Generation of Molecular Recognition Sites in Electrospun Polymer Nanofibers via Molecular Imprinting

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Received September 27, 2005; Revised Manuscript Received November 7, 2005

ABSTRACT: A simple method for the formation of molecular recognition sites in polymer nanofibers was developed. Electrospun nanofibers were prepared from a solution mixture of poly(ethylene terephthalate) (PET) and polyallylamine in the presence of a template molecule, 2,4-dichlorophenoxyacetic acid (2,4-D). The polyamine was used to provide functional groups that interact with the template during the electrospinning process, and PET acted as a major supporting matrix to ensure easy fiber formation and to minimize the conformational change of the polymers when the nanofibers were subjected to different solvent treatments. When the template was removed by solvent extraction, imprinted binding sites were left in the nanofiber materials that are capable of selectively rebinding the target molecule. The molecularly imprinted nanofibers were characterized by SEM, FT-IR, and radioligand binding analysis.

Introduction

The electrospinning process produces continuous polymer nanofibers with diameters ranging from a few nanometers to several micrometers (usually between 50 and 500 nm) through the action of an external electric field imposed on a polymer solution or melt.[1−8] At a voltage sufficient to overcome surface tension, fine jets of liquid are formed and ejected toward a grounded target. The single jet initially formed is divided into multiple filaments by radial charge repulsion, a process known as "splaying", which results in the formation of solidified nanodiameter fibers. By regulating the process parameters and the feeding polymer composition, the electrospinning process can be adjusted to control the fiber morphology (diameter, shape, porosity) and the physical and chemical properties of the resulting nanofibers.[5,6,13]

Electrospun nanofibers have very high surface area, mechanical strength, and flexibility. When collected on a plain target plate, they can easily form defined three-dimensional mats with well-controlled pore sizes. This makes electrospun nanofiber mats ideal candidates for industrial filtration purposes.[14] It is highly desirable for selective molecular recognition sites to be able to be introduced into nanofiber materials as they may provide highly efficient compound separation involving only a simple filtration step. In this work we investigated the possibility of preparing electrospun nanofibers that contain molecularly imprinted binding sites directly from a mixture of polymer solution containing a molecular template.

Molecular imprinting is a synthetic process that leads to the formation of template-defined binding sites in synthetic polymers.[15−20] Typically, this involves free radical copolymerization of functional monomer and cross-linker in the presence of a target molecule that acts as a molecular template. Subsequent removal of the template molecule from the solid polymer matrix reveals an imprinted recognition site (complementary to the template in shape and position of functional groups) that is selective for the original molecular template and its structural analogues (Figure 1). The bond between the template and the functional monomer or polymer can be either covalent[21,22] or noncovalent.[23]

Considerable advantages of molecularly imprinted polymers (MIPs) are their high chemical and physical stability. MIPs have been used as antibody and enzyme mimics in an increasing number of applications in which selective target recognition plays an important role. These include the use of MIPs in affinity-based separations, ligand binding assays, screening of combinatorial libraries, biomimetic sensors, and controlled organic synthesis.[24−31]

In this work we attempted to combine appropriate functional polymers with a supporting material to generate template-defined binding sites directly by electrospinning. The herbicide that has been of some environmental concern, 2,4-dichlorophenoxyacetic acid (2,4-D), was used as a model compound. Instead of using highly cross-linked polymer network to stabilize imprinted recognition sites, the present study exploited the relatively strong dipole−dipole interactions between the π-electron systems of the benzene rings and the carbonyl groups in the PET backbone to maintain the recognition sites during the process of template removal. To our knowledge, this is the fisrt example of molecular imprinting recognition sites by electrospinning.

Experimental Section

Materials. Poly(ethylene terephthalate) (PET) was from Wellman International Ltd. (Ireland). Analytical grade trifluoreacetic acid (TFA), dichloromethane (DCM), 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4-dichlorophenoxyacetic acid-carboxy-^{14}C (^{14}C-2,4-D, specific activity 15.7 mCi mmol^{-1}), and poly(allylamine) (MW 65 kDa, 20% solution in water) were purchased from Sigma-Aldrich (Figure 2). The poly(allylamine) solution was concentrated to 50 wt % in a 40 °C oven prior to use.

