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Functionalized bicomponent polymer membranes as supports for covalent immobilization of enzymes

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ABSTRACT

The present work was aimed at developing new reactive polymer materials suitable to be used as supports for enzyme covalent immobilization. Thus, new bicomponent polymer membranes were developed using poly(acrylonitrile-*co*-vinyl acetate) (PAN-*co*-PVAc) in mixture with polyvinyl alcohol (PVA). First of all, PAN-*co*-PVAc/PVA blends were dissolved in DMSO, until a homogenous polymer solution was obtained. To prepare membranes, these solutions were cast on a glass plate followed by the immersion of this plate in a coagulation bath containing a 50%–50% (volume %) water–isopropyl alcohol mixture. In this way membranes containing OH functional groups were obtained. Before tyrosinase immobilization, membranes were functionalized with glutardialdehyde; hence CHO binding sites were inserted and membrane became reactive for the enzyme. In order to prove that both, functionalization and immobilization reactions, were successful, the modifications produced by these reactions were investigated by various techniques i.e. Fourier Transform Infrared Spectrometry, Thermal Gravimetric Analysis, Differential Scanning Calorimetry and Atomic Force Microscopy. The occurrence of important changes in membrane features confirmed the success of both reactions. Furthermore, the activity of bonded enzyme was determined by pyrocatechol method and compared to the activity of the free enzyme.

1. Introduction

Wet phase inversion method is very often involved in membranes preparation. Organic polymers, inorganic materials or composite materials are widely used to produce membranes. Nowadays, there is an important interest in polymers modifying or in new polymer synthesis. The use of polymer blends as membrane material allows obtaining materials with designed properties, cheaper than developing new polymers [1–7].

Polyacrylonitrile exhibits several noteworthy features (good stability and solubility, excellent film-forming ability) being widely used to obtain membranes [8–14]. The use of polyacrylonitrile copolymers, instead of the homopolymer, enhances membranes properties by overcoming the drawbacks of the latter referring to its low hydrophilicity, biocompatibility, water flux (permeability) or pervaporation properties. To this matter, polyvinyl alcohol (PVA) might be of certain help, being often employed in membranes preparation because of its (I) high water solubility, (II) lack of toxicity, (III) increased hydrophilicity, biodegradability, biocompatibility, (IV) excellent film forming character and (V) a great ability to be transformed in order to make it convenient for various applications [15–19].

Natural enzymes are widely applied in various fields (catalysts, biosensors, chemical industry, analytical devices, food industry, environment protection), due to their good specificity, but their short catalytic lifetimes dramatically restrict their use. Enzyme immobilization, carried out by adsorption, entrapment, microencapsulation or covalent bonding, has emerged to increase enzyme stability and to facilitate its separation from the final reaction medium. Thus, expensive biocatalysts may be reused. After immobilization, enzymes, catalysts or even whole cells are successfully used in various processes. Polymer membranes, organic polymers, biopolymers, hydrogels, and smart polymers are the most important immobilization supports [20–27]. Membranes are often used as supports in enzyme immobilization because they offer certain special advantages, such as high specific surface and both bio-catalysis and separation functions are efficiently integrated in one structure [28].

Tyrosinase (Tyr), a copper containing enzyme, exhibits both monophenolase and diphenolase activity and may be immobilized on a wide range of supports (bentonite, carbon nanotubes, polyethylene

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oxide/polypyrrole or polypyrrole itself). Immobilized Tyr may be further used either in water pollution control or in biosensors production [29–34].

Considering the innovative multi-functionality of membrane supports brought by the synergistical use of PAN-*co*-PVAc and PVA, as well as the glutardialdehyde functionalization, the present paper was aimed at developing enzymatic composites by covalent immobilization of Tyr on these tailored bicomponent membranes. Such alternative of using two polymers instead of a single one is due to the fact that nitrile and acetate functional groups of PAN-*co*-PVAc cannot be used by themselves in enzyme immobilization and the subsequent reaction needed to turn them in binding site occurs very slowly and with low yields. For this purpose, PVA provides OH groups, which are available in the functionalization reaction with glutardialdehyde, generating reactive binding sites for enzyme immobilization. A functional polymer represents either a polymer with reactive functional groups or a polymer performing a specific function for which it is produced and used [35]. Herein, both meanings match, because functionalized membranes exhibit reactive groups (available in functionalization reaction with glutardialdehyde) and they play, also, a determined role, being the immobilization support. After functionalization, membranes reactivity is enhanced. Moreover, the use of two polymers results in pursued pores formation due to their rather different coagulation speeds. PVA improves, also, the hydrophilic character of membranes. Hydrophilicity becomes an important issue, since water ensures the required microenvironment for the enzyme. To the best of our knowledge the use of this kind of bicomponent membranes in enzyme immobilization has not been reported before. The obtained enzymatic composites will be further used in waste water ultrafiltration with simultaneous biocatalytic decomposition of phenolic compounds (resulted from detergents degradation). Hence, the achieved separation capacity of membranes is successfully coupled with the catalytic features of the enzyme.

2. Experimental

2.1. Materials

Monomers for PAN-*co*-PVAc synthesis: acrylonitrile (AN) and vinyl acetate (VAc), supplied by Merck (Darmstadt, Germany), were distilled to remove the inhibitor. Potassium peroxydisulfate (KPS) and sodium metabisulfite (MS), the components of redox initiation system, received from "Reactivul" (Bucharest, Romania) were used for PAN-*co*-PVAc synthesis without further purification. Sulfuric acid (p.a. 98%, "Reactivul") was used as received to ensure a proper pH in the copolymerization reaction.

