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Covalent molecular imprinting made easy: a case study of mannose imprinted polymer

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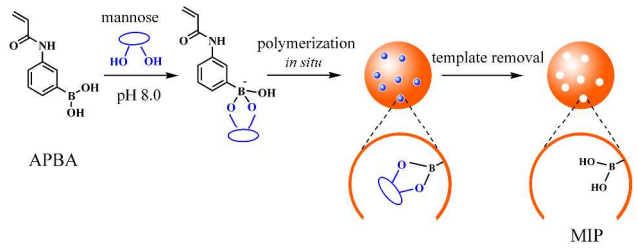
Covalently mannose imprinted polymers were prepared by simple one-pot method in aqueous medium for the first time. This new imprinted polymer showed high imprinting efficiency and fast kinetic binding for template in water phase.

Molecular imprinting is a versatile and straightforward method to produce materials with molecular recognition properties due to its low cost, simple preparation, and high stability in comparison with the complicated process of biological antigen-antibody systems.[1,2] Generally, molecularly imprinted polymers (MIPs) were formed either by covalent[3] or non-covalent approach[4] according to the interactions between target templates and functional monomers. Between the two methods, non-covalent imprinting is by far the most popular and general strategy used for MIP synthesis due to its experimental simplicity. Typically, only mixing of templates and monomers in a suitable solvent for several minutes is required and a huge variety of monomers were available and able to interact with almost any kind of templates. Yet non-selective binding sites may arise from this method owing to the excess of free functional monomers and their random incorporation into the polymeric matrix.[5] While covalent imprinted polymers can form more homogenous population of binding sites minimizing the number of nonspecific sites and has superb water-compatibility due to the high stability and distinct definition of template-monomer interactions provided by the covalent bonds.[6] The main disadvantage of this method is that the synthesis process is much more complicated since the covalent template-monomer prepolymers need to be synthesized firstly, which is complex and time-consuming.[7]

In this study, we demonstrated a facile one-pot approach for preparation of covalent MIPs aiming to promote convenience and efficiency of covalent molecular imprinting. Mannose, a common monosaccharide, was employed as a model template. So far, a large variety of monosaccharide imprinted polymers were prepared via covalent approach by azeotropic distillation reaction with boronic acid derivates and carbohydrates. [8-12] Unfortunately, as discussed above, these sugar-phenylboronic acid esters need to be synthesized firstly in organic solvent with pyridine or N,N-dimethylformamide at high temperature for several hours. Moreover, purifying of the product is particularly difficult. Some researchers reported monosaccharide imprinted polymers prepared in water phase without a special step for the preparation of covalent template-monomer complex. [13-16] However, research of this field is still insufficient. So far, covalently mannose imprinted polymers prepared by simple methods are not yet reported. Herein, an efficient one-pot synthesis of covalently mannose imprinted polymers was developed in this work as illustrated in Scheme 1. Sugar-phenylboronic acid ester was formed in alkaline aqueous solution at room temperature by adding excess mannose. Then precipitation polymerization was conducted *in situ* in the presence of cross-linker and initiator. After that, the template was cleaved and the imprinting cavities were capable of rebinding the target molecule by re-establishment of the covalent bond. No organic solvents and surfactants are involved during the preparation, and the whole procedure is simple, mild, and efficient.



Scheme 1 Schematic illustration of the one-pot synthesis of mannose imprinted polymers.

Boronic acids are known to bind with compounds containing diol moieties with strong affinity through reversible ester formation and the stability of the boronate ester is highly pH dependent.[17] In this work, 3-acrylamidophenylboronic acid (APBA) was used as the functional monomer and the synthesis of APBA was described in Electronic Supplementary Information. To investigate the reactivity of APBA with mannose, ^{11}B NMR spectra of the APBA/mannose mixture in the aqueous medium at various pH values was studied. As shown in Fig. 1a, at pH 5.5, only the peak from free APBA was observed at 28.9 ppm, indicating that no detectable mannose/APBA ester was formed under such pH conditions. With the pH increasing to 7.0, the other peak located at 8.0 ppm emerged which can be assigned to the boronate ester. When the pH is up to 8.0, only the peak from boronate ester was remained and the peak from free APBA disappeared, suggesting that all APBA has reacted with

mannose completely under this pH values. However, when the pH value was adjusted from 8.0 to 5.0, only the peak from free APBA was reappeared at 28.9 ppm. This result clearly demonstrated that the reactivity of mannose with APBA is reversible depending on the pH value, which is highly desirable for covalent imprinting system.