Electrospinning. PET was dissolved in a 1:1 mixture of DCM and TFA (v/v). This was mixed with polyallylamine solution (20 wt % of PET) and stirred for 3 h until complete dissolution. Finally, 2,4-D (20 wt % of PET) was added, and the solution was stirred overnight at room temperature. No phase separation was observed for all the solutions tested prior to electrospinning.

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10.1021/ma052091w CCC: $33.50
Published on Web 12/09/2005

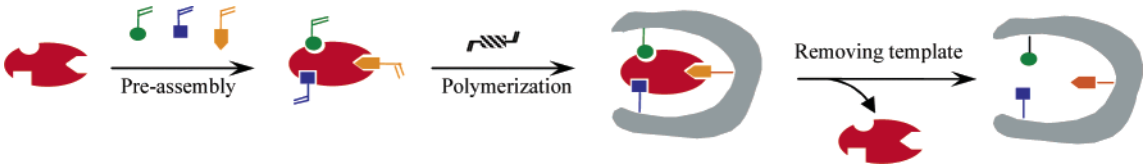
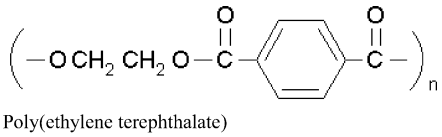
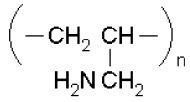


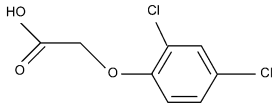
Figure 1. Schematic representation of molecular imprinting. Preassembly of functional monomers is driven by their molecular interactions with the template. Copolymerization with a cross-linker "freezes" binding groups to form a template-defined "cavity". Removal of the template by solvent extraction or chemical cleavage affords binding sites specific to the original template.



Poly(ethylene terephthalate)



Poly(allylamine)



2,4-Dichlorophenoxyacetic acid (2,4-D)

Figure 2. Chemical structures of matrix polymer (PET), functional polymer (polyallylamine), and templeate molecule (2,4-D) used in this study.

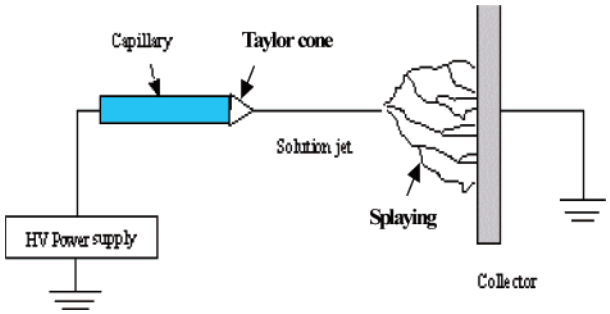


Figure 3. Scheme of the electrospinning equipment used.

The polymer solution was electrospun at room temperature using the setup shown in Figure 3, at a driving voltage of 30 kV (HV Power Supply, Gamma High Voltage Research, Ormond, FL). The syringe used in this experiment had a capillary tip diameter of 0.8 mm that contained an attached copper wire used as the positive electrode. A grounded aluminum foil used as the counter electrode was placed 20 cm from the tip of the capillary. Continuous nanofibers were collected on the aluminum foil to form a nonwoven, fibrous mat. The nanofiber mats were treated with methanol in a Soxhlet apparatus for 15 h to remove the template. The mats were then placed in a vacuum chamber for at least 4 h and kept in a desiccator until analysis. As a control, nonimprinted nanofiber was prepared in the same way except that no template was used during the electrospinning process.

Scanning Electron Microscopy. The morphology and diameter of the PET nanofibers were determined with a scanning electron microscope SEM (JEOL JSM-T300). A small section of the fiber mat was placed on the SEM sample holder and sputter-coated with gold.

