Atrazine-imprinted Microspheres Prepared Using a Microfluidic Device

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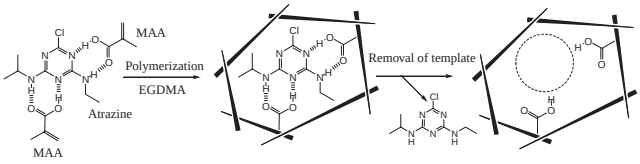
Atrazine-imprinted microspheres were prepared using a microfluidic device. With a dispersed phase containing atrazine, methacrylic acid, ethylene glycol dimethacrylate, and 2,2-azobis(2,4-dimethylvaleronitrile) and a continuous phase containing poly(vinyl alcohol), droplets could be generated by shearing force of continuous phase in a Y-junction microfluidic device. Monodisperse droplets were polymerized by UV irradiation to yield the imprinted polymer that showed selective binding for atrazine.

Molecular imprinting has been known as a useful technique for the preparation of molecular recognition materials. Molecular imprinted polymers (MIPs) are prepared by co-polymerization of complexes formed from a template and polymerizable monomer(s) interacting with the template through covalent and/or non-covalent bonds with a cross-linking agent. After the template is removed from the resulting polymer matrices, the binding sites having size and shape complementary to the template were generated.[1] Commonly, MIPs have been prepared by bulk polymerization. Subsequently, the polymers have been ground and sieved for classification of the polymer size. However, these processes are tedious, and often result in large losses of the material. In addition, the shapes of the resulting polymers are irregular. To address some of these issues, preparation of uniformly sized MIPs has received great interest.[2]

Atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) is widespread as a herbicide used to control grassy weeds. It has been suspected of an endocrine disrupter, thus the development of adsorbents for atrazine would be desirable in the field of environmental science. Bulk MIPs that can adsorb atrazine specifically have been previously reported.[3,4] However, the preparation of the MIPs-included time-consuming grinding/sieving steps that lead to irregular particles.

Recently, there have been intensive studies of microfluidic techniques for producing highly uniform droplets.[5] In particular, polymer microspheres obtained from droplets by shearing force of continuous phase using a microchannel has been developed owing to wide applications to ion exchange resin, LCD spacers, stationary phases in liquid chromatography, etc. Herein, we propose a new preparation method of atrazine-imprinted microspheres by using such microfluidic device. We employed a Y-junction microchannel for the formation of droplets, resulting in MIPs (Figure 1a). There are two advantages for the preparation of MIPs by using microfluidic devices. Firstly, the obtained polymers are spherical and monodisperse, and particle diameter can be controlled by flow speeds, width and depth of paths, and mixed ratio of organic phase and aqueous phase.[6] Secondly, as required amount of materials is a little, it is possible to reduce both cost and influences on the environment.

The preparation of the atrazine-imprinted polymer is illustrated in Scheme 1. As an organic phase, atrazine (0.73 mmol), methacrylic acid (MAA; 2.9 mmol), ethylene glycol dimethacrylate (EGDMA; 19 mmol), and 2,2-azobis(2,4-dimethylvaleronitrile) (ADVN; 80 mg) were dissolved in mesitylene (11 mL). In the previous report of atrazine-imprinted polymers, mesitylene was described as a good solvent to prepare the imprinted polymers.[7] As an aqueous phase, poly(vinyl alcohol) aqueous solution (1.0 wt %) was prepared. To prevent the microchannel plugging, both mixtures were filtered with syringe filters (0.2 μm). Flow speeds of the organic phase and the aqueous phase in the channel was kept 8 and 50 μL min^{-1} by syringe pumps, respectively. Oil in water (O/W) droplets from the outlet were collected (Figure 1b), and were polymerized by UV irradiation with stirring for 12 h. In order to remove atrazine templates, the imprinted microspheres were washed with methanol for 24 h by Soxhlet extraction. The amount of atrazine recovered was approximately 67% of total contained in the polymers.[8] The diameter for the resultant polymer is about 50 μm (Figure 1c). The specific surface area and pore size of the poly-



Scheme 1. Schematic representation of atrazine-imprinted polymers using MAA as a functional monomer and EGDMA as a cross-linking agent.