For PVA preparation, via polymer analogs reaction, the following compounds were used: VAc and MeOH (for polyvinyl acetate synthesis, which was further used for PVA preparation), AIBN (azobisisobutyronitrile, the initiator of VAc polymerization, p.a. Merck) and NaOH (the catalyst in the reaction between polyvinyl acetate and MeOH). MeOH (p.a. Merck), AIBN (p. a. Merck) and NaOH (delivered as pellets by Sigma Aldrich, St. Louis) were used as received.

Dimethylsulfoxide (DMSO) (solvent) and isopropyl alcohol (non solvent) were provided by Merck and used without further purification.

Glutardialdehyde (GA), aqueous solution 50%, provided by Merck, was used without further purification as functionalization agent.

The enzyme, tyrosinase (Tyr, E. C. 1.14.18.1; activity of 1.050 μ/DW; molecular weight of 100 kD), supplied by Worthington (Lakewood), was used as received.

Pyrocatechol (PC, 1,2-dihydroxybenzene, $C_6H_6O_2$, Merck), the substrate used in enzymatic assays, was used as received.

A borate–phosphate buffer was prepared using $Na_2B_4O_7{*}10H_2O$ 0.1 M (Reactivul, Bucharest, Romania) and KH_2PO_4 0.1 M (Reactivul, Bucharest, Romania) and further involved in Tyr and PC dissolution.

2.2. Synthesis

To obtain the membrane-based enzymatic composites several stages were accomplished, as follows: (I) PAN-*co*-PVAc synthesis (AN/VAc = 70/30), (II) PVA synthesis (polymerization degree of 920, hydrolysis degree of 99.2%, which means an acetate groups/hydroxyl groups ratio of 0.8/99.2, and content in sodium acetate of 1.73% relative to copolymer total weight), (III) membranes preparation (using PAN-*co*-PVAc in mixture with PVA), (IV) membrane functionalization with glutardialdehyde and, finally, (V) enzyme (Tyr) immobilization onto the functionalized membrane. Copolymer (C) and PVA synthesis, as well as membrane preparation conditions, were detailed in a previous paper [36].

2.2.1. Membranes preparation

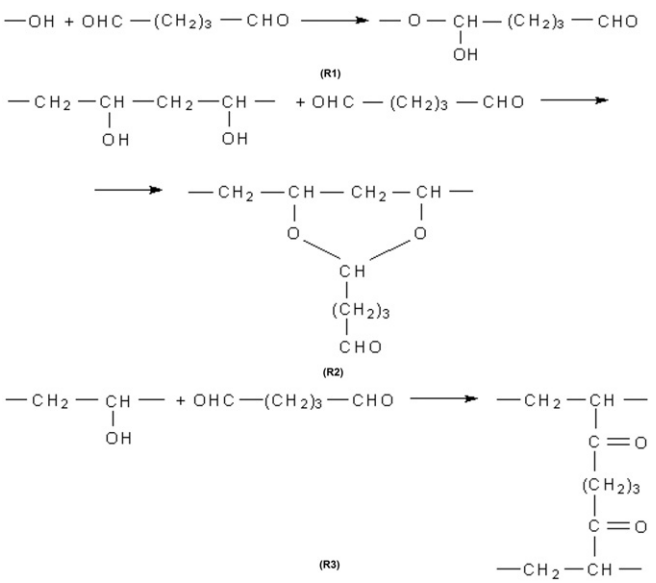
In order to produce the membranes (noted hereafter M1 and M2, respectively), two polymer solutions were prepared using C-PVA mixtures, with content in PVA of 20 and 10%, respectively. Then the solutions were cast on a glass plate support, followed by the support immersion in a water–isopropyl alcohol mixture (50:50 volume ratios). Membranes were kept for 10 h in the coagulation bath in order to stabilize their structure (finalize the precipitation process of polymer blend).

2.2.2. Membranes functionalization with glutardialdehyde (GA)

For membranes functionalization, a liquid phase (32 mL H_2O, 8 mL GA aqueous solution 50% and 0.560 mL H_2SO_4 98%) was prepared and contacted with 0.4 g wet membrane for 30 min at 70 °C. Functionalization may occur at PVA component from the membrane in three different manners (Scheme 1), leading to semiacetals (R1) or acetals (R2, R3).

2.2.3. Enzyme (Tyr) immobilization

For Tyr covalent immobilization step, an enzyme solution (1 mg/mL) was first prepared in borate–phosphate buffer 0.1 M pH = 6.5. The immobilization stage consisted of contacting the functionalized membrane surface (0.1 mg wet M1-GA or M2-GA sample) with the prepared enzyme solution at 4 °C. After 10 h, the membrane was intensively washed with distilled water and buffer to remove the absorbed and adsorbed enzyme. The traces of absorbed and adsorbed free Tyr were insignificant, under the detection limit of the UV–vis instrument, therefore it was postulated that the immobilization yield was close to 100%. Immobilization may occur at NH_2 group of the enzyme amino-acids in



Scheme 1. Mechanism of membrane functionalization reaction.