Infrared Spectroscopy. The equipment used was an FT-IR microscope with the AutoIMAGE System supplied by Perkin-Elmer. The measurements were made in triplicate.

Radioligand Binding Analysis. The imprinted and nonimprinted nanofiber mats were cut into 3 × 0.5 cm strips and placed in 2 mL polypropylene vials. To each vial were added 1.3 mL of deionized water and radioactively labeled 2,4-D (360 pmol) dissolved in 0.2 mL of ethanol. Samples were incubated at ambient temperature for 4 h. After the incubation, a clear solution (0.65 mL) was taken from each vial, mixed with 10 mL of scintillation liquid Ecoscint A (National Diagostics, Atlanta, GA), and counted for 1 min using a model 2119 Rackbeta β-radiation counter (LKB Wallac, Sollentuna, Sweden). The amount of radioactive 2,4-D bound to the nanofiber mats was calculated using the following equation:

$$\text{bound (\%)} = 100 \times [[\text{CPM(total)} - \text{CPM(free)}]/\text{CPM(total)}] \quad (1)$$

Competitive Binding Assays. The imprinted and nonimprinted (reference) nanofiber mats were cut into 3 × 0.5 cm strips and placed in 2 mL polypropylene vials. To each vial was added 1.3 mL of the following solvents: deionized water and 20 mM sodium formate (pH 3.0) or 20 mM sodium phosphate (pH 6.0). Radioactively labeled 2,4-D (360 pmol) dissolved in 0.2 mL of ethanol was added to the above solution. Samples were incubated at room temperature for 4 h. A small portion (0.1 mL) of clear solution was then withdrawn from each sample vial, mixed with 10 mL of scintillation liquid, and counted for 1 min to measure the amount of bound labeled 2,4-D. Following this, 0.1 mL of 30 mM unlabeled 2,4-D dissolved in ethanol was added to the remaining contents in the sample vial. Samples were further incubated at room temperature for 16 h. After this period, 0.1 mL of clear solution was taken from each sample vial, mixed with 10 mL of the scintillation liquid, and counted for 1 min to measure the amount of labeled 2,4-D still bound to the fiber mats.

Results and Discussion

Electrospinning of Nanofibers. PET was selected as the supporting fiber matrix because it could be easily electrospun to ultrafine nanofibers. The concentration (correspondingly viscosity) of PET solutions was one of the most effective variables for controlling the fiber morphology. Electrospray took place at a polymer concentration of 5 wt %, as chain entanglements are insufficient to stabilize the jet and lead to a droplet spray that coalesces into ill-defined shapes (data not shown). However, continuous nanofiber structures were obtained by electrospinning from 10 wt % polymer concentration (Figure 4a,b). The long, electrospun PET nanofibers were randomly distributed in a fibrous mat with very uniform and dense structures. The fibers exhibited cylindrical morphology with an average fiber diameter in the range of 150 ± 50 nm. The addition of polyallylamine or 2,4-D template molecule to the starting polymer solution caused no noticeable change in morphology in the PET nanofibers (Figure 4a,b).

The imprinted composite nanofibers presumably contained evenly blended structures of PET and polyallylamine. This is based on the fact that our electrospinning started from a homogeneous polymer solution containing PET, polyallylamine, and the 2,4-D template. As electrospinning produces structured polymer nanofibers on a millisecond time scale, it is unlikely to have phase separation during this extremely short period. The molecular interaction between polyallylamine and the template carboxyl group became dominant when the solvent completely evaporated at the end of the electrospinning process. The PET moeity provided additional interpolymer chain interactions that maintained the binding site fidelity, as well as good fiber matrix

