Grafting of Acrylonitrile Copolymer Membranes with Hydrophilic Monomers for Immobilization of Glucose Oxidase

T. GODJEVARGOVA* and A. DIMOV

Higher Institute of Chemical Technology, 8010 Bourgas, Bulgaria

SYNOPSIS

Poly(acrylonitrile-methylmethacrylate-sodium vinylsulphonate) membranes were subjected to modification by grafting of 2-dimethylaminoethyl methacrylate (DMAEM) and 2-acrylamido-2-methylpropanesulphonic acid (AMPSA). An Fe^{2+} H_2O_2 system was used to initiate the polymerization. Reaction time, temperature, pH, and concentrations of an initiator and modifying agent were the variable parameters. Tertiary amino groups, quaternary ammonium groups, and sulfo groups were determined quantitatively by potentiometric titration. The degree of grafting and hydrophilicity and charge density of the modified membranes were studied. Glucose oxidase was immobilized onto modified acrylonitrile copolymer membranes with DMAEM and AMPSA and showed high relative activity. The amount of bound protein and storage of the activity of the immobilized enzyme were determined. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Polymer membranes are widely used for immobilization of enzymes.[1-6] Additional modification of polymer membranes increases their suitability as enzyme carriers. In our previous work[7] we discussed the immobilization of glucose oxidase onto acrylonitrile copolymer membranes surface-modified with hydroxylamine, sodium hydroxide, 1,6-hexamethylendiamine, and hydrazine hydrate. This article describes the modification of acrylonitrile copolymer membranes by grafting with 2-dimethylaminoethyl methacrylate (DMAEM) and 2-acrylamido-2-methylpropanesulfonic acid (AMPSA).

The graft copolymerization of DMAEM and AMPSA onto cellulose and nylon has been studied in a number of articles.[8-12] Miyama and coworkers[13-15] reported that dimethyl-aminated nylon-containing tertiary amino groups is a good material for immobilization of enzymes after being quaternized.

The aim of this work was to study the graft copolymerization of DMAEM and AMPSA onto acrylonitrile copolymer membranes and their use for immobilization of glucose oxidase.

EXPERIMENTAL

Materials

Poly(acrylonitrile-methylmethacrylate-sodium vinylsulphonate) (PAN) membranes, molecular weight cutoff 10,000, supplied by Spartak Co., Bulgaria, was used. The ternary copolymer contained 91.03 mass % acrylonitrile, 7.3 mass % methylmethacrylate, and 1.4 mass % sodium vinylsulphonate. The following agents were used for the modification of the membranes: 2-dimethylaminoethyl methacrylate, 2-acrylamido-2-methylpropanesulfonic acid, benzyl chloride, pure, by Fluka, Switzerland; sodium hydroxide, hydrogen peroxide, and nitric acid, pure, from Bulgaria; ferrous ammonium sulfate, pure, from Reachim, Russia. The glucose oxidase immobilized onto the membranes was of specific activity of 190 U/mg, a product of Bioprogres Co., Bulgaria.

* To whom correspondence should be addressed.

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Modification of PAN Membranes

Modification by Grafting of DMAEM

PAN membranes were partially hydrolyzed with 6 wt % aqueous solution of NaOH at 333 K for 1 h. The hydrolyzed membranes were treated subsequently with a dilute solution of HCl and 2 L distilled water. Then, they were immersed in a 0.5 wt % aqueous solution of ferrous ammonium sulfate (pH = 5) for 10 min at room temperature and washed with distilled water (pH = 5.5). After that, the membranes were immersed in a 2–10 wt % aqueous solution of 2-dimethylaminoethyl methacrylate for 15 min. The monomer was neutralized by acid prior to the reaction. Hydrogen peroxide (0.01–0.1 wt %) was added to the reaction mixture, and the pH dropped to about 4. The modification was carried out at 303–333 K for a reaction time of 1–4 h. Modified membranes were thoroughly washed with distilled water to remove soluble homopolymers.

