Imparting Affinity Sites for Adenosine Triphosphate on the Surface of Polyurethane Through Molecular Imprinting

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ABSTRACT: A simple methodology for imparting affinity sites for adenosine triphosphate (ATP) on the surface of polyurethane (PU) film is discussed. It is widely known that, through oxidation, a thin layer conjugated polymer can be coated onto many polymeric substrates. This possibility is explored in this report. 3-Amino phenyl boronic acid was polymerized in the presence of ATP as template using ammonium per sulfate. A thin layer of polyamino phenyl boronic acid was coated onto the surface of PU film immersed in the solution during the reaction. The coated layer showed remarkably high affinity toward ATP and equilibrium adsorption was attained rapidly in contrast to free standing molecularly imprinted polymers in which equilibrium adsorption is normally attained after several hours. The imprinted layer also showed a high degree of selectivity toward ATP compared to adenosine diphosphate (ADP). The approach is simple and affinity sites for any water soluble molecules can virtually be created on the surface of polymers of interest. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 94: 2088–2090, 2004

Key words: molecular imprinting; ATP; polyurethane; 3-aminophenyl boronic acid

INTRODUCTION

Molecularly imprinted polymers have aroused considerable interest, particularly in recent years. The growing interest in these classes of materials is associated with their possible potential applications as artificial antibodies, sorbents in solid phase extraction, sensor elements, and stationary phases in chromatography, specifically in chiral separations.[1–5]

Among all of these applications, much emphasis is given to the optimization of molecularly imprinted polymers (MIPs) as recognition elements in sensor devises. MIPs as sensor elements have tremendous advantages over biologically derived elements such as antibodies and enzymes.[6,7]

Despite the extensive efforts, obstacles are still there, particularly in the preparation of the imprinted polymers in the form of thin films. Extensive crosslinking, which is mandatory in the technique of molecular imprinting, makes the polymer opaque and mechanically inferior. In recent years several authors attempted to coat imprinted layers on various substrates such as electrodes, glass slides, etc.[8–10] It would be useful if an imprinted layer could be coated on the surface of mechanically strong polymers having medical applications without affecting other features. The imprinted layers could hold molecules, such as drugs, peptides, etc., to impart specific properties such as improved blood compatibility.

Bossi et al.[11] have shown that amino phenyl boronic acid can easily be oxidized using ammonium per sulfate to form polyaminophenyl boronic acid and coated onto hydrophobic surfaces such as polystyrene. They have shown that affinity sites for proteins on the coated surface can be created through a noncovalent imprinting strategy by simply adding proteins as templates during the oxidative reaction. It appears advantageous to use such a simple approach to create affinity sites for smaller water-soluble molecules on the surface of polymeric substrates. An approach of this kind is proposed as a simple methodology for the preparation of imprinted layers with molecular recognition properties on the surface of widely available polymers such as polyurethane (PU). This communication explores this possibility and discusses the creation of affinity sites for ATP, a widely known clinically relevant molecule on the surface of PU.

EXPERIMENTAL

Methods

Polyurethane used in this study is Tecoflex 60 D obtained from Thermidic Inc, Woburn, MA, USA. The polymer was dissolved in tetrahydrofuran (THF) and precipitated by adding water. The precipitated poly-

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TABLE I
Summary of ESCA Analysis

Peak	Position (eV)
C1s	288.80
O1s	533.10
N1s	400.28
B1s	191.46

mer was collected, extracted with methanol, and redissolved in THF. The polymer solution was transferred to a glass Petri dish and evaporated slowly to get a film of thickness of 1 mm. 3-Aminophenyl boronic acid, ammonium per sulfate, adenosine triphosphate (ATP), and adenosine diphosphate (ADP) were from Sigma Chemicals Co, St. Louis, MO, USA.

Preparation of the imprinted layer

3-Amino phenyl boronic acid (100 mg) was dissolved in 10 mL distilled demonized water. APS (120 mg) and ATP (15 mg) were added to this solution. Strips of PU (2×2 cm^2) were placed in the solution. Care was taken to avoid touching the sides of the beaker with the polymer strips. The solution was kept at room temperature (28°C) overnight. Polymer strips were also kept in solution without ATP to serve as control. The transparent film turned a dark brown color after the surface modification. These films were washed with distilled water to remove loosely adhered entities and then kept in 0.1*M* hydrochloric acid to remove any complexed ATP with the boronic acid moieties. The extraction was continued until there was no absorption at 254 nm. The control strips were also subjected to the same extraction cycles.