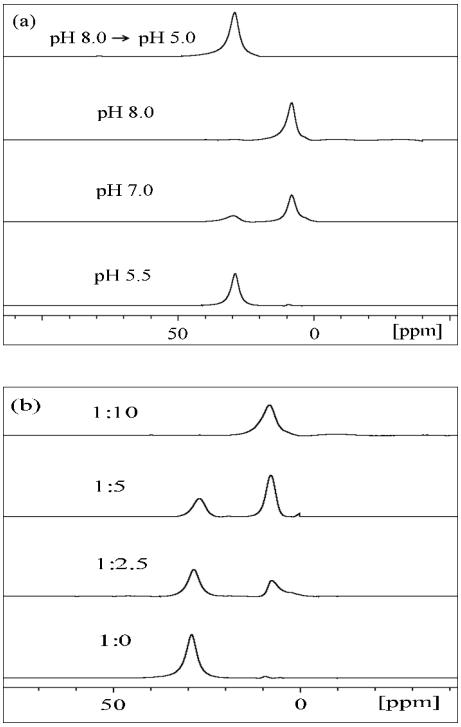


Fig. 1 ^{11}B NMR spectra of APBA/mannose mixture in the aqueous medium (a) at different pH values with the molar ratio of APBA/mannose at 1:10 and (b) with different APBA/mannose molar ratios at pH 8.0.

In addition, the effect of APBA/mannose ratios was evaluated and the result is shown in Fig. 1b. In general, with the amount of mannose increasing, the peak in the ^{11}B NMR spectra located at 28.9 was shifted gradually to 8.0. When 10-fold mannose to APBA was added, only the peak from mannose/APBA ester was observed. These results indicated that APBA could react thoroughly with mannose at pH 8.0 by adding excess mannose. After polymerization in situ and removal of template by acidic solution, all functional monomer APBA were immobilized in the imprinting cavities leaving large amount of homogeneous specific binding sites. Since the residual mannose didn't interact with the functional monomer, these parts of cavities after removal of mannose would not generate binding sites for the template. FT-IR spectra of MIPs containing bound template, NIPs and MIPs lacking template are presented in Fig. S2, and these results demonstrated that the template mannose was washed out effectively leaving empty imprinting sites for further rebinding.

The recognition ability of the MIPs to template (mannose) was investigated. MIPs or NIPs was added to a solution of mannose in the alkaline aqueous solution. After incubating for 30 min at room temperature, the polymers were isolated by filtration. The concentrations of mannose in the supernatants were monitored using a UV-vis spectrophotometer by 3,5-dinitrosalicylic acid (DNS) assay.[18] The amount of mannose bound to the polymers was calculated by subtraction of the free fraction from the total amount added. Rebinding conditions were optimized and the experimental results were listed in supplementary materials (Fig. S3).

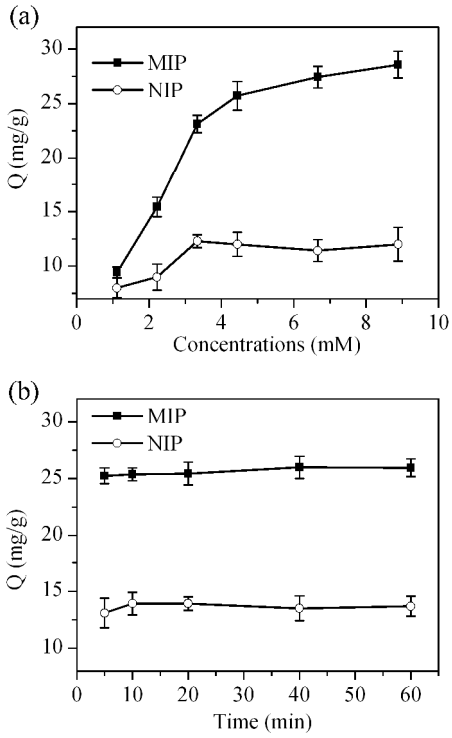


Fig. 2 Static (a) and dynamic (b) adsorption curves of the MIPs and NIPs for mannose.

Fig. 2a shows the amount of mannose bound to the MIPs and NIPs at various initial concentrations. The adsorption capacity of the MIPs was much higher than that of the NIPs for each of the different individual concentration values of mannose. The maximum adsorption capacities of the MIPs and NIPs were 28.1 and 12.5 mg g^{-1}, respectively. The superior rebinding ability of the MIPs was generated by the imprinting cavities, which had a memory of the shape, size and chemical functionality towards the template molecule. Furthermore, the kinetic uptake of mannose by the MIPs and NIPs was investigated. In the rebinding test, the concentrations of sugar in the supernatants at certain time intervals (5 to 60 min) were monitored using a UV-vis spectrophotometer by DNS assay. As shown in Fig. 2b, the adsorption equilibrium was achieved within 5 min after the addition of mannose, which was much faster than that of typical MIPs.[19-21] This result was attributed to the fast esterification between mannose and APBA.

