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Matrine- and oxymatrine-imprinted monodisperse polymers prepared by precipitation polymerization and their applications for the selective extraction of matrine-type alkaloids from *Sophora flavescens* Aiton

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ABSTRACT

Matrine (MT)- and oxymatrine (OMT)-imprinted monodisperse polymers have been prepared by precipitation polymerization. The prepared molecularly imprinted polymers (MIPs) for MT and OMT, MIP_{MT} and MIP_{OMT}, were monodispersed microspheres of 3.3 and 3.9 μm in diameter, respectively. Binding experiments and Scatchard analyses revealed that two classes of binding sites were formed on MIP_{MT} and MIP_{OMT}. In addition to shape recognition, ionic and hydrophobic interactions seemed to affect the retention and recognition of MT and OMT on MIP_{MT} and MIP_{OMT}, respectively, in low acetonitrile content, and ionic and hydrophilic interactions affected these properties in high acetonitrile content. MIP_{MT} was used to selectively extract MT and sophocarpine (13,14-dehydromatrine) from *Sophora flavescens* root, while MIP_{OMT} was used to extract OMT and oxysophocarpine (13,14-dehydrooxymatrine).

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1. Introduction

Molecularly imprinted polymers (MIPs), which have selective recognition site(s) for a target compound or its structurally related analog(s), have been used for chromatographic media, such as LC stationary phases or adsorbents for solid-phase extraction (SPE) and solid-phase microextraction [1–3]. MIPs are generally prepared by a bulk polymerization method in non-aqueous solvents, where the resultant monoliths must be crushed, ground and sieved to obtain microparticles for their applications [4]. When MIPs are used as LC stationary phases or SPE media, it is desirable to prepare spherical and monodispersed particles [1]. The preparation methods for microspherical MIPs include suspension polymerization in water [4], liquid perfluorocarbon [5] and mineral oil [6], seed polymerization (multi-step swelling and polymerization) [7] and precipitation polymerization [8]. Among those methods, the latter two yield monodispersed MIPs.

Sophora root, which is a folk medicine in Japan and China, is the dried root of *Sophora flavescens* Aiton (Leguminosae) and includes matrine (MT) and oxymatrine (OMT) as its primary components [9–11]. It was reported that MT and OMT exhibit sedative, depressant, anti-tumor, antipyretic, cardiotonic and anti-hepatitis B virus activities [9,12–14]. Previously, the MIPs for MT were prepared by suspension polymerization [15] or bulk polymerization [16,17] methods to extract MT from *S. flavescens* or *S. tonkinensis*. However, a MIP for OMT has not yet been prepared. Furthermore, a monodispersed MIP for neither MT nor OMT has been prepared.

In this study, we prepared MIPs for MT and OMT, termed MIP_{MT} and MIP_{OMT}, by a precipitation polymerization method, which yielded the spherical and monodispersed particles with high yields in one step. The binding properties of MIP_{MT} and MIP_{OMT} were evaluated for MT and OMT by binding experiments and Scatchard analyses. The retention and molecular recognition properties of MIP_{MT} and MIP_{OMT} were evaluated for MT, OMT and their structurally related compounds, sophocarpine (SC) and oxysophocarpine (OSC), with LC. Furthermore, MIP_{MT} and MIP_{OMT} were applied to extract not only MT and OMT but also their structurally related compounds, SC and OSC, from Sophora root.

2. Experimental

2.1. Materials

Methacrylic acid (MAA), divinylbenzene (DVB) (95.7%, mixture of isomers) and 2,2′-azobis(isobutyronitrile) (AIBN) were purchased from Wako Pure Chemicals (Osaka, Japan). Polyvinyl alcohol (dp = 500, saponification value = 86.5–89 mol%) was purchased from Nacalai Tesque (Kyoto, Japan). MT and OMT were

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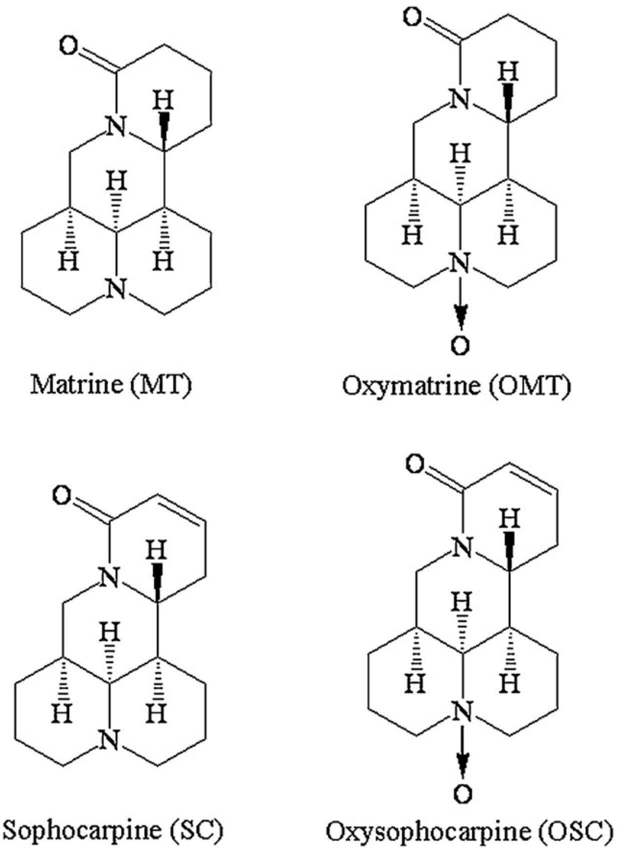

Fig. 1. Structures of MT, OMT, SC and OSC.

purchased from Xi'an Honson Biotechnology (Xi'an, China). SC and OSC were purchased from AvaChem Scientific (San Antonio, TX, USA). The roots of *S. flavescens* Aiton were purchased from Tochimoto Tenkaido (Osaka, Japan). The structures of the MT-type alkaloids (MT, OMT, SC and OSC) used in this study are illustrated in Fig. 1.

