

Chemical Modifications of Electrospun Non-woven Hydroxypropyl Cellulose Fabrics for Immobilization of Aminoacylase-I

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Abstract

Because of its wide solubility range, Hydroxypropyl Cellulose (HPC) is suitable for fabricating fine-fiber materials *via* electrospinning. The resulting electrospun non-woven fabric (HPC-ESNW) requires an appropriate post-spinning treatment for applications in aqueous environments. In the present study, we examined the insolubilization of HPC-ESNW *via* cross-linking using bifunctional isocyanates. Modification of the fine-fiber surfaces with NCO groups enables introduction of cationic functionalities; we found that cationic Diethylaminoethyl (DEAE) groups are suitable for immobilization of aminoacylase-I onto these fine-fiber surfaces under mild conditions. The NCO groups can be also converted to amino groups, followed by activation with bifunctional *N*-hydroxysuccinimide (NHS) esters. The NHS-ESNW can chemically bind the aminoacylase-I. Two kinds of immobilized enzyme were tested for stereospecific recognition of a substrate and for immobilized activity yields. The results suggest that NCO-ESNW is a multi-purpose intermediate for chemical modification of ESNW and that the hydrophilic HPC-ESNW is a promising material for use as a matrix for enzyme immobilization.

Keywords: Aminoacylase; Chemical modification; Electrospinning; Hydroxypropyl cellulose; Immobilization

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1 Introduction

Electrospinning is a versatile method for fabrication of fine fibers directly from polymer solution and has been widely studied on spinning mechanisms and applications [1]. The fibers are typically recovered as non-woven sheets and often called as electrospun non-woven fabrics (ESNWs), which are composed of fine fibers having diameters of submicro- (200–600 nm) to nano- (> 100 nm) scales [2]. Applied research of the ESNWs encompasses biomaterial, filtration, catalyst support, composite, and so on [3]. Most of these focus the available greater surface on the ESNWs for material functions, as compared with other shapes such as films, membranes, or gels [4].

In a series of previous studies, we have described preparative methods of ESNWs using polysaccharides, chitosan [5, 6] and cellulose [7]. We also have reported several applied research works on the ESNWs prepared from polysaccharide [7, 8] and synthetic poly(amino acid)s under watery environments [9, 10]. Hydroxypropyl cellulose (HPC) is a soluble derivative of cellulose, which has propylene oxide units bound to the hydroxyl groups of the parent cellulose molecules (Scheme 1). Electrospinning of HPC has been already described by Shukla [11]. Due to widely ranged solubility of HPC, on the other hands, a post-spun treatment is required for insolubilization of the HPC-ESNWs for further usage, namely, insolubilization of HPC-ESNW allows it to be utilized for application in watery environments. One of appropriate post-spun treatments is chemical cross-linking using bifunctional cross-linker molecules, which is reactive towards the side chain hydroxyl groups of HPC.

The cross-linked HPC-ESNW has still hydrophilic fiber surfaces, which can be adopted for biological molecules to interact with the large surface area. In order to design a reasonable application associated with the material properties of HPC-ESNWs, here, we concern to evaluate the HPC-ESNW as a support matrix for biological catalysts, *i.e.*, enzymes. Aminoacylases and lipases have been studied as immobilized enzymes for a prolonged period [12–17] and in the present study, aminoacylase-I (E.C. 3.5.1.14) was selected as the first attempt for immobilization onto the HPC-ESNW, since the enzyme is the earliest catalyst for industrial production of optically pure amino acids [12]. The aminoacylase-I catalyses hydrolysis of N^α -acetyl-L-amino acids to yield free L-amino acids and acetates. In the case of aminoacylase-I from hog kidney, the enzyme has stereo-specificity for N^α -acetyl-L-amino acids and does not hydrolyze N^α -acetyl-D-amino acid [17] Methods for immobilization of aminoacylases have been already described mainly by Chibata *et al.*, utilizing electrostatic interaction between the enzyme and cationic diethylamino ethyl (DEAE-) cellulose solids [12] and DEAE-Sephadex hydrated beads [13]. In order to adopt their concepts to the application of HPC-ESNW, requirements for efficient immobilization of aminoacylase-I is to find out a chemical modification procedure to give a cationic HPC-ESNW matrix, which should well coincide with the chemical cross-linking method of HPC-ESNW. In the present study, hexamethylene diisocyanate (HMDI) was used for cross-linking the HPC molecules in as-spun ESNW, resulting in insolubilization, and the unreacted NCO groups were further available for addition reaction with *N,N*-(diethyl)ethylene diamine to introduce DEAE groups on the HPC-ESNW matrix (Scheme 2).

In addition to that, there have been reported the immobilization procedures using chemically activated matrix, which allows condensation reaction between the carboxyl groups on matrix and the side chain amino groups of enzyme molecules, leading to covalent bond formations between the matrix surfaces and enzyme molecules [16, 17]. The chemical binding for enzyme immobilization can be applied for the enzymes which does not adsorb onto the cationic matrix, and thus the matrix is used for immobilizing more wide range of enzyme species. As described above,