Immobilization of Glucose Oxidase onto Membranes of Modified Acrylonitrile Copolymer

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SYNOPSIS

Acrylonitrile copolymer was modified with sodium hydroxide and 1,6-hexamethylen diamine (1) and with hydroxylamine (2). The amount of amine and carboxylic groups was studied as a function of the modification conditions. Membranes were prepared from the modified copolymer by the phase-inversion method. They were used as matrix for covalent immobilization of glucose oxidase by using glutaraldehyde. The amount of bound protein, relative activity, and storage of the activity of the immobilized enzyme were determined. The results were compared with those obtained with glucose oxidase immobilized onto the surface-modified membrane of acrylonitrile copolymer with sodium hydroxide and 1,6-hexamethylendiamine and with hydroxylamine. The results were proved by scanning electron microscopy. © 1994 John Wiley & Sons, Inc.

INTRODUCTION

Polymer membranes are widely used for immobilization of enzymes.[1-3] They possess some specific characteristics, such as exact chemical composition and physical structure, fixed porosity and hydrophilic–hydrophobic balance, which provide a possibility to carry out quantitatively defined immobilization of enzymes. The additional modification of polymer membranes increases their suitability as enzyme carriers. In our previous article, we discussed the immobilization of glucose oxidase onto surface-modified membranes of acrylonitrile copolymer. The modifications were performed by using hydroxylamine; sodium hydroxide and 1,6-hexamethylendiamine; hydrasine hydrate, etc. The best results for immobilized glucose oxidase were obtained with hydroxylamine and with sodium hydroxide and 1,6-hexamethylendiamine.

In the present study, the modification of acrylonitrile copolymer with sodium hydroxide and 1,6-hexamethylenediamine (1) and with hydroxylamine (2) was carried out in bulk. Membranes were then prepared from the modified copolymer as described in the Experimental section. These membranes were used as carriers for covalent immobilization of glucose oxidase. The basic characteristics of the immobilized glucose oxidase were studied and compared with the results reported earlier, using membranes that had only been surface-modified rather than bulk-modified.

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EXPERIMENTAL

Materials

Poly(acrylonitrile-methylmethacrylate-sodium vinylsulphonate) supplied by Neftochim Co., Bulgaria, was used for our experiments. The modification of acrylonitrile (AN) copolymer was carried out with the following chemical agents: sodium hydroxide (NaOH), p.a., Bulgaria; hydroxylamine (HA), 1,6-hexamethylendiamine (HMDA), p.a., glutaraldehyde, p.a., Fluka Chemie AG, Switzerland. The immobilization was carried out with glucose oxidase with specific activity of 190 U/mg, a commercial product of Bioprogress Co., Bulgaria.

Modification of AN Copolymer

Modification with Sodium Hydroxide and 1,6-Hexamethylendiamine

The AN copolymer was partially hydrolysed with 0–15 wt % aqueous solution of NaOH at a temperature of 323 K for reaction time of 60 min. Then it

was immersed in 10 wt % aqueous solution of HMDA for 60 min at room temperature.[5]

Modification with Hydroxylamine

The AN copolymer was swelled in 5 wt % aqueous solution of dimethylformamide for 30 min at room temperature. Then it was immersed in 10 wt % aqueous solution of HA (acid salty) and cured in a thermal chamber at 313 K for 120 min.[6]

Immobilization of Glucose Oxidase

Membranes prepared from modified AN copolymer were immersed in 25 wt % aqueous solution of glutaraldehyde at a temperature of 277 K for 60 min. Then they were washed with 1 *M* phosphate buffer, pH 7.0. After that the membranes were swelled in 0.1 *M* phosphate buffer (pH 7) containing 0.1 wt % glucose oxidase (190 U/mg) at 277 K for 16 h and washed with 0.1 *M* phosphate buffer (pH 7).