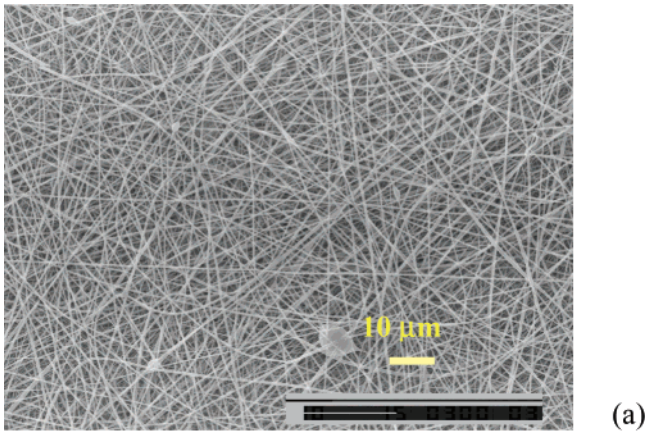


Figure 4. SEM micrographs of electrospun nanofibers onto aluminum foil from a solution consisting of 10 wt % PET and 2 wt % polyallylamine in TFA/DCM (1:1): (a) nonimprinted nanofiber, 1000× magnification; (b) nonimprinted nanofiber, 5000× magnification.

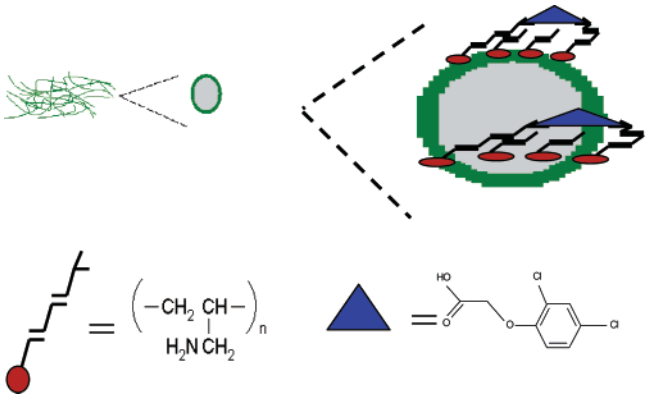


Figure 5. Schematic represenatation of molecularly imprinted nanofibers with binding sites specific for 2,4-D template molecules.

strength. Figure 5 depicts a possible binding site model for the present 2,4-D imprinted nanofiber.

Template Removal. In the present system we utilized the noncovalent strategy to simplify template removal. This was easily achieved by a simple solvent extraction treatment. The FT-IR spectra in Figure 6 indicated that extraction with methanol using a Soxhlet apparatus could provide efficient removal of the 2,4-D template. The characteristic peaks of the 2,4-D molecule at 1769 and 1479 cm^{-1} (as shown at spectra b) disappeared completely after the extraction treatment, and the IR spectrum became identical to that of the nonimprinted PET/poly(allylamine) nanofiber (spectra a). Moreover, as illustrated in Figure 7, the solvent extraction did not cause any noticeable

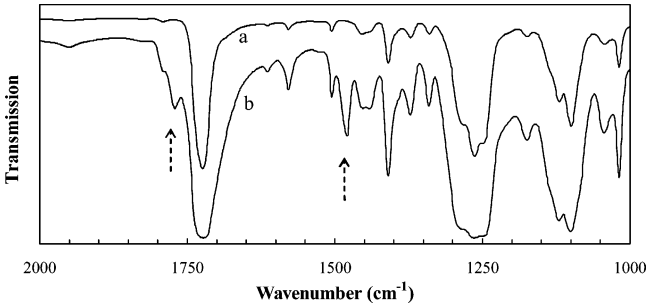


Figure 6. Microscopy FTIR spectra for (a) nonimprinted nanofiber prepared from 10 wt % PET and 2 wt % polyallylamine in TFA/DCM (1:1) and (b) imprinted nanofiber prepared from 10 wt % PET, 2 wt % polyallylamine and 2 wt % 2,4-D in TFA/DCM (1:1). For clarity, only the spectra between 2000 and 1000 cm^{-1} are presented.

