Facile Preparation of Core−Shell Type Molecularly Imprinted Particles: Molecular Imprinting into Aromatic Polyimide Coated on Silica Spheres

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ABSTRACT: Core−shell type imprinted spherical particles were prepared by using a conventional polymer solution coating method. A poly(amic acid) was synthesized by step-reaction polymerization of the diamines (1:19 mole ratio of the diamine monomer−template complex:4,4′-oxydianiline) and the pyromellitic dianhydride in *N*,*N*-dimethylacetamide at ambient temperature. The diamine monomer−template complex was synthesized by the reaction of the diamine having an isocyanato group and estrone (template) having a phenol moiety, in which the template was connected to the monomer by means of a thermally reversible urethane bond. The poly(amic acid) containing the template (estrone) molecule was used for the coating of a silica sphere, of which surface was functionalized with amino groups to increase the adhesion property of the poly(amic acid) film on the silica. The thermal imidization resulted in a spherical silica particle coated with a polyimide (thickness of polyimide: about 100 nm). The template molecules were removed from the polyimide layer by heating the particles in 1,4-dioxane in the presence of water or aniline. In the process, the isocyanato groups, which were generated by dissociation of thermally reversible urethane bonds, were converted to amino or phenyl urea groups by the reaction with water or aniline, respectively. The imprinted silica particles were used as a stationary phase in HPLC mode and their specific recognition ability for estrone and its structural analogues were evaluated.

Introduction

Mechanically stable materials fabricated by a molecular imprinting method are a subject of great interest due to their potential application such as stationary phases for high-performance chromatography, chemosensors, catalysts, and membranes for separating toxic chemicals.[1] In the molecular imprinting process, a template is complexed with a functional monomer covalently or noncovalently and then frozen into a matrix by polymerization. The removal of the template leaves cavities with a similar shape and complementary functional groups to the template molecule.

The advantages of molecularly imprinted materials, as compared to biological receptors, include their mechanical and chemical stability, low cost of preparation, and wide range of operating conditions. However, they suffer from some drawbacks in certain applications, such as the heterogeneous distribution of the binding sites, low capacity and selectivity, and poor site accessibility. Core−shell structured imprinted spherical particles with rebinding sites in the outer shell have been studied in an effort to improve accessibility and capacity[2] and found especially useful as stationary phases in chromatographic mode. They show fast binding kinetics, which is related to diffusion efficiency. In addition, the spherical particles with regular sizes are suitable for column packing, and thus wasteful grinding and sieving process can be avoided.[3] One drawback of the core−shell approach is the low content of molecularly imprinted materials in a particle, which might be overcome by using a nanosized core.

Molecularly imprinted core−shell particles can be prepared by emulsion polymerization or grafting polymerization at the surface of the supporting beads.[4] In the latter, the polymer chains are grafted onto the surface of the supporting beads either by initiation of the polymerization from the surface or by end-capping of a growing polymer chain to the surface. Most polymeric matrices constituting a shell are vinyl polymers, having cross-linked network structures, so as to prevent structural changes of the cavity occurring by relaxation or diffusion of the matrix molecules. They are generally obtained by radical polymerization of vinyl monomers in the presence of a cross-linking agent. One of the problems with this approach is that it is not easy to control the cross-linking reaction, resulting in inhomogeneous morphology. Moreover, the polymers cannot be processed further due to their network structures.

In this paper, we report a facile method for preparing core−shell type imprinted spherical particles. We prepared polyimide-coated silica particles by using a conventional polymer solution coating method[5] and carried out molecular imprinting into the polyimide layer. Previously, we reported an aromatic polyimide, which is a non-cross-linked high-performance polymer, as an imprinted matrix.[6] An aromatic polyimide is obtained by polymerization of an aromatic dianhydride and an aromatic diamine. A poly(amic acid) is first formed and subsequent intramolecular condensation reaction results in an aromatic polyimide. A poly(amic acid) is soluble in polar organic solvents, while an aromatic polyimide is generally insoluble and infusible due to the strong interactions between the polymer chains. A poly-

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(amic acid) has good film-forming property and is smoothly coated on various substrates. We used a silica sphere for coating with a poly(amic acid) containing the template (estrone) molecules. The silica sphere surface was functionalized with amino groups in advance to increase the adhesion property of the polymer film on the silica. After the coating, the poly(amic acid) was thermally imidized to yield the polyimide-coated silica particle.

