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Preparation of polyvinyl alcohol-polyacrylamide composite polymer membrane by γ-irradiation for entrapment of urease

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Abstract

Composite polymer membrane of polyvinyl alcohol (PVA) and acrylamide was prepared on cheesecloth support by γ-irradiation induced free radical polymerization. The enzyme urease was entrapped in the membrane during polymerization and was cross-linked within the matrix using glutaraldehyde. The membranes could be reused a number of times without significant loss of urease activity.

Keywords: Polyvinyl alcohol; Polyacrylamide; γ-irradiation; Urease; Polymer membrane; Entrapment

Biocatalysts have been immobilized on a variety of supports using different techniques [1,2]. One of the extensively used synthetic polymers has been polyacrylamide (PAA) [3,4]. The major advantage is that it can be polymerized either chemically or by using radiation. Advantages of γ-ray polymerization against chemical polymerization is that the polymerization can be carried out even under frozen conditions thus allowing the matrix to be molded to any form such as beads or membranes [5–7]. However one of the major drawbacks of this polymer especially in a membranous form is its brittleness. Other

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synthetic polymer that has been extensively used for immobilization of biocatalysts in a membranous form is polyvinyl alcohol (PVA). As compared to polyacrylamide, PVA is more hydrophilic and having adhesive property with better tensile strength in dry conditions. But it has high swelling index and dissolves readily in water when not cross-linked. PVA can be cross-linked using a variety of reagents including γ-rays [8]. The present studies delineates a simple method for the preparation of mechanically stable conjugate membranes of polyacrylamide and PVA for immobilization of biocatalysts so as to obviate some of the limitations of individual polymeric membranes.

PVA of degree of polymerization [1700–1800] of degree of hydrolysis [98–99 mol%] and glutaraldehyde (25% aqueous solution) were procured from Loba Chemie, Mumbai; urease (EC. 3.5.1.5) was purchased from Sigma Chemical; acrylamide (extra pure) was procured from SISCO Research Laboratories, Mumbai.

PVA–PAA conjugate membranes containing urease were prepared as follows. Polymerization mixture contained PVA (5%), acrylamide (10%) and *bis*-acrylamide (3%) in 10 mM sodium phosphate buffer of pH 7.4. The components were dissolved by heating in boiling water bath for 10 min followed by cooling to room temperature. Crushed urease tablets (2.5g), dithiothretol (1 mM), β-mercaptoethanol (1 mM) and 125 μl glycerol were mixed with 50 ml of this viscous slurry and homogenized. Ten 100 cm^2 cheesecloth pieces were soaked in the slurry, removed and were frozen in toluene-dry ice bath maintained at around −78 °C. These were then exposed to 1.2 kGy of Co^{60} γ-rays in a gamma-cell 220 (Atomic Energy of Canada, Ottawa, Canada) at a dose rate of 10 Gy/min using air as the gas phase (at −78 °C). After irradiation cloth pieces were soaked in phosphate buffer (pH 7.4) containing 0.2 % glutaraldehyde for 20 min and were finally washed extensively with phosphate buffer. The membranes were partially dried at room temperature and stored at 4 °C.

For the estimation of urease activity membrane of size 5 cm^2 (2 cm×2.5 cm) were cut and were soaked in phosphate buffer containing 50 mM urea for 1 min. 20 μl aliquot of the sample was removed and the ammonia liberated was estimated colorimetrically by the phenol-hypochloride method [9].

Polymerization of acrylamide depends on the free radical mechanism initiated by γ-rays. PVA also gets cross-linked via similar mechanism. Membranes with optimal activity and mechanical stability were obtained when immobilization was carried out as described above. A dose of 1.2 kGy of γ-rays was found to be optimal. Polyacrylamide when polymerized under frozen conditions is known to result in highly porous structure, which is essential for better mass transfer in the membrane [10]. Inclusion of dithiothretol, β-mercaptoethanol and glycerol in the polymerization mixture helped in enhancing the protection/stability of the enzyme. During polymerization enzyme was found to get entrapped in the membrane. However on soaking in buffer or during reuse a slow leakage of the enzyme is seen. This was obviated by cross-linking the enzyme within the membrane using glutaraldehyde. Cross-linking carried out with 0.2–0.4% glutaraldehyde in 10 mM phosphate buffer for 20 min in the cold was found to be optimal for retention of maximum enzyme activity (Fig. 1).

The K_m and V_{max} for entrapped urease were calculated by Lineweaver-Burk double reciprocal plot as 4.36 mM urea 1.2 U/cm^2 membrane respectively which was similar to the unimmobilized enzyme.

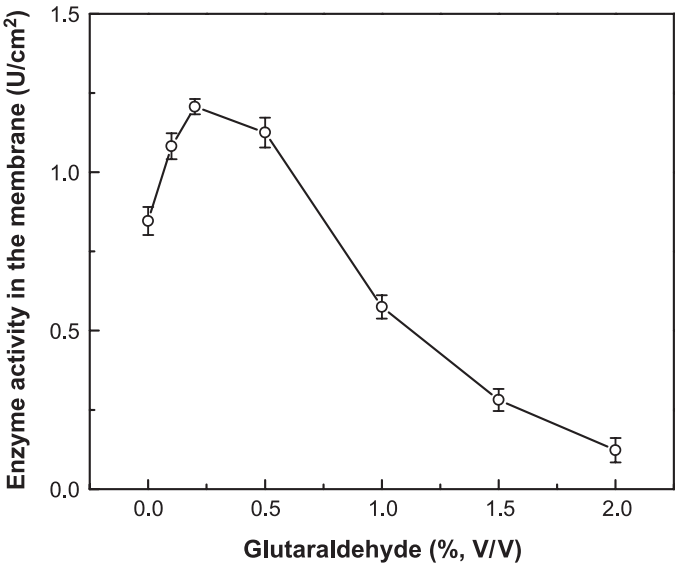

Fig. 1. Retention of urease activity in the membrane after cross-linking with increasing concentration of glutaraldehyde.

The membranes were stored moistened with buffer containing DTT, glycerol and β-ME at 4 °C. Moistening was necessary as the PAA-PVA gels upon complete dehydration yields a hard solid when kept in low humidity environment [11]. Addition of glycerol delayed the drying of the membrane. DTT and β-ME are known as thiol group protective for the enzyme [12]. Addition of these to the polymerization mixture helps in retaining the enzyme activity [12]. The enzyme activity in the membrane prepared without adding β-ME and DTT during polymerization reduced continuously with time even when stored at optimum storage condition. Casting of these membranes in cheesecloth helped greatly in enhancing its mechanical stability and helped in the ease of handling. Reuse stability of the membranes were studied by incubating them in the reaction mixture for 5 min. The supernatant was decanted off for estimation of urea hydrolyzed. The membranes were then put in another batch. The process was repeated a number of times while retaining over 90% of enzyme activity till 8 reuses.

Biocatalysts have been immobilized on a variety of supports obtained in different geometries based on their ultimate applications. Beaded forms are more useful for use in column reactors, whereas membrane forms are very useful in the fabrication of biosensors and or in membrane bioreactors. One of the important criteria for such applications of the membranes is their mechanical stability for reuse. Preparation of porous, mechanically stable membranes is also a prerequisite in the fabrication of biosensors or in membrane reactors. The membrane prepared by the method described above can find applications in some of these areas. Formation of the membranes on cloth not only enhanced its mechanical handling characteristics but also is amenable to be folded in any form for use on transducers or in annular or other membrane-based bioreactors.

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