Membranes modified by grafting of DMAEM were immersed in a 50 wt % solution of benzyl chloride in ethanol at 323 K for 7 h to quaternize the tertiary amino groups. Then the membranes were washed with distilled water.

Modification by Grafting of AMPSA

The grafting of AMPSA onto partially hydrolyzed PAN membranes was carried out under reaction conditions similar to those used for the grafting of DMAEM. Because AMPSA is a strong acid, it was completely neutralized to the sodium salt and was then copolymerized at an initial pH of 7. Concentration varied between 0.1 and 20 wt %. The modification was carried out at 293–333 K, with a reaction time of 1–4 h.

Immobilization of Glucose Oxidase

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

Free and immobilized glucose oxidase activities were measured spectrophotometrically (Specol 11, Carl Zeiss Jena) at 460 nm,[16] and bound protein was measured by the method of Lowry et al.[17]

Determination of the Degree of Grafting, Hydrophilicity and Concentration of Fixed Charge Groups (Charge Density) of Modified Membranes

The degree of grafting (X, %) was determined by the difference of membrane mass before (G_1) and after (G_2) the grafting,[10] according to the formula:

$$X = \frac{G_2 - G_1}{G_1} \cdot 100\%.$$

Membrane hydrophilicity was expressed by the weight difference between the water-swollen and dry membrane (water content) per unit membrane weight.[18] The charge density was determined as the ratio of the concentration of the amino and sulfo groups (mEq/g of dry membrane) to water volume in the membrane.[19] The contents of tertiary amino and sulfo groups and quaternary ammonium groups were proved by residual potentiometric titration in heterogeneous medium.[20] A Radelkis pH-meter (Hungary) was used for these measurements.

RESULTS AND DISCUSSION

Grafting of DMAEM and AMPSA onto PAN membranes was carried out by using the system Fe^{2+}/H_2O_2. Fe^{2+} ions were sorbed by the carboxylic groups of partially hydrolyzed (with 6 wt % NaOH) PAN membrane. After washing the excess of Fe^{2+} ions, the membrane was immersed in a water solution of the monomer containing H_2O_2 in an amount insufficient to initiate polymerization without Fe^{2+}. In this case, homopolymerization has not been observed.[12] The chain propagation is initiated by the hydroxyl radicals generated immediately on the partially hydrolyzed PAN membrane. These radicals interact with the membrane to give the free macroradicals, which in turn, initiate the polymerization of the grafted branches.

The optimum conditions for graft copolymerization of DMAEM were determined, by which physicomechanical and structural properties of the initial membrane were preserved and high degrees of grafting were achieved.

The effect of DMAEM concentration on the degree of grafting was studied. Curve 1 in Figure 1 shows that the degree of grafting increases with monomer concentration up to 10 wt %, probably due to an increase of initiation effectiveness, as well as to an increase of the macroradical propagation rate. Above this concentration, the degree of grafting remains almost the same.

The effect of initiator concentration (hydrogen peroxide) on the degree of grafting was studied. The curve shows a maximum at 0.6 wt % aqueous solution of H_2O_2 (Fig. 1, curve 2). The decrease of the degree of grafting with the increase of the concentration of H_2O_2 can be explained by the termination of the grafted chain by interaction of the propagating

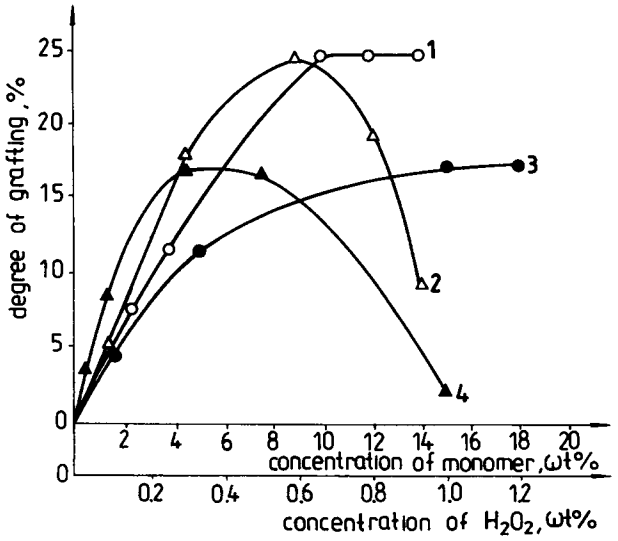

Figure 1 Influence of the concentration of DMAEM, AMPSA, and H_2O_2 (*) on the degree of grafting of DMAEM (1, 2*) and AMPSA (3, 4*) on PAN membranes. Reaction temperature: for DMAEM—318 K, for AMPSA—303 K. Reaction time—4 h.

