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Urease-carrying electrospun polyacrylonitrile mat for urea hydrolysis

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ABSTRACT

Electrospinning was used to fabricate beadless microfibrous polyacrylonitrile (ePAN) mats with an average fiber diameter of 1448 ± 380 nm from a 10 wt.% PAN in dimethylformamide (DMF) dope solution at applied voltage of 18 kV and 20 cm fiber collection distance. Urease (EC 3.5.1.5) was then covalently immobilized on dispersed microfibrous ePAN mats following the chemical treatment of fibers with ethylenediamine (EDA) and glutaraldehyde (GA). The optimal concentration of GA for immobilization was 5%. The amount of loaded urease reached 157 μg/mg mat, exhibiting 54% of the free urease activity. The surface chemistry of as-spun and chemically treated fibers was examined with Fourier transform infrared (FTIR) spectroscopy. Field emission scanning electron microscopy (FESEM) was used to study the morphology and diameter of the pristine, chemically treated, and urease-immobilized microfibrous mats. Immobilized urease showed increased temperature for maximum activity (from 37 to 50 °C for free and immobilized urease, respectively) and improved storage stability (20 days). The immobilized urease was also less sensitive to the changes in pH, especially in acid conditions. In addition, nearly 70% of initial activity of the immobilized urease was retained after 15 cycles of reuse, which proved the applicability of the electrospun fibers as successful enzyme carriers.

1. Introduction

In quest of a reliable way for daily removal of blood urea from patients suffering from permanent renal failure, artificial wearable or portable kidney machines have recently been developed [1,2]. In order to maintain the device within an acceptable working condition, the dialysate solution, which is mainly contaminated with urea, should be regenerated continuously. Urea is a remarkably stable molecule with degradation half-life of 3.6 years at 38 °C when dissolved in water. Due to its non-ionic polar nature, it can hardly be removed from water by ion-exchange media, activated carbon granules, or reverse osmosis membranes [3]. Thus, it seems that the most effective method for regeneration of the dialysate solution in the wearable kidney machine is the utilization of urease [4], a nickel-based enzyme that catalyzes urea hydrolysis to ammonia and carbon dioxide 10^{14} times faster than uncatalyzed urea hydrolysis.

To overcome the intrinsic vulnerability of free enzymes to the changes that intermittently happen in the reaction medium such as changes in pH and temperature, the immobilized form application of enzymes is preferable. More importantly, the immobilized enzyme provides a steady process, easy removal of the products, and convenient recovery of the catalyst from the stream. Three major methods are scrutinized for immobilization of different sorts of enzymes, i.e. adsorption, covalent binding, and encapsulation (entrapment) [5]. Among these techniques, covalent immobilization is widely studied as a durable technique for localization of enzymes on solid supports due to irreversibility of the bonds formed between the bioactive compartments and the support surface.

The choice of the support material and its conformation significantly affects the performance of the immobilized enzyme. Ideally, it should be inert, fairly inexpensive, and possess acceptable mechanical strength. In addition, its chemistry should allow easily incorporating of functional groups essential for direct or indirect covalent attachment of enzymes.

Polyacrylonitrile (PAN) is an important engineering polymer with good film and fiber forming characteristics which has widely been utilized for biomedical applications and enzyme

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immobilization. The presence of nitrile groups (C≡N) in the backbone of the polymer chains provides the possibility for incorporation of new reactive functional groups, macromolecules, or biofriendly species making PAN films or fibers hydrophilic and biocompatible [6]. Different PAN-based membranes has long been studied for direct or indirect (use of coupling agents) covalent immobilization of different sorts of enzymes. Poly (acrylonitrile-*co*-methylmethacrylate-*co*-sodium vinylsulfate) was activated with NaOH and hexamethylenediamine (HMDA) or hydroxylamine. Glucose oxidase (EC 1.1.3.4) was then covalently attached to the surface of membrane using glutaraldehyde (GA) as the coupling agent [7]. Similar procedure was also used for immobilization of urease (EC 3.5.1.5) on PAN hollow fibrous membranes [8].

The support conformation should pose minimum diffusional limitation and provide utmost surface area per unit mass for high enzyme loading [9]. Electrospun polymeric fibers are promising for enzyme immobilization [10–13] because the electrospun mats are more easily produced, dispersed in and recovered from the reaction media than other choices of nanoscaled geometries, e.g. nanoparticles and nanotubes [14]. Furthermore, electrospun fibers with high specific surface area could be prepared from a wide choice of polymers with disparate chemical characteristics, which render possibilities for different modes of enzyme immobilization [15–17].

Recently, enzyme immobilization on PAN-based nanofibrous membranes has become increasingly interesting because of the relatively easy procedures for introducing necessary functional groups on the fiber surface prior to immobilization and for fiber production. Lipase (EC 3.1.1.3) and cellulase (EC 3.2.1.4) have been successfully immobilized on electrospun PAN (ePAN) fibers through an amidination activation of nitrile groups and reaction with amine pendant groups of the enzymes [18–20]. Others have used PAN derivatives and tethered the electrospun fibers prior to immobilization with biomacromolecules such as collagen or protein hydrolysate from egg skin in order to circumvent the hydrophobic nature of PAN and to make it biocompatible [21,22].