Instrumental

A Shimadzu model ESCA 3400 S Electron Spectrometer was used to obtain surface composition of the films. The samples were placed in a vacuum for 24 h prior to the analysis. A Hitachi model S-2400 scanning electron microscope (SEM) 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-Visible spectrophotometer was used for the estimation of adsorbed ATP and ADP. Calibration plots were constructed between the amount of the components and the absorption at 254 nm. These plots were used to quantify the extent of uptake of the two molecules by the modified polymers. The extent of adsorption of the molecules by the polymers was determined from the differences in absorptions at 254 nm of the solutions before and after placing the polymer films.

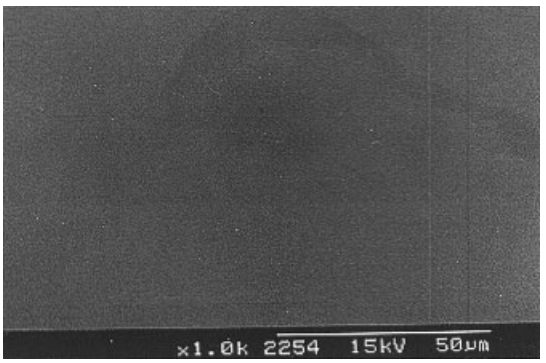

Figure 1 Scanning electron photomicrograph of modified surface.

Interaction of the polymer strips with the print molecules

Polymer strips having a surface area of 4 cm^2 were placed in solutions containing known quantities of ATP and ADP, respectively. The absorption of the solutions at regular time intervals was measured and from the differences in the values of the absorptions before and after placing the polymer strips the amount adsorbed was determined.

RESULTS AND DISCUSSION

The imprinted PU strips appeared as a brown color. The results of the ESCA analysis are summarized in Table I. In addition to the C, N, and O bands, ESCA showed a band at 191.46 eV characteristic of BIS, reflecting that the surface layer is indeed due to polyaminophenyl boronic acid. Koskinen[12] has shown that normally the thickness of the layer is 100 nm. It is also reasoned that the strong hydrophobic interactions between the phenyl ring of PBA and the hydrophobic polymer surface stabilizes the adsorbed layer.

Figure 1 shows the SEM microphotograph of the modified PU. The modified surface looks smooth and a distinctive phase separation or aggregation could not be seen, indicating that the modified layer is uniform.

Figure 2 depicts the time bound uptake of the print molecule by the modified PU. It can be seen that the

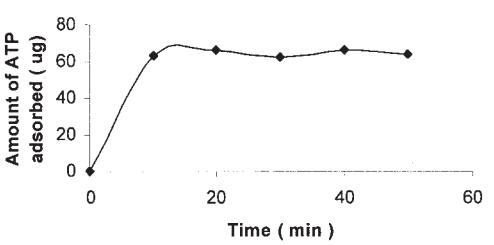

Figure 2 Time bound uptake of the ATP by the imprinted surface.

TABLE II
Extent of Uptake of ATP and ADP by the Imprinted and Nonimprinted Polymer Strips

Material	Amount of ATP adsorbed ($\mu g/cm^2$)	Amount of ADP adsorbed ($\mu g/cm^2$)
Imprinted for ATP	16.46 ± 0.32	4.22 ± 0.06
Nonimprinted	4.03 ± 0.02	3.50 ± 0.05

equilibrium establishes rapidly. The extent of interaction of the polymer with ADP is used to assess the selectivity in the interaction of the imprint layer with the print molecule. Table II summarizes the extent of adsorption of the ATP and ADP by the imprinted and nonimprinted polymers. The extent of uptake of ATP by the nonimprinted polymer is much less and it is comparable to the uptake of ADP, indicating that the adsorption is due to nonspecific interactions. An interesting aspect of the study is the considerably low uptake of ADP by the imprinted surface though ADP differed from ATP by one phosphate group. There is nearly a fourfold increase in the uptake of ATP by the imprinted polymer compared to the nonimprinted polymer, indicating the creation of affinity sites on the surface resulted from imprinting. The enhanced adsorption of ATP by the imprinted surface compared to the uptake of ADP further reflects the ability of the surface to discriminate the print molecules from other structurally similar entities.

One of the serious drawbacks of the conventional imprinted polymers is the slowness in attaining the equilibrium, which in fact limits their utility particularly in sensing. In the present case, the equilibrium is attained rapidly (~ 20 min) and, due to this, these materials could be considered in sensing applications.

The adsorbed entities can be removed by rinsing in dilute hydrochloric acid and the films can be reused, which are perhaps added advantages of the present approach. The preliminary study indicates that the imprinted layer for small molecules can be formed on the surface of widely used polymers such as PU, which could be used in affinity separation and other biomedical applications. The lack of extensive crosslinking is the prominent feature of the method.

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