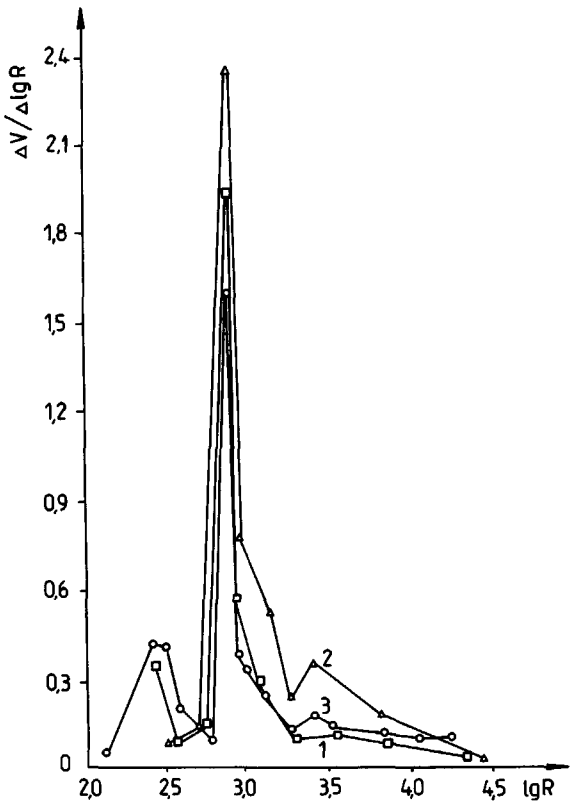

Figure 1 Differential pore size distribution of the unmodified membrane (1); membrane prepared from AN copolymer modified in bulk (2) with 6% NaOH, 60 min, 323 K and 10 wt % HMDA, 60 min, 298 K; membrane prepared from AN copolymer modified in bulk (3) with 10 wt % HA, 313 K, 120 min.

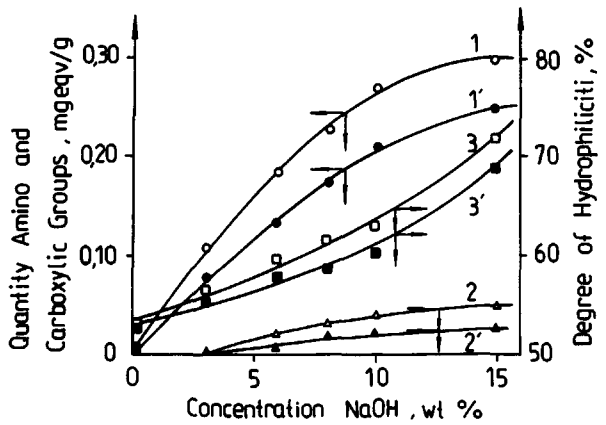

Figure 2 Effect of NaOH concentration on formation of amine (1) and carboxylic (2) groups and the degree of hydrophility (3) for membranes prepared from AN copolymer modified in bulk (1–3) with NaOH (60 min, 323 K) and 10 wt % HMDA (60 min, 298 K) and surface-modified membranes (1′, 2′, 3′) at the same conditions.

The unmodified PAN membranes were prepared by the same method, but without glutaraldehyde (immobilization by adsorption). The bound protein was measured by the method of Lowry et al.,[7] and free and immobilized glucose oxidase activities were measured spectrophotometrically (Specol 11, Carl Zeiss Jena) at 460 nm.[8]

RESULTS AND DISCUSSION

In this study, the initial acrylonitrile copolymer was modified in bulk by two methods: (1) with NaOH + HMDA, and (2) with HA. The copolymer was partially hydrolysed in aqueous NaOH to produce the corresponding carboxylic groups. The latter were made to react with HMDA to produce the corresponding amine groups.

Membranes were prepared by the phase-inversion method[9] from AN copolymer modified in bulk with NaOH and HMDA (1) and HA (2). The porosities of these membranes were essentially the same as those prepared from unmodified AN copolymer (Fig. 1).

The quantities of amine and carboxylic groups in the membranes of AN copolymer modified with NaOH and HMDA were determined by potentiometric titration[10] at different degrees of modification (Fig. 2, curves 1 and 2). These results were compared to those obtained under the same conditions for surface-modified membranes of AN copolymer (curves 1′ and 2′).