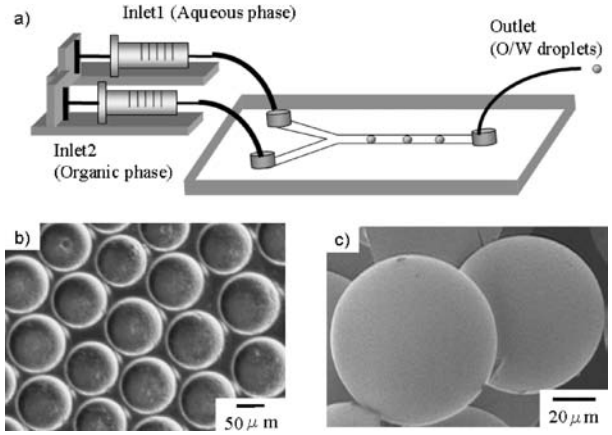


Figure 1. a) Schematic drawing Y-junction microchannel. The microchannel (70 × 38 × 2 mm) has paths with 153.1 μm width and 80.0 μm depth. b) Microscope image of oil in water (O/W) droplets (containing atrazine, MAA, EGDMA, and ADVN in mesitylene) before polymerization. c) Scanning electron micrograph of atrazine-imprinted polymer.

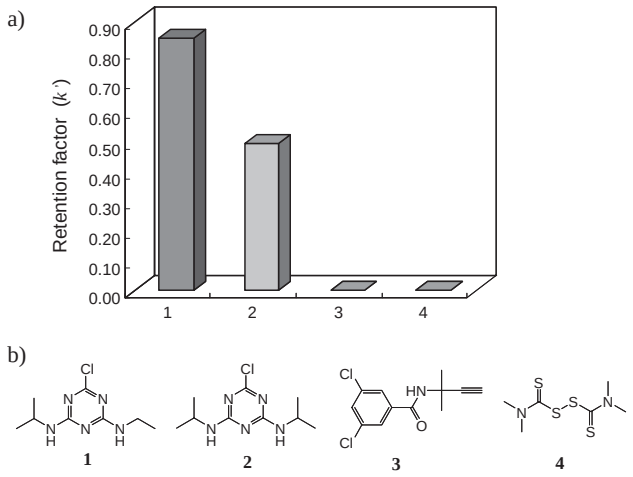


Figure 2. a) Retention factors (k') of the tested samples in atrazine-imprinted microspheres. Retention times were measured in triplicate by HPLC with the imprinted microspheres packed column. b) Chemical structures of samples used in this work. (**1**) atrazine; (**2**) propazine; (**3**) propyzamide; (**4**) thiuram.

mer were estimated to be 128 m^2/g (BET method) and <5 nm pores (DFT method) by nitrogen gas adsorption/desorption.[9] The shape was keeping spherical form and monodispersity in the both before and after the imprinting process (Figures 1b and 1c).

Subsequently, we carried out chromatographic experiments to evaluate the selectivity of the resulting polymer microspheres. The washed polymer microspheres were dispersed in chloroform–acetonitrile (1/1, v/v), and packed in a stainless steel column (50 × 4.6 mm, i.d.) with methanol by a pump (Waters 510). Atrazine and three reference compounds (propazine, propyzamide, and thiuram) were injected into an HPLC system.[10] Retention times of the analytes and a void marker (acetone) were measured, and retention factors k' were calculated by an equation, $k' = (t_s - t_0)/t_0$, where t_s is a retention time of the sample, t_0 is a retention time of the void marker.

The retention factor of atrazine is obviously higher than those of other herbicides (Figure 2). This means that the atrazine-imprinted microspheres prepared by the microfluidic device show the selectivity for atrazine. While propyzamide and thiuram that are not structurally related to atrazine have no interaction to the polymer, propazine was weakly adsorbed under the conditions employed. It is difficult to distinguish between propazine and atrazine, because propazine has a similar triazine structure except for an isopropyl group at 2-position. The retention factor to atrazine of this polymer was not high. This may be due to the heterogeneity for the inside of the imprinted polymer. Therefore, the composition of monomers in the MIPs is necessary to improve.

In conclusion, a novel method for the preparation of atrazine-imprinted microspheres by using the microfluidic device has been developed. The obtained MIP microspheres showed the specific adsorption for atrazine. They were spherical and narrow size distribution. When the proposed preparation method for MIP microspheres is used, the polymer microspheres would be developed to various applications such as LC, solid-phase extraction,[11] and chemical sensors,[12] would be easily achieved without crash and sieving of MIPs.

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8 The amount of atrazine recovered was determined by HPLC with a column, SUPELCO SIL LC-8-DB (150 × 4.6 mm, i.d.), a mobile phase of water–methanol (4/6, v/v), 1 mL min^{-1}.

9 The surface area and pore size distributions of MIP were measured by NOVA-1000 YUASA IONICS. The surface area was determined by Multiplot BET method. And the pore size distribution was determined by DFT method (Density Functional Theory) used for analysis on porous materials.

10 HPLC conditions; eluent: hexane–chloroform (8.5/1.5, v/v), flow rate: 1 mL min^{-1}, sample concentration: 1.0 mM, injection volume: 10 μL, UV–vis detection: 263 nm.

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