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Preparation of molecularly imprinted polymers for warfarin and coumachlor by multi-step swelling and polymerization method and their imprinting effects

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ABSTRACT

Monodisperse molecularly imprinted polymers (MIPs) for warfarin (WF) and coumachlor (CC), MIP_{WF} and MIP_{CC}, respectively, were prepared using 4-vinylpyridine (4-VPY) as a functional monomer and ethylene glycole dimethacrylate (EDMA) as a crosslinker by multi-step swelling and polymerization. Six kinds of MIP_{WF}, MIP_{WF1} – MIP_{WF6}, were prepared varying the concentrations of WF and 4-VPY, while maintaining the EDMA concentration constant, and their retention and molecular recognition properties were evaluated using a mixture of sodium phosphate buffer and acetonitrile as a mobile phase in LC. In addition to shape recognition, hydrogen bonding, ionic and hydrophobic interactions could affect the retention and molecular recognition of WF on MIP_{WF}, and ionic interactions seem to govern the retention and molecular recognition of WF above mobile phase pH 6 associated with higher molar ratio of 4-VPY to EDMA. Furthermore, MIP_{CC} was prepared under the same conditions with MIP_{WF6}, which gave the highest imprinting factor for WF. WF could be recognized more strongly on MIP_{CC} than MIP_{WF6}, and the imprinting factors of WF on MIP_{WF6} and MIP_{CC}, respectively, are 2.68 and 5.03 using 20 mM sodium phosphate buffer – acetonitrile (30/70, v/v)(final pH 6.1) as the mobile phase. This result indicates that the use of CC as a template molecule instead of WF could be useful for getting a higher imprinting factor for WF and for avoiding the leakage problem in the assay of WF in LC.

1. Introduction

Molecular imprinting technologies are attractive for easy introduction of a selective recognition site(s) for a target compound and its structurally related compounds on polymerized materials [1]. Molecularly imprinted polymers (MIPs) have been used for separation, extraction and bioapplication as affinity media [2–5]. MIPs are generally prepared in non-aqueous solvents by bulk polymerization, which gives irregular particles after grinding and sieving [1], while suspension polymerization [6], multi-step swelling and polymerization [7] and precipitation polymerization [8] give microspherical beads. Among those, the latter two methods could yield monodisperse MIPs. Recently, we prepared monodisperse MIPs for hydrophilic compounds by a modified precipitation polymerization method [9]. In the method, a hydrophilic compound was first dissolved in a small portion of water, methanol or dimethyl sulfoxide, and then a MIP for the hydrophilic compound was prepared by precipitation polymerization. Since the hydrophilic compound is first dissolved in a hydrophilic solvent, the method is called modified precipitation polymerization.

Nicholls and his group prepared MIPs for warfarin (WF), MIP_{WF}, using methacrylic acid (MAA) or 4-vinylpyridine (4-VPY) as a functional monomer, ethylene glycol dimethacrylate (EDMA) as a crosslinker and chloroform as a porogen by bulk polymerization [10]. MAA-*co*-EDMA MIP_{WF} showed the molecular recognition ability for WF, while 4-VPY-*co*-EDMA MIP_{WF} had no molecular recognition ability. MAA-*co*-EDMA MIP_{WF} gave the imprinting factors of 2.1 and 1.2 for WF in chloroform and acetonitrile, respectively. They concluded that a large number of a specific binding sites were present in MAA-*co*-EDMA MIP_{WF} based on hydrogen bonding interactions, while in 4-VPY-*co*-EDMA MIP_{WF} high degree of nonspecific bindings occurred because of electrostatic interactions. A

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direct comparison of MAA-*co*-EDMA MIP_{WF} binding characteristics with those of the human serum albumin Sudlow I binding site was made, and similarities in site population and affinities were observed [10]. Furthermore, they prepared MAA-*co*-EDMA MIP_{WF} varying the concentration of MAA, while maintaining WF and EDMA concentrations constant [11]. Higher degrees of crosslinking resulted in the co-existence of specific and non-specific bindings, while polymers with lower degrees of crosslinking afforded higher capacity with non-specific bindings. MIPs for (*S*)-WF, $MIP_{(S)\text{-}WF}$, were designed computationally [12]. The results indicated that MAA as a functional monomer and acetonitrile as a solvent had the highest interaction energies for the pre-polymerization adducts, and that the optimal molar ratio of (*S*)-WF to MAA was 1:3. $MIP_{(S)\text{-}WF}$ was synthesized by bulk polymerization and applied for solid-phase extraction (SPE) of (*S*)-WF from plasma. Furthermore, MIP_{WF} was prepared using pyrrole as a functional monomer and vinyl triethoxysilane as a crosslinker and applied for SPE of WF in human plasma and urine [13].

An electrochemical sensor for WF was prepared based on molecular imprinting technology by electropolymerization of o-phenylenediamine on a glassy carbon electrode, on which multiwall carbon nanotubes containing a carboxy functional group were introduced [14]. The prepared sensor was successfully applied for the assay of WF in human serum sample. Li and his group fabricated an electrochemical sensor for WF sodium by coupling nanoporous gold leaf with MIP prepared using resorcin as a functional monomer by electropolymerization. The sensor was used for the determination of WF in human blood [15]. Furthermore, they used the MIP-based Au-Ag alloy microwire electrode for detecting WF on a microfluidic chip in therapeutic drug monitoring of WF [16].

