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Molecularly imprinted nanoparticles with nontailing peaks in capillary electrochromatography

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ABSTRACT

The combination of microparticles of molecularly imprinted polymers (MIPs) with partial filling capillary electrochromatography (CEC) has previously been demonstrated for the enantiomer separation. In this paper, precipitation polymerization was used to prepare *d*-zopiclone imprinted nanoparticles (50–80 nm) by a strategy of the dilution of pre-polymerization mixtures. The influence of some important parameters on the preparation of MIPs nanoparticles, including template to monomer ratio, type and amount of cross-linking monomer, and functional monomer composition ratio were investigated. In addition, the effect of separation condition, e.g., organic modifier content, pH value and salt concentration of buffer, on the electrochromatographic behavior of the MIP nanoparticles were studied. In spite of lower selectivity factor (1.11), high column performance (theoretical plates 41,400) of template was obtained and the resolution of enantiomers separation was 4.75 under the optimized conditions. Compared to the previously reported MIP microparticles, the MIP nanoparticles showed good peak symmetry and an ability of high speed separation (<15 min) in CEC mode.

1. Introduction

Molecular imprinting technology (MIT) is a very effective and promising method to prepare artificial materials that can achieve specific recognition at a molecular level [1,2]. The resultant polymers, i.e., molecularly imprinting polymers (MIPs), demonstrate high selectivity for the used template molecule [3–6]. Due to their favorable molecular recognition capability and stability, as well as low cost, applications of MIPs have been found in broad areas, such as catalysis [7], bio-mimetic sensors [8], drug delivery system [9], solid-phase extraction [10] and chromatography [11,12].

Up to now, the most widely used method for MIPs preparation is bulk polymerization [13]. Because the obtained MIPs need to be crushed, ground and sieved, this procedure is time-consuming and the irregularity of shape of such MIP particles results in decreased efficiency in chromatography. An attractive and reliable method to avoid these drawbacks is precipitation polymerization [5,14,15]. This approach involves the formation of MIP nano- or micro-particles in an excess of suitable solvent by interrupting polymerization to prevent particles coalescence. The size of these particles can be varied by alterations in monomer concentrations and types. The molecularly imprinted microsphere prepared with the approach was first developed by Ye et al. [16] and used for competitive radioassay studies.

Direct utilization of MIP nanoparticles (MINP) as stationary phase for HPLC is limited high back pressure due to its small sizes [17]. In contrast, using the partial-filling technique, MINP (200–300 nm) can be used as additive in the background electrolyte and have been shown to be successful pseudo-stationary phase in capillary electrochromatography (CEC) [18–23]. However, these CEC-based systems suffered from low column efficiency and peak tailing for template, which is due to slow association dissociation kinetics between template and imprinted sites.

MIP coating protocol for CEC has been explored for the fabrication of imprinted stationary phase without peak tailing. 4-Styrenesulfonic acid introduced in the monomer mixture can result in nontailing peaks of template [24,25]. Indeed, we recently reported MIP coating in capillary with improved column efficiency in CEC, prepared with a new crosslinking functional [26] or liquid crystal monomer [27] to imprint the memory of a template at low levels of cross-linking (20 mol%). In addition, high column efficiency of template in CEC was demonstrated using MINP with sizes of 80 nm prepared with miniemulsion polymerization [28]. In contrast to previous MIPs-based HPLC and CEC methods, there was no apparent tailing for the template with a baseline separation for enantiomers. However, this approach needs a synthesis of

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Table 1
Recipes of preparation for *d*-ZOP-imprinted nanoparticles.

Polymer	Template (mmol/L)	Functional monomers (mol/L)				EDMA (mol/L)	Time (min)
		MAA	AM	BMA	4-VP		
MIP 1	4.0	0.02				0.1	80
MIP 2	6.6	0.02				0.1	120
MIP 3	5.0	0.02				0.1	120
MIP 4	3.3	0.02				0.1	120
MIP 5	2.0	0.02				0.1	90
MIP 6	4.0	0.02				0.06	180
MIP 7	4.0	0.02				0.08	120
MIP 8	4.0	0.02				0.12	90
MIP 9	4.0	0.02				0.2	60
MIP 10	4.0	0.01	0.01				90
MIP 11	4.0	0.01		0.01			180
MIP 12	4.0	0.01			0.01		60
MIP 13	4.0	0.015			0.005		90

surfactant monomer to prepare MINP. In view of facts above, we decided to study the approach for MIP nanoparticles and evaluate these particles as a pseudo-phase in CEC to obtain non-tailed peaks.

This report relates our systematic effort to develop a highly effective separation system for CEC based on MINP. Provided that the imprinted materials were prepared in the form of nanostructures with a size small enough, improved binding capacity, binding kinetics, and site accessibility of imprinted materials can be expected. The principal aims of this study were to define the sizes of MINP that control the behaviors of CEC. In this work, MINP were controlled below 80 nm carefully in conventional precipitation polymerization by a strategy of the dilution of pre-polymerization mixtures. The effects of the polymerization variables, e.g., the molar ratio of the template to the monomers, the type and amount of functional and cross-linking monomers were also investigated.

