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Synthesis and evaluation of multiply templated molecularly imprinted polyaniline

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Abstract This manuscript demonstrates the possibility of creating affinity sites in polyaniline (PAN) through molecular imprinting for clinically relevant molecules namely, Adenosine triphosphate (ATP), Adenosine diphosphate (ADP) and Adenosine mono phosphate (AMP). The binding of these molecules by the substrate was demonstrated indicating the feasibility of enriching multiple components at a time enabling the subsequent estimation. ATP, ADP and AMP were used as template molecule and aniline as the monomer. The equilibrium uptake was found to vary as ATP > ADP > AMP.

Introduction

The effort to synthesis artificial receptors capable of binding a target analyate with comparable affinity and selectivity to antibodies has been followed through all these years. One approach that is widely practiced for the creation of such systems is molecular imprinting [1–8].

Molecular imprinting is a process where functional monomers and cross linkers are copolymerized in the presence of a template molecule. It is assumed that functional monomers form a complex with the template molecule prior to polymerization. After the polymerization process, the functional groups are held in position by the highly cross linked polymeric structure. The removal of the template molecules from the cross linked matrix forms binding sites that is complementary in size and shape to the template. The resultant polymers subsequently recognize and bind the template molecules. Molecularly imprinted polymers (MIPs) are synthesized either by covalent approach or non-covalent approach. One of the important features of molecular imprinting method is the possibility of synthesizing MIPs for a wide array of molecules ranging metal ions, smaller organic and complex biomolecules like proteins.

The process of imprinting particularly by the non-covalent approach is carried out in organic phases to improve the interaction between the monomers and template molecules. In aqueous media these types of interactions are affected. Many biomolecules are insoluble in organic phases which, in fact, curtail the possibility of preparing the imprinted polymers for such species.

The synthesis and characterization of conductive polymers have become one of the most important topics during fast decades [9]. Among these, polymers based on aniline are of particular interest due their many desirable features such as stability, electrical conductivity etc [10, 11]. PAN is known to have the ability to interact with many components through hydrogen bonding and π–π stacking [12]. It seems that PAN is an interesting matrix to create affinity sites for water soluble molecules. Recently Bossi et al. have used amino phenyl boronic acid for the preparation of imprinted layer on the surface of polystyrene to recognize proteins [13]. Additionally conjugated polymers were described as promising materials suited for MIP formation. Such conjugated polymer is easily synthesized by chemical oxidation of aniline and its derivatives. These polymers are known to adhere tightly particularly on the surfaces of hydrophobic polymers. Imprinted layer coated on the polymeric substrates such as polystyrene and polyurethane

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has shown remarkable recognition ability towards template molecules in aqueous media [13, 14].

Though the MIPs have been used in varied applications, their utility as adsorbent for more than one component at a time has not been explored widely. The first effort towards this direction was reported from the author's laboratory [15, 16]. Very recently Nilsson et al. have reported the usage of MIPs for such application [17]. The syntheses of the MIPs are carried out in organic phases using vinyl monomers. Detection of multicomponents simultaneously appears to have several advantages in clinical diagnosis and environmental monitoring. This communication discusses the creation of affinity sites for ATP, ADP and AMP in PAN through molecular imprinting and the reaction was carried out in aqueous medium.

Experimental

Chemicals

Analytical grade Ammonium per sulfate (APS), ATP, ADP and AMP were obtained from Sigma Chemicals St. Louis, USA. Other chemicals, analytical grade or chromatographic grade, were from E Merck, Mumbai, India.

Preparation of polymers

The methodologies for the synthesis of PAN are widely known [18]. Chemical method using the reaction between aniline and ammonium per sulfate was used in this study. Briefly 2 g of aniline was placed in 5 ml distilled deionized water and added 1 M hydrochloric acid drop wise to dissolve the aniline. About 100 mg of each of the template molecules, namely ATP, ADP and AMP were added to the solution and stirred to dissolve. About 2.28 g of ammonium per sulfate dissolved in 5 mL distilled deionized water was added drop wise under stirring. The solution was kept at 25 °C overnight. The dark colored polymer powder was isolated by filtration and washed with plenty of distilled water until the wash doesn't have any absorption in the range of 300–220 nm. Non imprinted polymer (NIP) was prepared in a similar fashion without the addition of templates.