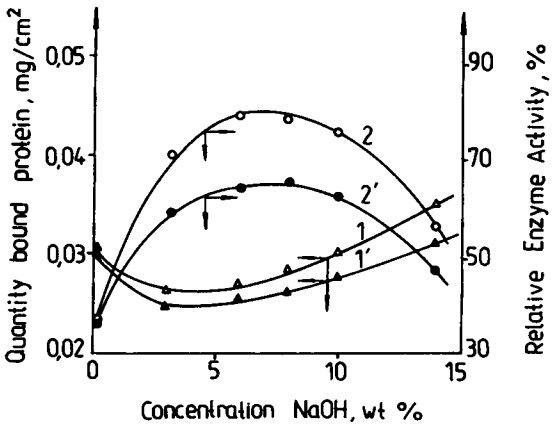

Figure 3 Effect of NaOH concentration on bound protein (1) and relative activity of glucose oxidase (2) immobilized on membranes prepared from AN copolymer modified in bulk (1, 2) with NaOH (60 min, 323 K) and 10 wt % HMDA (60 min, 298 K) and surface-modified membranes (1′, 2′) at the same conditions.

The comparison shows that the conversion of CN to the corresponding $-CO_2^{(-)}$ and $-CONHRNH_2$ groups was considerably less extensive in the case of the surface modification. This is due to the smaller degree of partial hydrolysis of the surface-treated PAN membranes, compared with the bulk-modified AN copolymer membranes treated with NaOH solutions.

The degree of hydrophilicity of both types of membranes was investigated. It was expressed by the weight difference between the water swollen and the dry membrane (water content per unit membrane weight).[11] Obviously, the degree of hydrophilicity (curves 3 and 3′) increases with the increase of the modifier concentration, and it is also higher in the case of AN copolymer modified preliminarily in bulk.

The quantity of bound protein and the activity of the immobilized glucose oxidase were studied (Fig. 3). The quantity of bound protein was found to increase with the increase of the quantity of amine groups and the degree of hydrophility.

The dependence degree of modification–relative activity (as ratio of immobilized enzyme activity to total activity of the free enzyme used for the immobilization) shows a maximum (curves 2 and 2′, Fig. 3). The optimum modification conditions for covalent immobilization of glucose oxidase in both cases were found to be at NaOH concentration between 3 and 10 wt %. Above this value, the relative activity abruptly decreases because of steric disturbances for the penetration of the substrate to the active centers of the immobilized glucose oxidase. On membranes of unmodified AN copolymer, the immobilization is accomplished by adsorption. It is well known that a matrix with higher hydrophobity adsorbs more protein.[12] Since membranes of unmodified AN copolymer are more hydrophobic compared with the other membranes studied, the quantity of the adsorbed protein is high and the relative activity of the glucose oxidase is low (Fig. 3).

Obviously, better results for the bound protein and the relative activity of the immobilized glucose oxidase were obtained by bulk modification of AN copolymer with NaOH and HMDA, rather than for the surface-modified membranes. Figure 4 shows

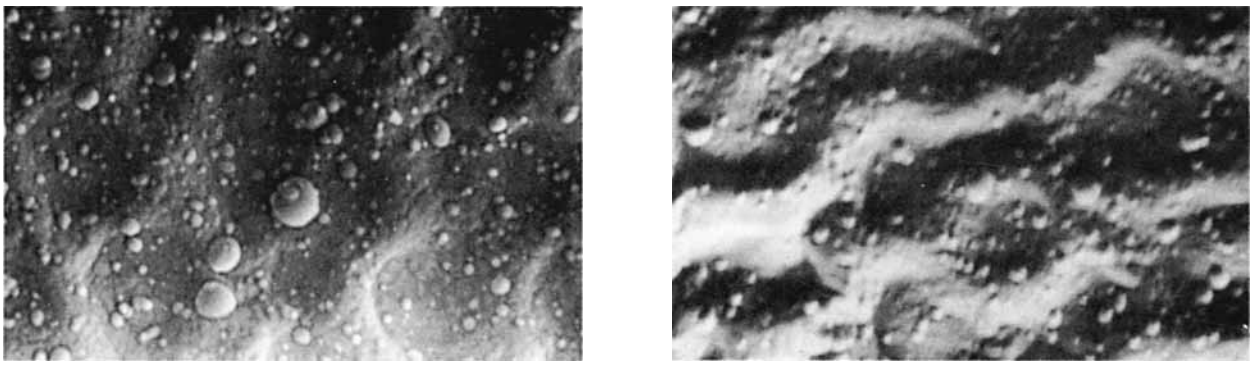

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Figure 4 Electron micrographs of glucose oxidase immobilized onto membrane of AN copolymer modified in bulk with 6% NaOH (60 min, 323 K) (1) and surface-modified membranes at the same conditions (2).