The template molecule, estrone, was connected to the polymer chain by means of a urethane bond. The urethane bond was stable at room temperature but was cleaved at elevated temperature.[7] The use of a thermally reversible bond has certain advantages in that it is possible to easily remove the template molecule from the matrix and to introduce various functional groups into the cavities.[7] We extended this approach to modify the nature of functional groups inside binding sites and thereby to control the specific recognition abilities of the imprinted materials.[8] Estrone is one of several naturally occurring estrogens. It influences the normal development and maturation of the female. Estrone has been suspected of having carcinogenic properties and adverse environmental effects.[9]

Experimental Section

Materials and Measurement. 3,5-Dinitrobenzoic acid, sodium azide, 1,2,4,5-benzenetetracarboxylic dianhydride, 4,4′-oxydianiline, potassium hydrogen sulfate, dibutyltin dilaurate, trifluoroacetic acid, palladium (3 wt % on activated carbon), and 3-aminopropyltriethoxysilane, chlorotrimethylsilane, phenanthrene, anthracene, and naphthalene were purchased from Aldrich Co. and used as received. Estrone, testosterone, testosterone propionate, ethyl chloroformate, and 3,5-dinitrobenzoyl chloride were purchased from TCI and used as received. LiChrospher Si 100 (10 μm) was purchased from Merck. *N*,*N*-Dimethylacetamide (DMAc) was purchased from Junsei. Methylene chloride (MC) and toluene were purchased from J.T. Baker (HPLC grade) and used as received. Tetrahydrofuran (THF) and *n*-hexane (*n*-Hxn) were dried over sodium metal and distilled. Triethylamine (TEA) was purified by distillation after drying over K_2CO_3.

Transmission electron microscope (TEM) images were obtained by using a CM-20 transmission electron microscope at 200 kV (Philips Electron Optics). Samples for TEM characterization were first embedded in epoxy resin and cured at 60 °C overnight. Ultrathin sections (~80 nm) were microtomed at room temperature and picked up on 200 mesh copper grids. Field emission scanning electron microscope (FE-SEM) images were taken by using a JEOL JSM-6330F microscope. Extraction of template molecules was monitored by a SCINCO S-3150 UV/vis spectrophotometer. The solid-state ^{13}C NMR (100 MHz) and ^{29}Si NMR (80 MHz) spectra were obtained on an AVANCE 400 solid-state NMR spectrometer equipped with a CP-MAS probe (Bruker). Samples were spun in air at ~7 kHz. The polyimide-coated silica particles (PISi) were packed into a 50 × 4.6 mm i.d. stainless steel HPLC column by using a slurry packer (Alltech, model 1666). The slurry was made by mixing 1 g of the PISi with 20 mL of 2-propanol and degassed by sonication for 5 min. The HPLC system consisted of a Spectra SERIES P100 pump, a Spectra SERIES UV100 detector operated at 235 nm, and a Rheodyne 7125 injector with a 5 μL loop. Acetonitrile was used as a mobile phase and a void maker. The flow rate was 0.6 mL/min at room temperature.

