilized GOD was also studied (Table I). The activity of the immobilized GOD was maintained high for both types of modification. Slightly better storage of activity was observed with the GOD immobilized onto polymer membranes modified with DMAEM.

REFERENCES

1. G. Box, A. Chapiro, M. Huglin, A. M. Jendrychowska-Bonamour, and T. O'Neill, *J. Polym. Sci.* **C-22,** 493 (1968).
2. J. Andrichenko, T. Drijina, and C. Kuznetsova, *Chem. Fibres,* **1,** 22 (1992).
3. A. Chidijatov and Z. Rogovin, *Chem. Fibres,* **1,** 51 (1969).
4. C. McCormick and L. Park, *J. Polym. Sci.,* **22,** 49 (1984).
5. T. Graczyk and V. Hornoff, *J. Macromol. Sci., Chem.,* **A20,** 213 (1983).
6. A. Gulina and Z. Rogovina, *Chem. Fibres,* **3,** 29 (1965).
7. T. Godjevargova and A. Dimov, *J. Appl. Polym. Sci.* **57,** 487 (1995).
8. D. J. McDowall and B. S. Gupta, *Polym. J.,* **19,** 643 (1987).
9. H. Yesuda and C. Lamaze, *J. Polym. Sci.,* **9,** 1117 (1971).
10. K. Dimov, V. Sarmadjieva, and P. Pavlov, *Laboratory Practice on Technology of Synthetic Fibres,* Technica, Sofia, 1983.
11. H. Bergmayer and E. Berndt, *Methoden der Enzymatische Analyse,* Chemie, Wienheim, 1962.
12. H. Lowry, N. Roseborough, and H. Farr, *J. Biol. Chem.,* **193,** 265 (1951).
13. A. Jong, *J. Appl. Polym. Sci.,* **36,** 87 (1988).
14. J. Lai, T. Chang, Z. Wu, and T. Hsieh, *J. Appl. Polym. Sci.,* **32,** 4709 (1986).
15. A. Pikaev, *Contemporary Radiation Chemistry,* Mir, Moscow, 1987.

Received October 3, 1995
Accepted January 10, 1996