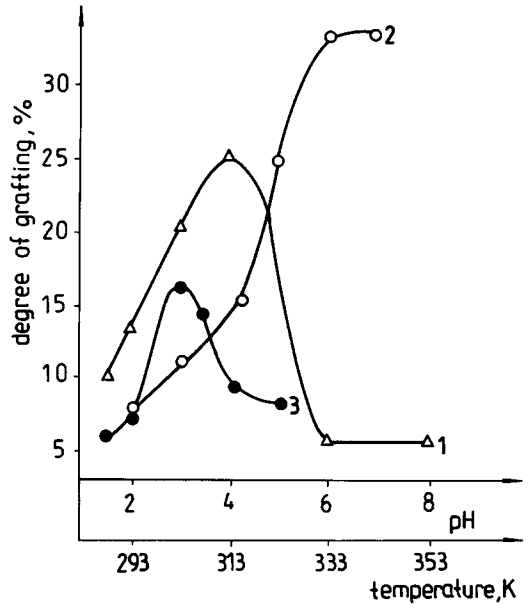

Figure 2 Influence of pH and temperature (*) on the degree of grafting of DMAEM (1, 2*) and AMPSA (3*) on PAN membranes. Concentration: 10 wt % DMAEM + 0.6 wt % H_2O_2 and 15 wt % AMPSA + 0.3 wt % H_2O_2.

macroradicals with $^{\cdot}OH$ generated by the decomposition of the H_2O_2.

Another factor affecting the amount of grafted copolymer is pH of the reaction medium. The DMAEM monomer is a base and it can be polymerized only in the form of a salt. Because its pK is 4.25,[21] it can be polymerized at pH $\leq$ 4.25. Fanta et al.[22] and Lepoutre et al.[21] grafted DMAEM to starch and cellulose, respectively, at pH = 2. In our case, the optimum pH was found to be 4 (Fig. 2, curve 1). The decrease of the degree of grafting of DMAEM at pH < 4 is probably due to desorption of Fe^{2+} and formation of homopolymer.[12]

The influence of the temperature on the degree of grafting was studied. The degree of grafting increases with temperature up to a certain value (Fig. 2, curve 2). At temperatures above 333 K the amount of grafted copolymer is practically the same.

The dependence of the reaction time on the degree of grafting was studied. The degree of grafting increases with the increased interval for contact of the membrane with the monomer. The optimum reaction time was found to be 4 h.

Modification of PAN membranes by grafting of DMAEM incorporates new groups in the membrane, tertiary amino groups. Their amount was determined by potentiometric titration. The amine groups content increases with the degree of grafting (Fig. 3, curve 1). A 2.3 mEq/g of tertiary amino groups were found at the highest degree of grafting of 35%