Synthesis of 3,5-Diaminophenylcarbamic Acid Estronyl Ester (1). This compound was synthesized according to our previous report.[6]

Synthesis of 3,5-Diaminobenzoyl Azide (2). This compound was synthesized according to the literature.[10]

Surface Modification of Silica Particle. Silica particles (d = 10 μm, 0.5 g) were suspended in toluene (10 mL) under stirring. 3-Aminopropyltriethoxysilane (4 mL) was added to the above suspension. After refluxing for 12 h, the silica particles were isolated by filtration, washed with toluene and chloroform, and dried in vacuo for 12 h at room temperature. Incorporation of propylamine was investigated by solid-state ^{29}Si CP- and DP-MAS NMR spectroscopy. Remaining silanol groups were reacted with trimethylchlorosilane. The silica particles (0.5 g) were dispersed in 10 mL of toluene. Trimethylchlorosilane (0.04 mL) was added, and the mixture was refluxed for 12 h. The silica particles were isolated by filtration, washed with toluene and chloroform, and dried in vacuo at 50 °C for 24 h. All procedures were performed under a nitrogen atmosphere.

Preparation of Polyimide-Coated Silica Particle (PISi). To a solution of PMDA (0.5 g, 2.29 mmol) in DMAc (2 mL), a solution of 3,5-diaminophenylcarbamic acid estronyl ester (**1**) (0.046 g, 0.11 mmol) in DMAc (1 mL) was added under nitrogen. After stirring at 0 °C for 0.5 h, a solution of 4,4′-oxydianiline (0.436 g, 2.17 mmol) in DMAc (2 mL) was added. The solution was stirred at 0 °C for 0.5 h and then at room temperature for 12 h. Surface-modified silica particles (1 g) were dispersed in the poly(amic acid) solution in DMAc under nitrogen. The mixture was stirred at room temperature for 12 h. Silica particles were isolated by centrifugation and dried in vacuo at room temperature for 24 h. Poly(amic acid) coated on a silica particle was thermally imidized by heating for 0.5 h each at 80, 130, 170, 220, and 270 °C.

Preparation of Control Polyimide-Coated Silica Particle (CPISi). CPISi was fabricated in the same manner as the estrone containing PISi. For the CPISi, 3,5-diaminobenzoyl azide was used as a diamine in place of 3,5-diaminophenylcarbamic acid estronyl ester.

Preparation of Imprinted Polyimide-Coated Silica Particle, IPISi-A. PISi (1.00 g) was added to a solution of 1,4-dioxane (40 mL) and water (5 mL). The mixture was refluxed for 24 h. The process of extraction was monitored by UV/vis spectroscopy. The extraction continued until the absorbance intensity at 282 nm for the dissociated estrone in the solution reached a constant. The particles were isolated by filtration, washed with 1,4-dioxane and THF, and dried in vacuo at room temperature for a week.

Preparation of Imprinted Polyimide-Coated Silica Particle, IPISi-B. IPISi-B was prepared by following the procedure for IPISi-A. PISi (1.00 g) was added to a solution of aniline (4 mL) in 1,4-dioxane (40 mL). The mixture was refluxed for 24 h. The particles were isolated by filtration, washed with 1,4-dioxane and THF, and dried in vacuo at room temperature for a week.

Results and Discussion

Fabrication of PISi. The poly(amic acid) having the template (estrone) molecules and the control polymer were prepared according to Scheme 1. Diamine monomer **1** was prepared according to our previous report.[6] 3,5-Diaminobenzoyl azide (**2**) was synthesized according to the literature.[10] Polymerization was carried out in *N*,*N*-dimethylacetamide (DMAc) at room temperature using a stoichiometric amount of the diamine [1:19 mole ratio of **1** (or **2**) to 4,4′-oxydianiline] and pyromellitic dianhydride.

The poly(amic acid)-coated silica particles were prepared by dipping silica particles into a solution of poly(amic acid) **3** in DMAc (Figure 1). The silica particle surface was previously functionalized with amino groups by the reaction with 3-aminopropyltriethoxysilane. Incorporation of propylamine was confirmed by solid-state ^{29}Si CPMAS and DPMAS NMR spectroscopy (Figure 2). The peaks for silicone atoms bonded to an aminopropyl group appeared at −59 (T^2) and −67 ppm (T^3), and their relative concentrations were calculated to be 7.7 and 8.4%, respectively, from the solid-state ^{29}Si DPMAS NMR spectrum (Table 1).[11] It is very likely that the poly(amic acid) chains constituting the most inner layer