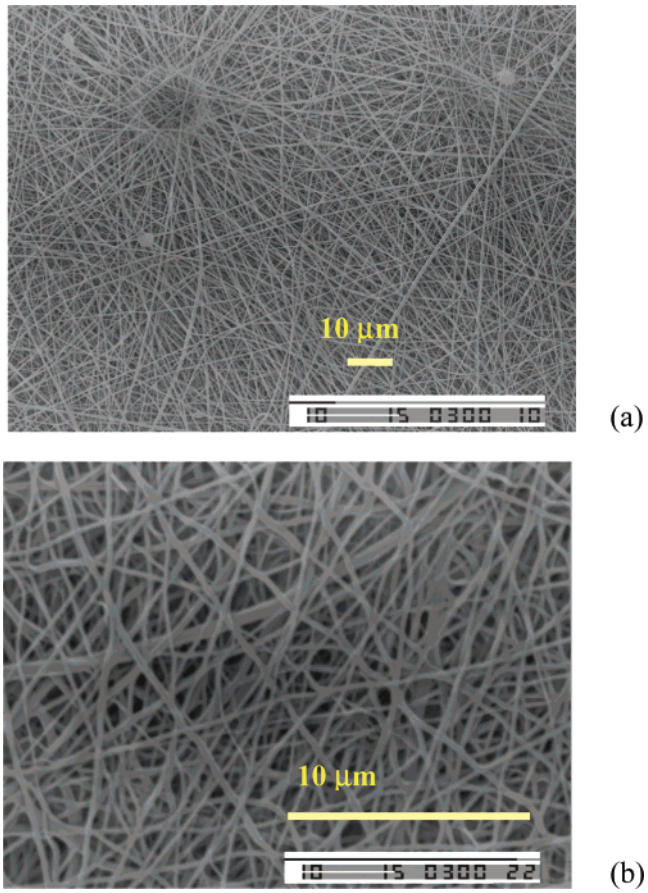


Figure 7. SEM micrographs of electrospun nanofibers onto aluminum foil from a solution consisting of 10 wt % PET, 2 wt % polyallylamine, and 2 wt % 2,4-D in TFA/DCM (1:1): (a) imprinted nanofiber after template removal by solvent extraction, 1000× magnification; (b) imprinted nanofiber after template removal by solvent extraction, 5000× magnification.

change of morphology for the imprinted PET/poly(allylamine) nanofibers.

Molecular Recognition. For future applications, we were interested in studying 2,4-D binding in an aqueous environment at a low concentration level. Radioligand binding analysis was used to characterize the target recognition property because of its high sensitivity. The sample strips used in the binding analysis contained ~3.5 mg of nanofiber mats. In preliminary experiment, the imprinted nanofiber mat bound 75% of the added 2,4-D (initial concentration 24 nM in water) within 4 h, whereas the nonimprinted nanofiber took up only 15% of the 2,4-D under the same conditions. Thus, the target binding with the imprinted nanofiber was 5-fold of that when the non-

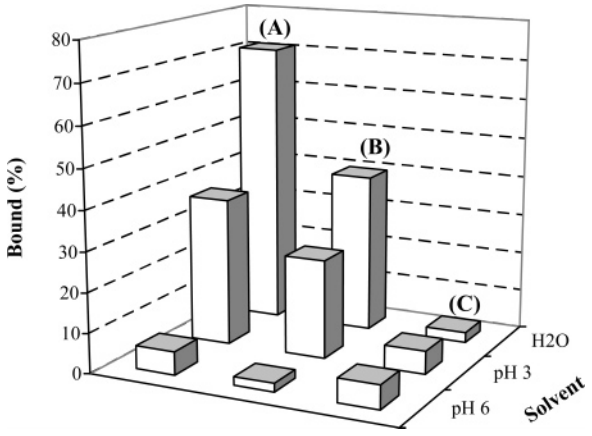


Figure 8. Radioligand binding analysis of imprinted and nonimprinted nanofibers in buffer (pH 6.0 and 3.0) and in pure water. The amount of ^{14}C-2,4-D bound to the nanofibers was measured with and without the addition of unlabeled 2,4-D. (A) columns stand for the imprinted fibers in the absence of unlabeled 2,4-D, (B) columns stand for the imprinted fibers in the presence of unlabeled 2,4-D (2 mM), and (C) columns stand for the nonimprinted fibers in the absence of unlabeled 2,4-D.