In this study, six kinds of monodisperse MIP_{WF}, MIP_{WF1} – MIP_{WF6}, were prepared using 4-VPY as a functional monomer and EDMA as a crosslinker by multi-step swelling and polymerization. They were prepared varying the concentrations of WF and 4-VPY, while maintaining the EDMA concentration constant. The retention and molecular recognition properties of MIP_{WF} toward WF, coumachlor (CC), coumarin and benzenesulfonic acid were evaluated using a mixture of sodium phosphate buffer and acetonitrile as a mobile phase in LC. Furthermore, monodisperse MIPs for CC, MIP_{CC}, were prepared using the same conditions with MIP_{WF6}, which gave the highest imprinting factor. The retention and molecular recognition properties of MIP_{WF} and MIP_{CC} were compared in reversed-phase mode, and MIP_{CC} was applied for selective extraction of WF in human serum samples as a pretreatment column by column-switching LC.

2. Experimental

2.1. Reagents and materials

EDMA and 4-VPY were purchased from Tokyo Chemical Industry (Tokyo, Japan). Polyvinyl alcohol (degree of polymerization = 500, saponification value = 86.5-89 mol%) and coumarin were purchased from Nacalai Tesque (Kyoto, Japan). 2,2′-Azobis(2,4-dimethyl valeronitrile) (ADVN) and benzenesulfonic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). WF, CC, coumatetralyl and coumafuryl were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Control human serum (Seiken Liquid Normal V Plus) was purchased from Denka Seiken (Tokyo, Japan). Other reagents and solvents were of analytical-reagent grade and were used without further purification. Water purified with a PURELAB Ultra (Organo, Tokyo, Japan) system was used to prepare mobile phases and sample solutions. The structures of WF, CC, coumatetralyl, coumafuryl and coumarin used in this study are shown in Fig. 1.

2.2. Preparation of MIPs

MIP_{WF}, MIP_{CC} and non-imprinted polymers (NIP_{WF} and NIP_{CC}) were prepared by multi-step swelling and polymerization according to the method reported previously [8]. The molar amounts of template molecule, functional monomer, crosslinker and initiator used for the preparation of MIP_{WF}, MIP_{CC}, NIP_{WF} and NIP_{CC} were shown in Table 1. The corresponding NIP was prepared similarly but without template molecule. Briefly, 0.085 mL of uniformly sized polystyrene seed particles (0.0924 g/mL) dispersed in water was mixed with microemulsion prepared from 0.02 g of sodium dodecyl sulfate, 10 mL of water and 0.24 mL of dibutyl phthalate as an activating solvent by sonication. This first swelling step was carried out at room temperature for 24 h with stirring at 125 rpm until the microdrops of oil completely disappeared. Next, the dispersion of ADVN as an initiator, 5.0 mL of toluene as a porogen, 12.5 mL of water and 10 mL of 4.8 % polyvinyl alcohol aqueous solution as a dispersion stabilizer was added to the dispersion of swollen particles. This second swelling step was carried out at room temperature for 20 h with stirring at 125 rpm. Next, the dispersion of WF or CC as a template molecule, 4-VPY as a functional monomer, EDMA as a crosslinker, 12.5 mL of water and 10 mL of 4.8 % polyvinyl alcohol aqueous solution was added to the dispersion of swollen particles. This third swelling step was carried out at room temperature for 6 h with stirring at 125 rpm. After the third swelling step was completed, the polymerization procedure was started at 50 °C under argon atmosphere with stirring at 160 rpm for 24 h. The resulting polymer particles were washed, collected and dried as reported previously [8].

2.3. Scanning electron micrographs and particle diameter measurement

Scanning electron micrographs (SEMs) were obtained using a Mighty-8 instrument (Technex, Machida, Japan). The average particle diameters of MIPs and NIPs were measured using ImageJ software (National institute of health, Bethesda, MD, USA, http://rsb.info.nih.gov/ij/) in triplicate.

2.4. Porosity measurements

The surface areas and porosity of MIPs and NIPs were measured by nitrogen adsorption porosimetry using a TriStar 3000 surface area and porosity analyzer (Micromeritics Instruments, Norcross, GA, USA). Prior to measurement, a 200 mg weight of the polymers was heated at 80 °C for 4 h in vacuo. The specific surface areas were calculated using the BET method, and the specific pore volumes and average pore diameters were calculated by the BJH method.

2.5. Evaluation of pK_a of WF in mobile phase

WF was dissolved in a mobile phase [20 mM sodium phosphate buffer – acetonitrile (50/50, v/v)] at a final concentration of 38 μM and the absorbance at 330 nm was measured using a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). The dataset of absorbance was plotted against an apparent pH of a mobile phase solution, and the pK_a value of WF in the mobile phase was determined by a graphical method.

2.6. Chromatographic evaluation of MIPs

To evaluate their retention and molecular-recognition properties, the obtained MIPs and NIPs were packed into stainless-steel columns (50 mm × 4.6 mm ID) using methanol-2-propanol (2/1, v/v) as the slurry solvent and methanol as the packing solvent at constant pressure of 9.8 MPa. The LC system was composed of an

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