Scheme 1

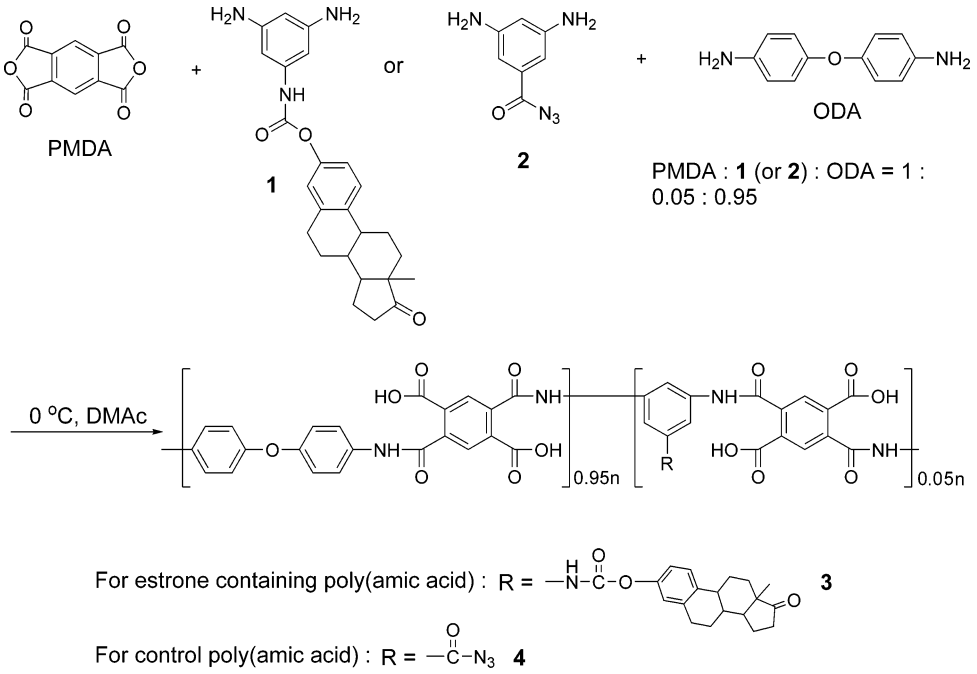

Table 1. ^{29}Si Chemical Shifts (δ_{Si}, in ppm) and Relative Concentrations of T^n and Q^n Groups (in %) of the Amino Group Functionalized Silica Particle Derived from the Solid-State ^{29}Si DPMAS NMR Spectrum

	T^2	T^3	Q^2	Q^3	Q^4
δ_{Si}	−59	−67	−91	−102	−111
%	7.7	8.4	0.1	9.1	74.7

were deposited on the silica particle through electrostatic interaction between carboxylic groups of the polymer chain and the amino groups on the silica particle surface, and outer layers were packed by strong $\pi-\pi$ interaction between the polymer chains. The poly(amic acid) coated on the silica particle was thermally imidized to yield PISi. In the thermal reaction, the intramolecular cyclization was expected to occur predominantly compared to the intermolecular amidation reaction between the carboxylic groups at the poly(amic acid) chain and the amino groups on the silica surface. In fact, no significant peaks corresponding to the amido groups were observed in the IR and the solid-state ^{13}C NMR spectra.

The core−shell structure of PISi was confirmed by FE-SEM and TEM (Figure 3). The average polyimide thickness was about 100 nm. CPISi, as a control particle with the same size and shape as those of IPISi, was also fabricated in the similar manner by using poly(amic acid) **4**, which did not contain estrone.