imprinted nanofiber was used. This indicated that the present imprinting by the electrospinning method indeed generated high affinity binding sites that were characteristically different from the randomly distributed functional groups in the nonimprinted nanofiber. It is possible that the amino groups from polyallylamine in the imprinted nanofiber were arranged in a template-defined orientation during nanofiber formation, so that, upon removal of the template, they remained in place to furnish a complementary binding site for the original template. In addition, PET may also contribute to assist the formation of the defined binding site via $\pi-\pi$ interaction with the aromatic moiety of the template. The relatively high rebinding activity may furthermore be explained in terms of the very high surface area and porosity of the nanofiber mats. In contrary to the conventional imprinted polymers containing highly cross-linked networks, the recognition sites in the present electrospun nanofibers were stabilized by the dipole−dipole interactions between the π-electron systems of the benzene rings and the carbonyl groups in the PET polymer backbone. It must be noted that the recognition sites in the imprinted nanofibers were stable enough to survive the long solvent extraction treatment. In addition, the template-removed nanofibers displayed the same binding performance after being stored at room temperature for more than 1 month.

Assuming that template binding took place in the imprinted sites, we expected that the labeled 2,4-D would be displaced when excess unlabeled template was added to the solution. This was based on the assumption that competition for binding only took place at a limited number of specific sites. No competition could be observed between labeled and unlabeled analytes at nonspecific sites that are normally widely spread.

As shown in Figure 8, the addition of 2 mM unlabeled 2,4-D caused the radioligand binding to decrease from the original 75% to about 40%. Considering that the addition of excess 2,4-D may change the pH of the solvent, the same experiment was carried out in buffered solution at two different pH values (pH 3.0 and pH 6.0). Specific 2,4-D binding on nanofibers at pH 3.0 was still clearly indicated. Under these conditions, addition of unlabeled 2,4-D (2 mM) caused the bound labeled 2,4-D to decrease from the original 35% to less than 25%. Thus, if the preferred adsorption of radioactive 2,4-D has been due to the different accessibility or porosity of the nanofiber matrix, we would not observe the actual competition effect from the unlabeled 2,4-D. These competitive binding results further confirmed the presence of specific recognition sites in the imprinted nanofiber mats.

Conclusions

The above results demonstrate for the first time that electrospun template directed molecular imprinting is a viable method for creating robust, molecularly imprinted nanofibers that can selectively rebind the target molecule. Molecular recognition sites in polymer nanofibers were prepared with a simple electrospinning method using a combination of structural and functional polymers as the starting material. The 2,4-D imprinted nanofibers displayed favorable binding characteristics in aqueous solution. The imprinted nanofibers also had a well-defined morphology and physical stability. Overall, the electrospun nanofibers provided a porous matrix with very high surface area-to-volume ratio, which is desirable for the selective rebinding of the target molecule. Considering nanofiber processing, the present electrospinning−imprinting method is promising for its simple operation and high efficiency.

We expect that molecularly imprinted nanofibers will find interesting applications in affinity-related separation areas. It should be pointed out that the choice of the functional polymers used for imprinting electrospinning must be made according to the targeted compounds. It is also possible to use postspinning cross-linking to further stabilize the imprinted sites by different chemical means. Furthermore, the electrospun nanofiber itself may be modified with an in situ generated MIP layer, for example, via the grafting-from approach using a surface-immobilized initiator system.

Acknowledgment. The financial support of the Swedish Agency for Innovation Systems (VINNOVA) and the European Commission (MASMICRO Integrated Project) to IFP Research is gratefully acknowledged.

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MA052091W