Instrumental

The infra red spectra of the materials were recorded using Nicollet Inc (Madison, USA) model Impact 410 FT-Infrared spectrophotometer at a resolution of 4 cm^{-1}. The total number of scans was 100.

The estimation of the phosphate was measured using the well known phosphovanado molybdate method [19].

A Waters Inc HPLC system (Milford, USA) consisting of 510 pump, Reodyne 7725i injector and model 486 tunable absorbance detector was used for the simultaneous estimation of ATP, ADP and AMP. The separation was effected on a μ-bondapak C18 column (Waters Inc) in conjunction with a mobile phase of water:methanol:acetic acid (78:20:2 V/V/V) at a flow rate of 1 mL/min. The column effluents were monitored at 254 nm. Standard solutions of the templates molecules were prepared by dissolving 10 mg each in 10 ml water. Calibration plots were constructed between peak height and concentration in the injected volume of the solutions. These plots were used for estimating the amount of the components adsorbed onto the polymers. The extent of uptake of these molecules by the polymers was estimated by measuring the absorbance of the solution before and after placing 25 mg polymers. The uptake of each of the molecules was estimated using individual solutions as well as the mixture. The absorbance was monitored at different time intervals to know the attainment of equilibrium and it is found that equilibrium is established within 1 h.

Results and discussion

Figure 1 shows the spectrum of PAN. Major vibrational bands can be seen around, 1599, 1506, 1307, and 835 cm^{-1}, respectively. In addition to these bands, peaks associated with –NH stretching can also be seen around 3300 cm^{-1}. These bands, characteristic of emarldine form of PAN, are in good agreement with the published results [20, 21].

Under the present chromatographic conditions, ATP, ADP and AMP eluted at a retention time of 1.7 ± 0.09 min, 2.4 ± 15 min and 2.9 ± 0.2 min, respectively. All the measurements were performed in triplicate.

Table 1 provides the equilibrium uptake of ATP, ADP and AMP when the template molecules are equilibrated

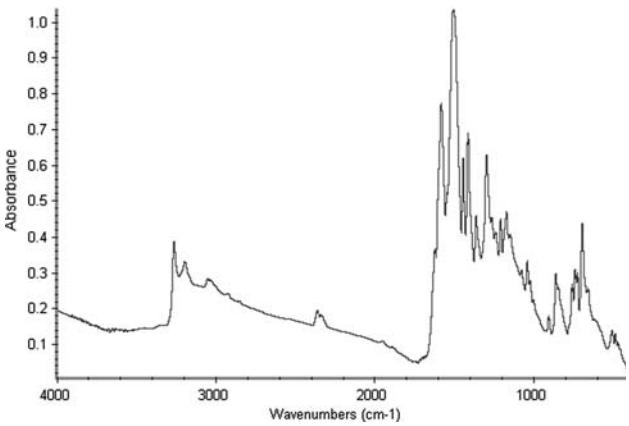

Fig. 1 Infra red spectrum of PAN

Table 1 Interaction of ATP, ADP, AMP and phosphate with MIP and non imprinted polymer (NIP): polymers treated with individual solutions

Compound	Equilibrium adsorption by MIP (μg/100 mg)	Equilibrium adsorption by NIP(μg/100 mg)
ATP	2682 ± 22	140 ± 11
ADP	1545 ± 19	159 ± 9
AMP	1132 ± 23	149 ± 16
Phosphate (monosodium hydrogen phosphate)	3228 ± 24	378 ± 14

Table 2 Interaction of ATP, ADP and AMP with MIP and NIP: polymers treated with the mixture of the components

Compound	Equilibrium adsorption by MIP (μg/100 mg)	Equilibrium adsorption by NIP (μg/100 mg)
ATP	1665 ± 18	120 ± 9
ADP	785 ± 12	109 ± 6
AMP	572 ± 9	131 ± 11

with the MIP individually. Table 2 shows the equilibrium uptake of the templates when the polymer is treated with templates mixture. It is interesting to see that the in both cases, uptake vary as ATP > ADP > AMP. Since these three molecules are used as templates, creation of more or less same number of affinity sites could be expected. Interestingly, ATP is adsorbed more reflecting the possibility of involvement of phosphate (PO4) groups in the process of imprinting. Additionally, the MIP adsorbs reasonably good amount of PO4 when it is treated with a solution of monosodium hydrogen phosphate. The uptake of the components by the non imprinted (NIP) polymer is much less indicating the creation of affinity sites for the phosphate groups in the polymer as a result of imprinting.