Generation of Recognition Sites. The estrone-containing PISi particles were refluxed in 1,4-dioxane in the presence of a nucleophile such as water or aniline. In the process, the isocyanato groups, which were

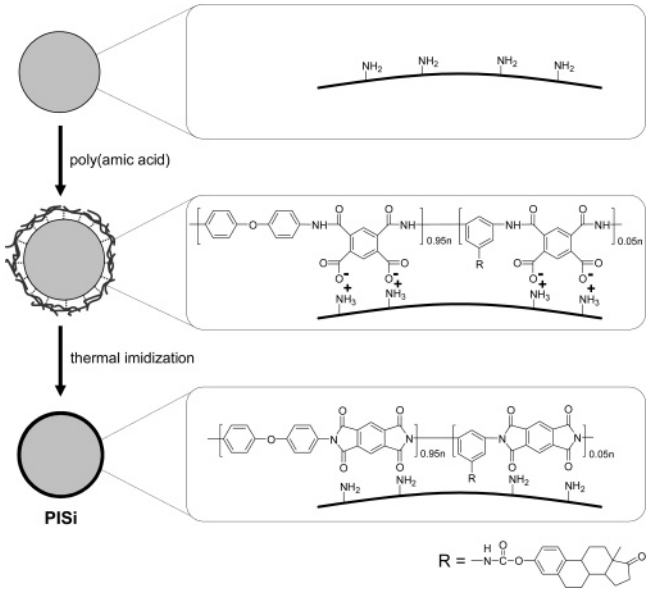

Figure 1. Schematic illustration for the fabrication of estrone containing PISi.

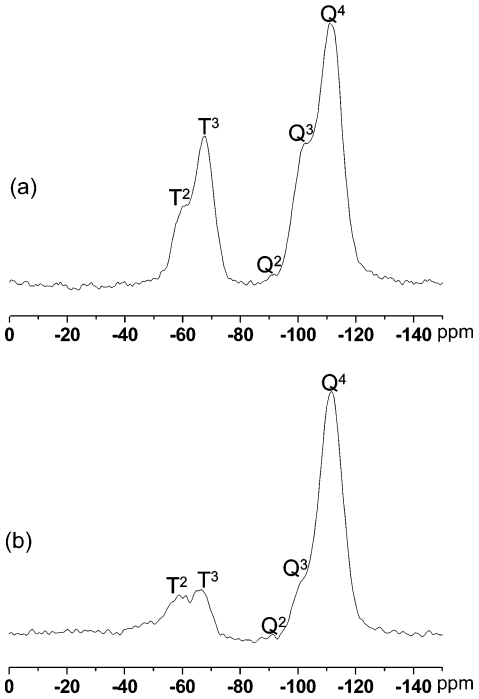

Figure 2. (a) ^{1}H → ^{29}Si CPMAS and (b) ^{29}Si DPMAS spectra of the amino group functionalized silica particle.

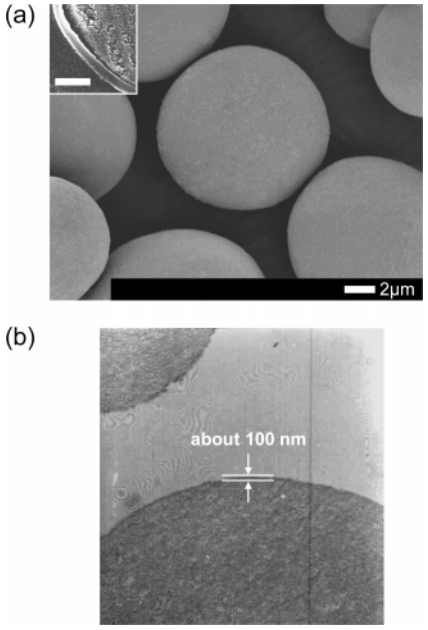

Figure 3. (a) FE-SEM images of PISi particles. The inset shows the FE-SEM image of the IPISi particle cross section (scale bar = 200 nm). (b) TEM image of PISi particle cross section.