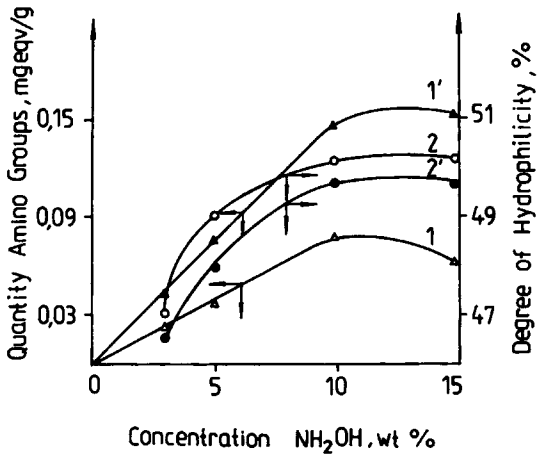

Figure 5 Effect of concentration of NH_2OH on the formation of amine groups (1) and the degree of hydrophilicity (2) for membranes prepared from AN copolymer modified in bulk (1, 2) at 313 K and 120 min and surface-modified membrane (1′, 2′) at the same conditions.

electron micrographs of glucose oxidase immobilized (1) onto membrane of AN copolymer modified with NaOH and HMDA, and (2) onto membrane surface modified with the same reagents. It can be seen that the distribution of the immobilized glucose oxidase over the surface of a membrane of AN copolymer modified in bulk is more uniform and enzyme quantity is higher.

Figure 5 shows the results obtained for the modification with HA to produce the corresponding amine and oxyme groups. The quantity of amine groups in membranes of bulk-modified copolymer was compared with that of surface-treated membranes. The amine groups' concentration increases slightly with the increase of the HA concentration up to 10 wt %, and above 10 wt % abruptly decreases. This is explained by the partial hydrolysis of amine and oxyme groups to produce carboxylic ones.[13] For the same reason, the quantity of amine groups in bulk-modified AN copolymer at all concentrations of HA was lower than that of the surface-treated membranes (curves 1 and 1′, Fig. 5). Therefore, better conditions for the process of hydrolysis were produced by the bulk modification. This is also proved by the higher degree of hydrophilicity of the membranes prepared from modified AN copolymer, regardless of their possessing fewer amine groups (curve 2, Fig. 5).

The dependence of the degree of modification on the relative activity shows a maximum (curves 2 and 2′, Fig. 6). The optimum modification conditions for covalent immobilization of glucose oxidase in both cases were found to be at HA concentration of 10 wt %. Above this value the relative activity decreases, probably because of the higher quantity of bound protein (curves 1 and 1′, Fig. 6).

In contrast to bulk modification with NaOH and HMDA, by the bulk modification with HA the relative activity and the bound protein of the immobilized glucose oxidase are lower, compared to those of the surface-modified membranes. As in former cases, this is proved by comparison of electron micrographs of glucose oxidase immobilized onto membrane prepared from AN copolymer modified with HA and onto a membrane surface modified with the same agent (Fig. 7).

The storage of the activity of the glucose oxidase immobilized onto membrane modified at the optimum conditions found was studied (Fig. 8). The best storage of the enzyme activity shows glucose oxidase immobilized onto membrane of AN copolymer modified with 6% NaOH (curve 2, Fig. 8) and membrane surface treated with the same concentration of NaOH (curve 2′, Fig. 8).