Direct amine functionalization of nanofibrous PAN mats have recently been studied for efficient heavy metal ion removal from aquatic environments [23–25]. Amine containing reagents such as ethanolamine, ethylenediamine [24,26], or hydroxylamine [25] react with PAN pendant nitrile groups through nucleophilic addition [24]. After the amine functionalization, bioactive compartments containing —NH_2 groups can be immobilized on the surface of aminated carrier by use of glutaraldehyde (GA) through the formation of stable amino-aldehyde bonds [27]. However, there are no references concerning the application of directly aminated PAN membranes or fibers for immobilization of enzymes.

In this work, the surface aminated electrospun polyacrylonitrile (NH_2-ePAN) mats were prepared by electrospinning of PAN dope solution followed by amination with ethylenediamine (EDA). Urease was then covalently immobilized on NH_2-ePAN mats using glutaraldehyde (GA). Properties of the immobilized urease were studied and compared to those of free enzyme. Results will possibly lead to the use of urease-immobilized ePAN mats (Urs-ePAN) in miniaturized wearable kidneys to regenerate the valuable dialysate fluid by preserving the catalytic activity for increasing the number of reuses.

2. Experimental

2.1. Materials

Jack bean urease type III (EC 3.5.1.5), PAN (MW = 150,000), and GA (25 wt.% aqueous solution) were purchased from Sigma (USA) and used as received. Dimethyl formamide (DMF) (analytical grade), ethylenediamine (EDA) (analytical grade), potassium dihydrogen phosphate (monobasic) (analytical grade), potassium monohydrogen phosphate (dibasic) (analytical grade), and urea (biology grade) were purchased from Merck (Germany). The activity of the native or immobilized urease was measured by Atlas Medical Berthelot urea kit. All other chemicals were of analytical grade. Deionized water (DI) (>18 MΩ cm) was used for washing or preparation of all aqueous solutions unless otherwise stated. The urea and urease solutions were prepared in 22 mM phosphate buffers (PBS) containing 1 mM ethylenediamine tetra acetic acid (EDTA) as an ion chelating compartment of the PBS.

2.2. Preparation of NH_2-ePAN mats

The 10 wt.% PAN dope solution was prepared by slowly adding of PAN powder to DMF followed by mechanical stirring for 24 h at ambient temperature. The solution was then loaded to a 10 ml glass syringe and was upheld for 2 h to remove the dispersed air bubbles. A metal needle spinneret (D = 0.7 mm) was attached to the syringe tip. Electrospinning was performed for 7 h to collect a detachable thickness of the fibers. A syringe pump (Cole-Parmer® 100 Touch Screen) was used to deliver the polymer solution to the tip of the needle at a constant flow rate of 1 ml/h. High voltage power supply (ES30P-5W-Gamma High Voltage Research, Florida, USA) with low current output (166 μA) was used as the electricity power source. Positive charge (18 kV) was applied to the needle tip and the produced electrospun fibers were collected on a grounded aluminum sheet. The distance between the needle tip and the collector was kept constant at 20 cm. Afterwards, the nonwoven sheets were cut into 2 × 2 cm^2 sheets which were dried overnight in a vacuum oven and weighed carefully after drying. The sheets were then soaked in 70% ethanol aqueous solution in small glass bottles, followed by shaking on a rocking shaker at 250 rpm for 24 h to disentangle the fibers by increasing their wettability. Further surface functionalization was carried out on the dispersed spun fibrous mats. The dispersed fibers were washed successively and kept immersed in 3 M aqueous ethylenediamine (EDA) solution at 99 ± 1 °C for 4 h. Those pieces were further washed to remove residual EDA. Aminated electrospun ePAN mats, called NH_2-ePAN, were stored in DI for later use.

2.3. Immobilization of the urease using glutaraldehyde

Aminated electrospun mats, NH_2-ePAN, were soaked in aqueous GA solutions at different concentrations, 0–10 wt.%, for 2 h at ambient temperature, followed by washing with a copious amount of water. GA treated mats (GA-ePAN) were further loaded in glass bottles containing 5 ml of urease solution in PBS (1 mg/ml) at pH 7. Then, the bottles were shaken for 1 h at ambient temperature. Afterwards, the bottles were transferred to a refrigerator for further urease covalent immobilization at 4 °C ± 1 °C for 24 h.

2.4. Characterization

The attenuated total reflection-FTIR (ATR-FTIR) (Thermo Nicolet Instrument Corporation, Madison, WI) was used to characterize the surface chemistry before and after the surface modification of the pristine electrospun PAN mats (ePAN).

The morphology and diameter of the pristine, the chemically treated, and the urease immobilized ePAN mats were investigated by field emission scanning electron microscope FESEM (JEOL JSM-6701F, Japan). The samples were prepared by placing 0.5 × 0.5 cm^2 pieces over FESEM specimen stabs, followed by sputtering a thin