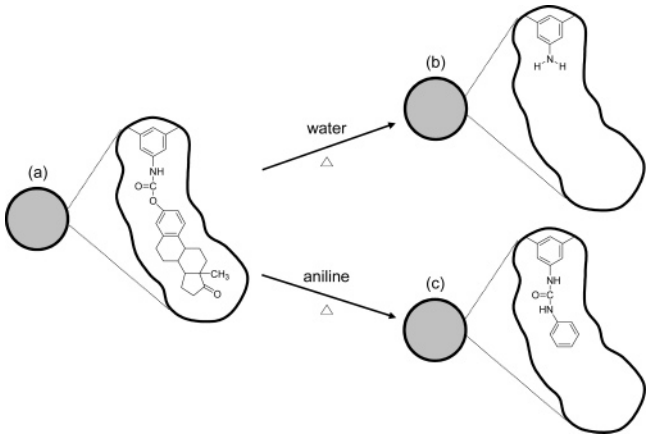

Figure 4. Schematic illustration for the removal of the estrone and generation of recognition sites: (a) PISi, (b) IPISi-A, and (c) IPISi-B.

generated by dissociation of thermally reversible urethane bonds, were converted to amino or phenyl urea groups by the reaction with water or aniline, respectively (Figure 4). The extraction process was monitored by UV/vis spectroscopy and continued until the absorption intensity at 282 nm, corresponding to dissociated estrone in the solution, became constant. The removal of estrone and the resultant generation of the recognition site were confirmed by solid-state ^{13}C NMR spectroscopy (Figure 5). The peaks corresponding to the carbonyl carbons of a urethane group and a phenyl moiety of estrone appeared at 157 and 127 ppm, respectively. These peaks disappeared after extraction of the template molecules. In the spectrum of the IPISi-B obtained by the reaction with aniline, a new peak for the urea carbonyl group showed up at 148 ppm, and the intensity of the phenyl carbon peaks increased. These results indicate that most template molecules were removed from the matrix, and recognition sites were generated inside the cavities successfully.

Evaluation of Recognition Abilities. Imprinted PISi particles were slurry packed in a 50 × 4.6 mm i.d. stainless steel chromatography column to evaluate their molecular recognition properties in HPLC mode. The retention factor (k') was calculated using the equation $k' = (t_R - t_0)/t_0$, where t_R is the retention time of sample and t_0 is the time taken to elute a void maker, i.e., acetonitrile. We define the imprinting factor as $I = k'_{imp}/k'_{con}$, where k'_{imp} and k'_{con} are the retention factors of the same compound on the IPISi and CPISi, respectively. The selectivity factor (S) was calculated from the equation $S = I_x/I_y$, where I_x and I_y are the imprinting factors of the template molecule and the competitor, respectively. The IPISi-A showed higher retention and imprinting factors for estrone than for any other structural analogues. Also, all selectivity factors for the analogues were larger than 1, suggesting that the IPISi-A had higher specific recognition ability for the template than for the analogues. Retention factors, imprinting factors, and selectivity factors of the template and its analogues are summarized in Table 2.

The elution profiles of estrone and its structural analogues (Figure 6), taken under same chromatographic conditions with IPISi-A, CPISi, and IPISi-B as stationary phases, are shown in Figure 7. On the IPISi-A, estrone was eluted with a retention factor of 1.40, while estrone was poorly retained on the CPISi with a retention factor of 0.76, indicating that the IPISi-A had a much higher binding ability than the CPISi. The peak

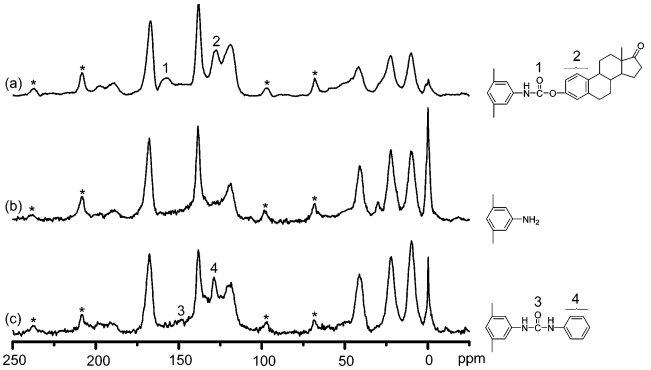

Figure 5. Solid-state ^{13}C NMR spectra of (a) PISi before removal of the template molecules, (b) IPISi-A, and (c) IPISi-B. The asterisk (*) denotes a spinning sideband.