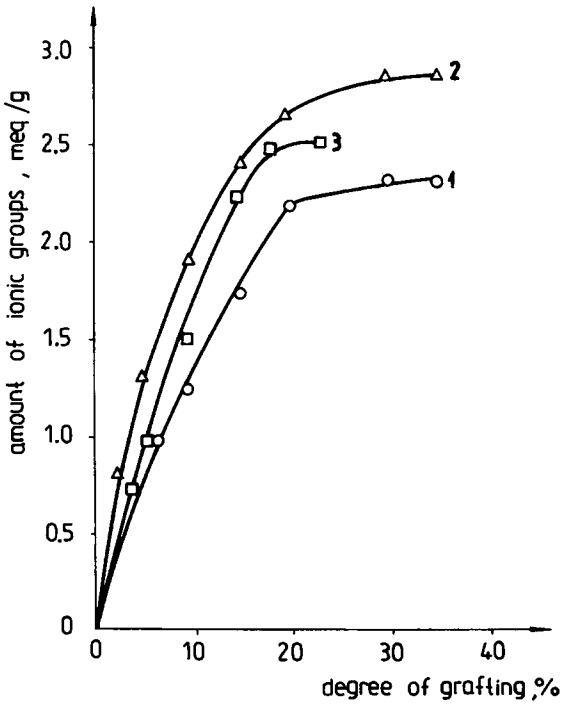

Figure 3 Influence of the degree of grafting on the amount of ionic groups: tertiary amino groups (1), quaternary ammonium groups (2), and sulfo groups (3) formed at the optimum conditions determined.

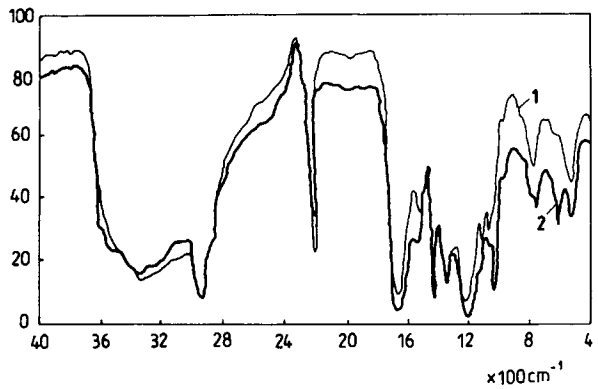

Figure 4 Infrared spectra of the initial (1) and modified PAN membrane (2) grafted with 15 wt % AMPSA and 0.3 wt % H_2O_2 at 303 K for 4 h.

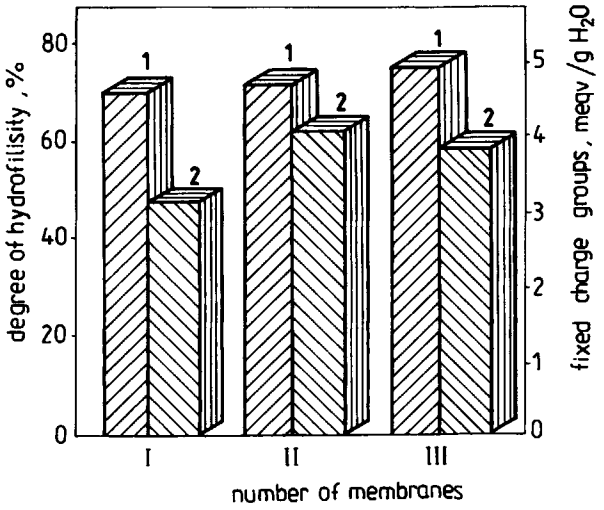

Figure 5 Degree of hydrophilicity (1) and fixed charge groups (2) of membranes modified at optimum conditions with: I—DMAEM, II—DMAEM and benzyl chloride, III—AMPSA.

achieved in our experiments. Tertiary amine groups were quaternized with benzyl chloride to obtain higher positively charged membranes, and the amount of the quaternized groups was measured to be 2.85 mEq/g at a 35% degree of grafting.

The optimum conditions for grafting of AMPSA onto PAN membranes were determined. The effect of monomer concentration is illustrated on Figure 1, curve 3. Up to 15 wt % AMPSA, the degree of grafting increases with the monomer concentration and then remains practically the same. Figure 1, curve 4, shows that the highest degree of grafting was achieved within the concentration interval from 0.3 to 0.4 wt % aqueous solution of H_2O_2.

Another factor affecting the degree of grafting of AMPSA is temperature. The curve showing the dependence of the degree of grafting on temperature has a maximum of 16% (Fig. 2, curve 3). The most suitable reaction time was found to be 4 h.