Effective interaction between polymer and the template molecules is mandatory for the binding of the analyte of interest in specific sites created by imprinting. Zheng et al. have shown extensive hydrogen bonding (H-bonding) in PAN between amine–imine groups which is said to be stronger than H-bonding between imine–imine groups [22]. These authors observed a shift in the H-bonding from amine-imine to –C=O... H.N– in the presence of traces of *N*-methyl-2-pyrrolidnone indicating that PAN has a stronger ability to form H-bonding with moieties containing oxygen (e.g. carbonyl groups). It is reasoned that PO4 groups can interact with PAN through H-bonding as well as through ionic interaction enabling the creation of affinity sites for this entity. Our earlier studies have shown that the modification of PVA with phosphate groups increased the water uptake significantly indicating the feasibility of forming H-bonding with water [23].

A typical infra red spectrum of imprinted PAN equilibrated with the monosodium hydrogen phosphate solution after subtracting the spectrum of polymer is shown in Fig. 2. The spectrum (broken line) compares well with the spectrum of the sodium hydrogen phosphate (Fig. 2, solid line).The characteristic peaks associated with Phosphate groups can be seen around 1170, 1044 and 965 cm^{-1} respectively.

One notable feature in the spectrum is the down ward shift in the peak position (peaks labeled with circle). The shift could be assigned to the interaction of the functional group with the polymer matrix. The presence of OH or NH groups in the vicinity of P=O groups is known to lower the P=O stretching frequency by 50–80 cm^{-1} [24]. These results suggest that affinity sites for phosphate groups are created in PAN as a result of imprinting. It is reasonable to presume that the interaction of imprinted PAN with the three molecules namely ATP, ADP and AMP are through phosphates groups present in these molecules.

The imprinted polymer adsorbs relatively higher quantity of ATP in spite of the fact that other molecules used also contain phosphate groups. It is reasoned that phosphate groups of ATP bind onto the polymer. Since it contain three phosphate groups, presumably it is held strongly simultaneously binding to three sites in the polymer. ADP contains two groups which can bind to two sites in the polymer. Due to steric hindrance, subsequent binding would be in other sites. As a result of this, many sites in the polymer are left unfilled. Interestingly the extent of uptake of AMP is still less comparing to the other two molecules. AMP contains only one phosphate group and its adsorption to the polymer could be through the interaction between the

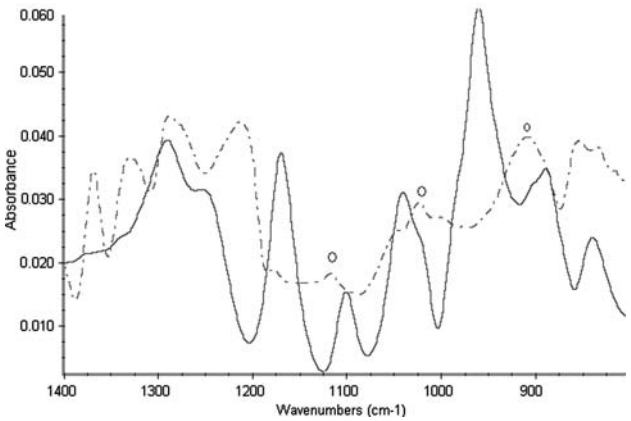

Fig. 2 Infra red spectrum of molecularly imprinted PAN equilibrated with sodium hydrogen phosphate after subtracting the spectrum of PAN (broken line). Infra red spectrum of sodium hydrogen phosphate (solid line)

phosphate specific sites in the polymer. As stated earlier, due to steric hindrance, further binding of the molecules would be at a distant point. As a result of this, many sites in the polymer are left vacant resulting in the reduced adsorption.

The higher uptake of phosphate when the polymer is treated with mono sodium hydrogen phosphate, in fact, indicates this possibility. The same aspect could be responsible for the variation in the uptake of the print molecules by the polymer when it is treated with template molecules individually and as a mixture.

Conclusion

The study shows that affinity sites for more than one component can be created in a polymer matrix through molecular imprinting in aqueous media. Such an approach may be useful for the creation of imprinted polymers as specific recognition elements for water soluble entities.

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