2. Materials and methods

2.1. Chemicals

d-Zopiclone (ZOP) and *rac*-zopiclone were obtained from Kaiyuan Minsheng Sci. & Tech. Corp. (Suzhou, China). Methacrylic acid (MAA) and butyl methacrylate (BMA) were from Beijing Donghuan Chemical Reagent (Beijing, China). Acrylamide (AM) was purchased from Beijing Pubo Biotech. (Beijing, China). 4-Vinyl pyridine (4-VP), ethylene glycol dimethacrylate (EDMA) and trimethylolpropane trimethacrylate (TRIM) were from Sigma (St. Louis, MO, USA). 2,2′-Azobis (2-isobutyronitrile) (AIBN) was purchased from Special Chemical Reagent Factory of Nankai University (Tianjin, China). Acetonitrile (ACN, HPLC grade) was from Fisher (NJ, USA). 3-(Trimethoxysilyl)propyl methacrylate (γ-MPS) was from Acros (Geel, Belgium). Other analytical reagents were obtained from Tianjin Chemical Reagent Co., Ltd. (Tianjin, China). Fused-silica capillaries with 100 μm ID and 375 μm OD were purchased from Xinnuo Optic Fiber Plant (Hebei, China).

2.2. Preparation of molecularly imprinted nanoparticles

The template molecule *d*-zopiclone, the radical initiator AIBN (4.3 mg/mL), functional monomers (MAA) and cross-linking monomers (EDMA) were dissolved in ACN in proportions stated in Table 1. The pre-polymerization mixture was sonicated for 15 min followed by degassing by a stream of nitrogen for 5 min. The flask was sealed and put into a water bath and heated at 53 °C. After completed polymerization, the suspension was washed by successive centrifugation (13,000 rpm for 15 min) and resuspension (sonication for 15 min) twice in ACN:acetic acid (2:1, v/v) and once in ACN. The corresponding blank polymers were prepared in the absence of template. Finally, the polymer nanoparticles were dried and stored at room temperature until use.

2.3. CEC

All CEC experiments were carried out on a K1050 system (Kaiao, Beijing, China) equipped with a UV detector. A Lenovo personal computer for data processing was used. In our work, since the migration of the MIP plug is partially determined by the EOF, a derivatized capillary with γ-MPS with reduced EOF [19] was used in order to be able to use a MIP plug of appropriate length and still have the analyte to reach the detection window prior to the MIP. The derivatization was performed by successively flushing the capillary with 1 mol/L NaOH, water, 0.1 mol/L HCl (to protonate the silanol groups and form Si–OH) and water for 2 h, followed by drying with a stream of nitrogen gas. Then the capillary was filled with a solution of toluene/γ-MPS (85:15, v/v), and the solution was kept in the capillary overnight. Finally the capillary was flushed with toluene and dried.

A γ-MPS-derivatized capillary (74.5 cm total length; 64.5 cm effective length) was used in CEC separation. The electrolyte used was composed of ACN/20 mmol/L acetate–sodium acetate buffer solution (pH 3.6) (85:15, v/v). All the electrolyte was made using double distilled water and filtered with 0.2 μm microporous film. MINP were suspended in the electrolyte to 8 g/L. The samples were prepared from 5 mmol/L ACN solutions diluted with electrolyte to give samples of 20 μmol/L concentration. All solutions were degassed by sonication. Before the CEC analysis every day, the capillary was rinsed with water and electrolyte for 10 min respectively. Between consecutive runs, the capillary was rinsed with electrolyte for 1 min. The MINP suspensions and the samples were introduced hydrodynamically for 5.0 s and 3.0 s, respectively. The separation voltage was 15 kV. UV detection was performed at 230 nm.

In this paper, because some of analytes are eluted prior EOF, separation factor is evaluated using α, which is calculated by [29]

$$\alpha = \frac{t_2}{t_1}$$

where t_1 and t_2 are the retention time of the first and second peaks. The degree of enantiomer separation was represented by a normalized separation index $\Delta t_R/t_{R1}$, where Δt_R is the difference in the elution times of the enantiomers at peak maximum and t_{R1} is the retention time of the first eluted enantiomer. The resolution (R_s) was calculated according $R_s = (t_2 - t_1)/0.5(W_2 + W_1)$, W is the width at the baseline between tangents drawn to inflection points for the peak.

2.4. Characterization of the MINP

Transmission electron microscopy (TEM) analysis was performed on a JEM100CXII UHR microscope from JEOL, with a 100 kV acceleration tension. The porosity of the MIP particles was measured at 77 K by nitrogen adsorption–desorption isotherms using a Micromeritics ASAP 2020 Surface Area and Porosity Analyzer (Micromeritics Instrument, Norcross, GA, USA).

3. Results and discussion

3.1. Synthesis and evaluation of d-zopiclone imprinted nanoparticles

To prepare smaller MINP, a strategy of the dilution of pre-polymerization mixtures was used in this study. Compared with pervious MINP [18,19], the pre-polymerization solution was therefore diluted five fold in acetonitrile. The nanometer size of the resultant MIP particles was demonstrated by TEM. As shown in