2.2. Preparation of MIP$_{MT}$ and MIP$_{OMT}$ by a precipitation polymerization method

MIP$_{MT}$, MIP$_{OMT}$ and a non-imprinted polymer (NIP) were prepared by precipitation polymerization according to a previously reported method [18]. MT or OMT (1.5 mmol) was used as a template molecule, MAA (4 mmol) was the functional monomer, DVB (28.8 mmol) was the cross-linker, and AIBN (1.9 mmol) was the initiator, and they were all dissolved in 128 mL of acetonitrile–toluene (3:1, v/v). The mixture was degassed with argon gas for 15 min and then rotated slowly at 16 rpm using a Shellspin VS-60 rotor (Taitec, Tokyo, Japan). The reaction temperature was increased from 25 °C to 60 °C for 2 h and then maintained at 60 °C for an additional 16 h. The generated polymers were washed and dried as previously reported [18].

2.3. Porosity measurements and scanning electron micrographs

The surface areas and porosity of MIP$_{MT}$, MIP$_{OMT}$ and NIP were measured by nitrogen sorption porosimetry using a TriStar surface area and porosity analyzer (Micrometrics Instrument, Norcross, GA, USA). The scanning electron micrographs (SEMs) were performed on MIP$_{MT}$, MIP$_{OMT}$ and NIP using a Mighty-8 instrument (Technex, Machida, Japan).

Table 1
Physical properties of MIP$_{MT}$, MIP$_{OMT}$ and NIP.

Polymer	Specific surface area (m^2/g)	Specific pore volume (cm^3/g)	Average pore diameter (nm)
MIP$_{MT}$	520	0.25	3.3
MIP$_{OMT}$	460	0.20	3.3
NIP	570	0.28	3.5

2.4. Binding experiments of MT and OMT

A total of 10 mg of MIP$_{MT}$, MIP$_{OMT}$ or NIP was added to 1 mL of an acetonitrile solution containing 0.2–7.0 mM MT or 1.0–8.0 mM OMT. After shaking at 25 °C for 10 h, an aliquot of each sample was taken and filtered through a 0.45-μm membrane filter. The concentration of free MT or OMT in the supernatant was determined by LC with a Cosmosil 5C$_{18}$-MS-II column (150 mm x 4.6 mm I.D.), a column temperature of 40 °C, a mobile phase of 50 mM phosphoric acid (pH 2.3)–acetonitrile–methanol (940:50:10, v/v/v) including 160 mM sodium perchlorate and a flow rate of 1.0 mL/min with detection at 220 nm. The dissociation constant (K_d) and the apparent maximum number of binding sites (Q_{max}) were determined by a Scatchard plot.

2.5. Chromatographic evaluation of MIP$_{MT}$ and MIP$_{OMT}$

The LC system used was composed of a LC-10ADvp pump, a SPD-10Avp spectrophotometer, a C-R6A integrator (all from Shimadzu, Kyoto, Japan), a Rheodyne 7125 injector with a 20 μL loop (Rheodyne, Cotati, CA, USA) and a CO-1093C column oven (Uniflows, Tokyo, Japan). The LC conditions used are specified in the legend of each figure.

The imprinting factor (*IF*) was calculated from the equation $IF = k_{MIP}/k_{NIP}$, where k_{MIP} and k_{NIP} are the retention factors of a solute on the MIP and NIP columns, respectively.

2.6. Application of MIP$_{MT}$ and MIP$_{OMT}$ for extraction of MT-type alkaloids in Sophora root

One gram of ground and sieved Sophora root was weighed and extracted with 100 mL of 50% methanol in an ultrasonic bath for 20 min. A 1-mL aliquot of the extract was passed through a 0.45-μm membrane filter and concentrated to approximately 100 μL. Then, a 20-μL aliquot of the concentrate was loaded onto MIP$_{MT}$, MIP$_{OMT}$ or NIP. The LC system used was the same as that used for the chromatographic evaluation of the MIPs. The LC conditions used a column size of 50 mm × 4.6 mm I.D., a column temperature of 40 °C, a mobile phase of 10 mM ammonium acetate (pH 6.8)–acetonitrile (25:75, v/v) and a flow rate of 1.0 mL/min with detection at 220 nm. After the isolation of several fractions, the concentrated fractions were analyzed under the same LC conditions as described in Section 2.4.

3. Results and discussion

3.1. Physical properties of MIP$_{MT}$ and MIP$_{OMT}$

We prepared MIP$_{MT}$ and MIP$_{OMT}$ by a precipitation polymerization method using MAA as a functional monomer and DVB as a cross-linker in a mixture of toluene and acetonitrile according to the method for the preparation of a MIP for (*S*)-nicotine [18]. Parts a–c of Fig. 2 show the SEMs of MIP$_{MT}$, MIP$_{OMT}$ and NIP, respectively. The particle diameters of MIP$_{MT}$, MIP$_{OMT}$ and NIP were 3.28 ± 0.07, 3.92 ± 0.09 and 3.44 ± 0.05 μm, respectively, and their size uniformity was observed. Table 1 shows the specific surface areas, specific pore volumes and average pore diameters of MIP$_{MT}$,

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