In this modification, sulfo groups were introduced into PAN membranes. The former were proved qualitatively by comparison of IR spectra of initial and modified PAN membranes (Fig. 4). The new absorptions at 630 and 1050 nm, corresponding to valency vibration of S—O bonds and valency symmetric vibration of $-SO_2$ groups prove the modification of the initial membranes with AMPSA. The sulfo groups contents in the initial (present due to the sodium vinylsulfonate) and modified membranes (a sum of the initial and additionally introduced sulfo groups during the grafting) was determined by potentiometric titration. The difference between these values gives the amount of the introduced sulfo groups (Fig. 3, curve 3). The sulfo group content increased with the degree of grafting. At the optimum degree of grafting of 16%, this corresponded to 2.5 mEq/g sulfo groups.

The degree of hydrophilicity of the modified membranes was determined (Fig. 5, 1). The highest degree of hydrophilicity was shown by membranes modified with AMPSA. The charge density of the modified membranes was determined by their hydrophilicity and the amount of ionogenic groups (Fig. 5, 2). Membranes containing quater-

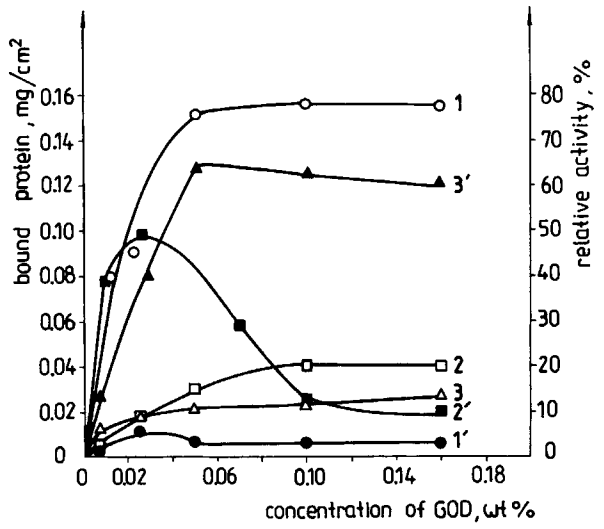

Figure 6 Influence of the concentration of glucose oxidase on the bound protein (1, 2, 3) and relative activity (1′, 2′, 3′) of glucose oxidase immobilized on membrane modified with: DMAEM (1), DMAEM and benzyl chloride (2), and AMPSA (3).

nary ammonium groups possess the highest charge density.

Glucose oxidase was immobilized on all three types of modified membranes containing essentially tertiary amine, quaternary ammonium, and sulfo groups. The immobilization was carried out by adsorption (on membranes containing tertiary amine groups) and by electrostatic interaction (on the other two types of membranes).

Figure 6 shows that the highest content of bound protein was obtained with membranes modified with DMAEM and containing tertiary amine groups, and the lowest with membranes modified with AMPSA. It is well known that a matrix with higher hydrophobicity adsorbs more protein.[23] The results obtained prove this fact.

The relative activity of immobilized glucose oxidase was determined as a ratio of immobilized enzyme activity to total activity of the free enzyme used for the immobilization (Fig. 6). The lowest relative activity was shown by membranes containing tertiary amine groups, because there were local aggregations of protein limiting the diffusion of substrate to the active centers of the immobilized glucose oxidase. Highest relative activity showed glucose oxidase immobilized on membranes containing sulfo groups (65%).

The amount of bound protein increased and the relative activity decreased after a certain maximum, with the increase of the concentration of the initial glucose oxidase used for the immobilization. This effect was most clear for glucose oxidase immobilized on membrane containing quaternary ammonium groups.

An important factor for the immobilized glucose oxidase is the storage of its activity. After 2 months, the activities of free and immobilized enzymes decreased: free enzyme down to 90.0% of the initial activity; immobilized enzyme on membrane modified with DMAEM to 91.0%, with DMAEM and benzyl chloride to 92.5%, and with AMPSA to 92.0%.

Best retention of activity was shown by glucose oxidase immobilized on membranes containing quaternary ammonium groups. This can be explained by the high charge density of this membrane and its good ionic bond to glucose oxidase.

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