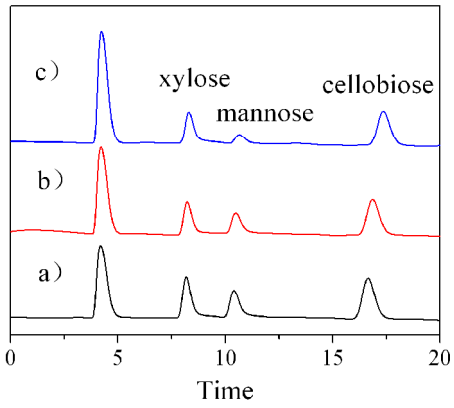


Fig. 3 HPLC chromatograms of mixtures of mannose, xylose, and cellobiose before (a) and after enrichment by NIPs (b) and MIPs (c).

Selectivity is an important factor to evaluate the imprinting efficiency of MIPs. In this study, xylose and cellobiose were employed as the competitive analogues of mannose. The rebinding test was carried out in the same manner as described above except that the concentrations of the three sugars were measured by HPLC-RID. Fig. 3 shows the mixtures' chromatograms before (a) and after enrichment by NIPs (b) and MIPs (c). It is evident that the peak area of the template mannose (retention time at 10.14 min) after enrichment by MIPs is much lower than the treatment of NIPs, while the peaks of xylose (retention time at 8.41 min) and cellobiose (retention time at 16.25 min) decreased slightly after both MIPs and NIPs enrichment. The amount of mannose bound to the polymers was calculated by subtraction of the free fraction from the initial concentrations according to the peak area of each sugar in HPLC chromatograms. The amount of template mannose adsorbed by the MIPs (32.0 mg g^{-1}) was found to be significantly higher than that of xylose (13.6 mg g^{-1}) and cellobiose (5.9 mg g^{-1}), while NIPs showed low capacities towards mannose (12.4 mg g^{-1}), xylose (14.1 mg g^{-1}), and cellobiose (6.5 mg g^{-1}). Compared with the maximum adsorption amount (28.1 mg g^{-1} in Fig. 2) without adding the competitive analogues, the adsorption amount was not reduced in the competing experiment. These results clearly demonstrated that the MIPs synthesized by one-pot method had high selectivity towards template molecule.

In summary, a totally new strategy was developed for synthesis of covalently mannose imprinted polymers by one-pot method in aqueous medium, which was as simple as non-covalent imprinting approach. ^{11}B NMR spectra validated the feasibility of this method. The resulting MIPs exhibited high imprinting efficiency and fast kinetic binding for template in water phase. Since mannose-phenylboronic acid is a typical case for preparing covalent imprinted polymers, we believe that this efficient method could also be extended to manufacture more covalent-bonding based MIPs.

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Notes and references

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† Electronic Supplementary Information (ESI) available: detailed experimental procedures and analysis, NMR data, FT-IR data. See DOI:10.1039/b000000x/

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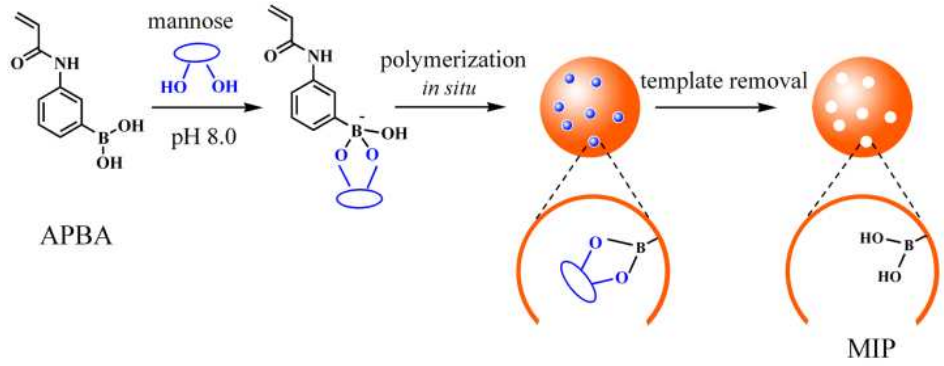

Covalently mannose imprinted polymers were synthesized by one-pot method in aqueous medium.