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Short communication

Surface imprinted polyurethane film as a chiral discriminator

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1. Introduction

Many of the drugs available in the market are administered as racemates. Since the biological recognition elements interact differently with the enantiomers, often one of the enantiomers of racemic drugs exerts pharmacologically different action, which may be unwanted too. Regulatory agencies such as FDA, therefore, insist that any drug formulation should be enantiomerically pure. Extensive research efforts are being made to develop methodologies for the separation of enantiomers. Several techniques based on chiral stationary phases, chiral mobile phases, asymmetric synthesis, and derivativization with chiral reagents etc. has been attempted to separate enantiomeric species [1–3].

An alternative approach to isolate chiral components is based on the technique of molecular imprinting [4–8]. In this approach, affinity sites are created by the copolymerization of monomers and cross-linkers in the presence of template (print) molecules. After the polymerization, the template is removed leaving behind the site complementary to the print molecules in shape and size. In molecular imprinting the usage of a high amount of cross-linking agent is mandatory to preserve the shape and size of the recognition sites intact. Due to this, the imprinted polymers are thick and fragile and often difficult to fabricate in the form of mechanically strong thin films. This limitation often introduces diffusion barriers for the association of the analytes with the imprinting sites, resulting in slow response times. Recently, generation of imprinted sites in monolayer or thin film assembles was suggested to eliminate diffusion barriers [9–12]. These approaches have largely been directed to develop suitable materials for sensing applications.

Synthetic polymers are used extensively in the contemporary health care managements for applications as diverse as the fabrication complex devices to simple dressing containing drugs for preventing infection [13]. It may be interesting to form an imprinted layer selective to specific molecules on the surface of widely used polymers without affecting the bulk features. It would be advantageous if such a surface could bind selectively a pharmacologically active enantiomer from a racemate. Such a film containing the active component on the surface could be employed in medical applications.

It has been demonstrated that a thin layer of conjugated polymers can be coated virtually on any support using either chemical grafting or electro polymerization [14]. The conjugated polymeric layers based on conductive polyaniline or its derivatives were described as suitable candidates for the formation of molecularly imprinted polymers (MIPs). Recently Piletsky et al. and Bossi et al. have shown that affinity sites can be imparted on the surface coating on the polymeric substrates such as polystyrene microlitre plates by adding molecules of interest as templates during the polymerization [15,16].

The effort in this communication is to create chiral specific affinity sites on the surface of polyurethane film. The imprinting layer was formed by coating a thin layer of polyaminophenyl boronic acid. L-Tyrosine and D-tyrosine were used as model compounds.

2. Experimental

Polyurethane used in this study was Tecoflex 60 D obtained from Thermidic Inc., MA, USA. The polymer was dissolved in tetrahydrofuran and precipitated by adding water to remove any additives present. The precipitated polymer

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was collected, extracted with methanol and redissolved in tetrahydrofuran (THF). The polymer solution was transferred to glass Petri dish and evaporated slowly to get a film of thickness of 1 mm. Amino phenyl boronic acid (ABA), ammonium per sulfate (APS), L-tyrosine (LT) and D-tyrosine (DT) were from Sigma Chemicals Co., St. Louis, USA. Other chemicals were obtained from E. Merck, Mumbai, India.

2.1. Preparation of the imprinted layer

ABA (100 mg) was dissolved in 10 ml distilled deionized water. Ammonium per sulfate (APS) (120 mg) and 10 mg L-tyrosine were added to this solution. Pre-weighed strips of PU ($1 \times 3\,\text{cm}^2$) were placed in the solution. Excess of APS was added to covert nearly the whole of the ABA to polymer. Polymerization is known to form a macromolecular stretches containing up to 1000 units [16]. Care was taken to avoid the touching of the polymer strips on the sides of the beaker. The solution was kept at room temperature (28 °C) overnight. Polymer strips were also kept in solution without the drug to serve as control. The transparent films were turned into brown color after the reaction. Polymer films were washed with distilled water to remove the loosely adhered entities. The extraction was continued until there was no absorption of the extract at 254 nm indicating that loosely held entities, if any, originated during the polymerization of ABA as well as the print molecules are completely removed from the surface. The non-imprinted strips were also subjected to the same extraction cycles. After the coating process, the polymer strips were dried in a vacuum oven at 50° C and weighed to constant weight. The weight gain of the PU strips were taken as the amount of polymer coated onto the surface.

2.2. Instrumental

A Shimadzu model ESCA 3400 S Electron Spectrometer was used to obtain surface composition of the films. The samples were placed in vacuum for 24 h prior to the analysis. A Hitachi model S-2400 Scanning electron microscope was used to visualize the surface features of the film. A thin layer of gold was coated before the SEM analysis. A Varian model 100 Bio UV–vis spectrophotometer was used for the estimation of the drug. Calibration plots were constructed between the amount of the drug and absorption at 254 nm. This plot was used to quantify the extent of uptake and the release of the drug. A Nicolet Inc. (Madison, WI, USA) model Impact 410 FT-IR in conjunction with horizontal ATR accessory with Germanium crystal was used to record the ATR spectra of the polymer. The number of scans was 100. The thickness of the coated layer was determined using the following equation [17]:

$$\text{Dp} = \lambda/2\pi n_1(\sin^2\theta - n_{21}^2)^{1/2} \qquad (1)$$

where $n_{21} = n_2/n_1$ = refractive index of sample/refractive index of the optical element θ the angle of incidence (45°), λ is the wavelength. In this case, the wavelength corresponding to the boron–oxygen stretching mode at 1300 cm^{-1} was taken. The reported value of the refractive index of PU was taken.