CONCLUSIONS

AN copolymer was bulk-modified with NaOH and HMDA (1) and with HA (2). The membranes prepared from the copolymer obtained possess the same porosity as the membranes of unmodified AN copolymer. The bulk modification of AN copolymer gave higher degree of modification compared with

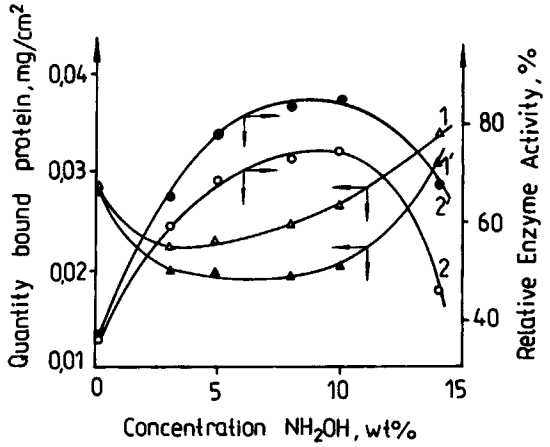

Figure 6 Effect of concentration of NH_2OH on the quantity of bound protein (1) and relative activity of glucose oxidase (2) immobilized on AN copolymer modified in bulk (1, 2) at 313 K and 120 min and surface-modified membrane (1′, 2′) at the same conditions.

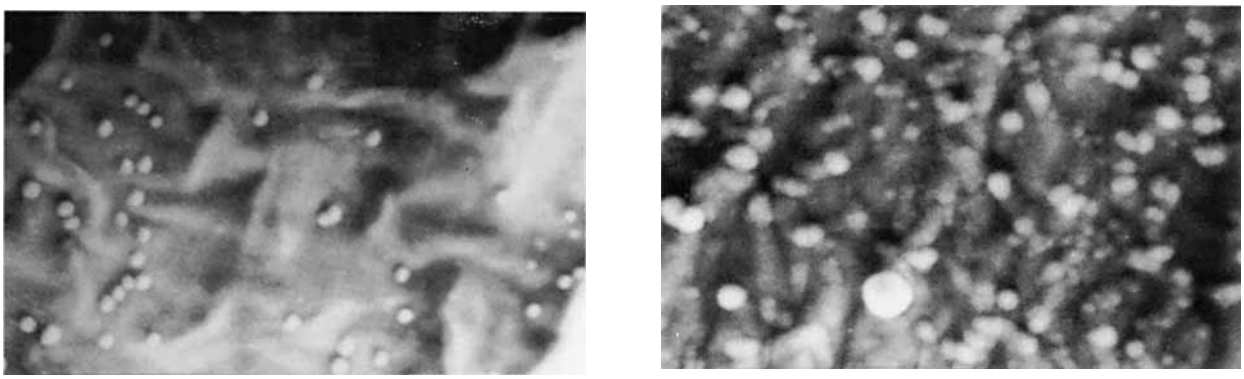

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Figure 7 Electron micrographs of glucose oxidase immobilized onto membrane of AN copolymer modified in bulk with 10% HA (120 min, 323 K) (1) and surface-modified membrane at the same conditions (2).

the surface-modified PAN membranes. Hence, the relative activity of glucose oxidase immobilized onto membranes of bulk-modified AN copolymer is higher (78% at 6 wt % NaOH), compared with that of the surface-modified membranes (62% at 6 wt % NaOH). The bulk modification of AN with HA is an exception. The amine group content decreases during the process of hydrolysis, which leads to a decrease in the relative activity of the immobilized glucose oxidase (from 85% for membranes surface-treated with 10 wt % HA to 75% for membranes of AN copolymer modified in bulk with the same concentration of HA).

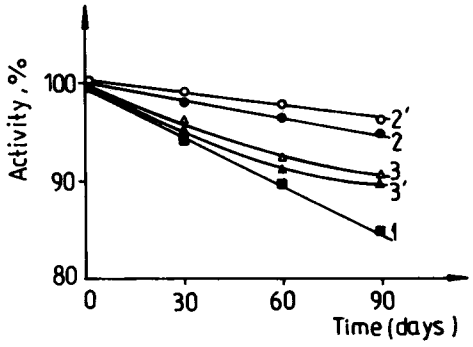

Figure 8 The effect of storage on the activity of free (1) and immobilized glucose oxidase on (2) membrane of AN copolymer modified with 6 wt % NaOH (60 min, 323 K) and 10 wt % HMDA (60 min, 298 K); (2′) surface-modified membrane with NaOH and HMDA at the same conditions; (3) membrane of AN copolymer modified with 10 wt % HA (120 min, 313 K); (3′) surface-modified membrane with HA at the same conditions. Free and immobilized glucose oxidase were stored at 277 K in distilled water.

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