2.3. Interaction of the polymer strips with the print molecules

Print molecule (400 μg) was dissolved in 50 ml of double-distilled water (pH 6.3). Polymer strips, $1 \times 3\,\text{cm}^2$, were placed in these solutions. The absorption of the solutions at regular time intervals was measured and from the differences in the values of absorption before and after placing the polymer strips the amount adsorbed was determined. In a similar fashion, the extent of uptake of DT was also estimated.

3. Results and discussion

In molecular imprinting, the formation of a complex between the monomer and template is mandatory for the creation of affinity sites in the resultant polymer. The structure of the template molecule (LT) is shown in Fig. 1. The functional groups can interact with ABA through hydrogen bonding and hydrophobic stabilization between aromatic rings is also possible. The creation of affinity sites for the amino acid is assumed to be due to the interaction of the drug molecules and the monomer prior to polymerization.

Polyaminophenyl boronic acid (PAPBA) can be coated onto several hydrophobic polymers and are known to stabilize through hydrophobic interaction [15]. The formation of dark brown color characteristic of the formation of conjugated structure by the oxidation of aniline and its derivatives indicates the polymerization of ABA. FT-IR spectrum showed peaks around 1595 and 1500 cm^{-1} indicating further the presence of quinone and benzene ring deformations reflecting the formation of a conjugated structure by the polymerization of the ABA. The presence of Boron at 191.46 eV confirms the formation of a thin layer of PAPBA on the surface.

ATR-FT-IR technique has been used widely to measure the thickness of polymeric layers coated onto the surfaces [18,19]. The thickness of the coated layer was estimated from the ATR spectra using Eq. (1) was found to be 146 ± 6 nm. Earlier studies on hydrophobic substrates have shown that the thickness of the layer is around 100 nm [16].

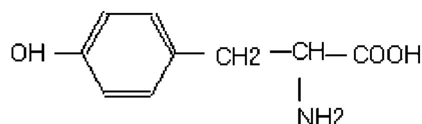

Fig. 1. Structure of tyrosine.

Fig. 2. Scanning electron microphotograph of the imprinted surface.

Fig. 2 shows the scanning electron micrograph of the imprinted surface. The coating appears to be uniform since the formations of any aggregates are not seen.

The time bound uptake of the print molecule (LT) by the imprinted and non-imprinted polymers are depicted in Fig. 3. The equilibrium is attained relatively faster (∼20 min) due to the fact that the affinity sites are on the surface. Table 1 summarizes the equilibrium uptake of the print molecule by the imprinted and non-imprinted surfaces. The adsorption of LT by imprinted surface is remarkably high indicating the formation affinity sites on the surface. The low uptake of LT by the non-imprinted polymer further points out that the major factor for the enhanced adsorption for the amino acid by imprinted surface is indeed due to the formation of affinity sites of the drug by the process of imprinting. The extent of uptake of DT by the polymers (average adsorption per unit area) is also summarized in Table 1. The equilibrium adsorption of DT by the MIP is considerably less comparing to the uptake of LT reflecting that the MIP has chiral selectivity.

The selectivity factor is calculated from:

$$\alpha = A_{\text{mip}} - A_{\text{nonmip}}/B_{\text{mip}} - B_{\text{nonmip}} \quad (2)$$

where A_{mip} extent of adsorption of LT by the MIP, A_{nonmip} the amount adsorbed by non-imprinted polymer, B_{mip} the amount

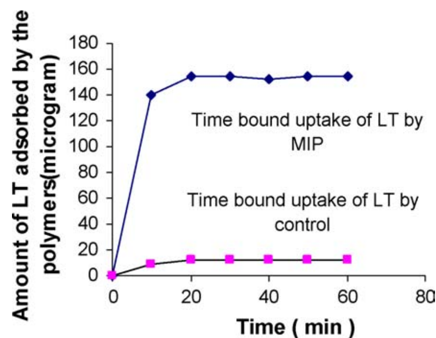

Fig. 3. Time bound adsorption of the print molecule by the polymers (total quantity adsorbed by the film of size 1 cm × 3 cm).

Table 1
Equilibrium adsorption of the amino acids by the polymers

Material	Amount adsorbed (μg/cm^2)	
	L-Tyrosine	D-Tyrosine
Imprinted surface	25.56 ± 0.32	3.61 ± 0.09
Non-imprinted surface	2.05 ± 0.18	2.64 ± 0.42

of DT adsorbed by the MIP and B_{nonmip} is the quantity of DT adsorbed by the non-imprinted polymer. The selectivity factor calculated using the data summarized in Table 1 is 24.24 which apparently indicate that enrichment of the print molecule on the surface is indeed possible. Though the polymer does not contain any asymmetric discriminating centers, the remarkable enantioselectivity is presumed be due to the creation of LT specific sites on the surface of the polymer through molecular imprinting.

The results summarized in this communication shows that selective concentration of isomer on an imprinted surface is possible. The enriched species could be recovered for further studies. The methodology is simple and modified surface could be used in applications as diverse as separation, sensing, medical uses, etc.

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