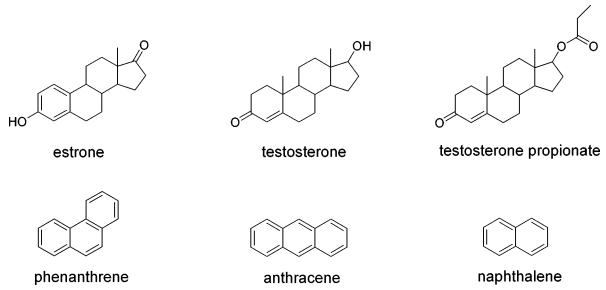

Figure 6. Template molecule (estrone) and its analogues.

Table 2. Results of HPLC Analysis for the Template and Its Structural Analogues on the IPISi-A, IPISi-B, and CPISi; Retention Factor (k'), [Imprinting Factor (I)], and {Selectivity Factor (S)}

	IPISi-A	CPISi	IPISi-B
estrone	1.40 [1.84] {1}	0.76	0.79
testosterone	0.73 [1.07] {1.72}	0.68	0.40
testosterone propionate	0.26 [0.87] {2.11}	0.30	0.19
phenanthrene	0.74 [0.72] {2.56}	1.03	1.06
anthracene	0.67 [0.80] {2.30}	0.84	0.87
naphthalene	0.14 [0.67] {2.75}	0.21	0.22

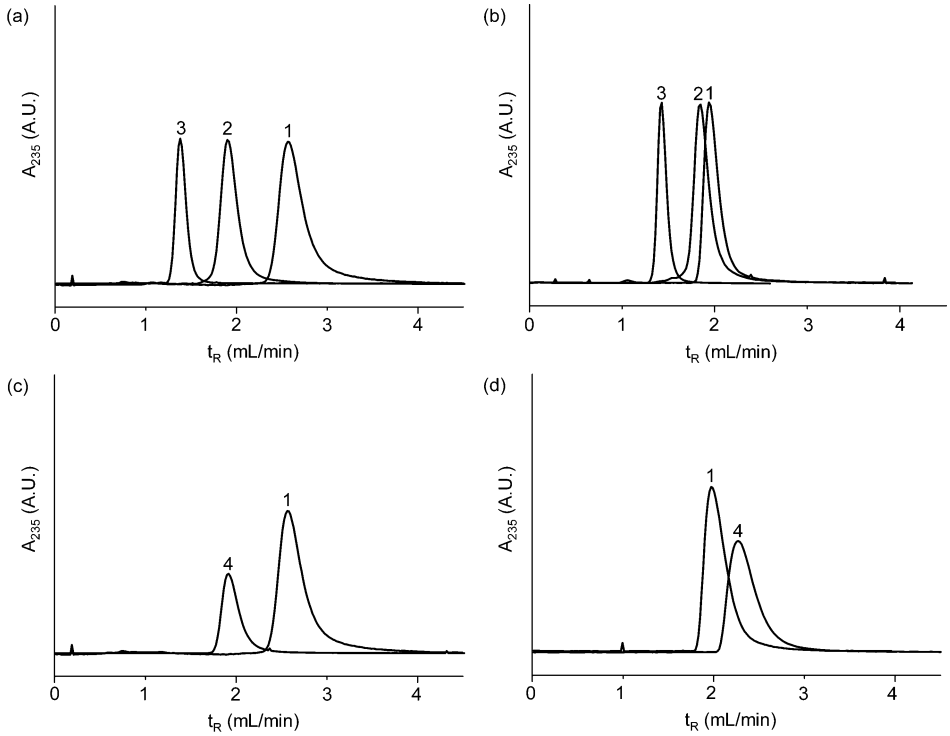

Figure 7. Chromatograms of estrone (1), testosterone (2), testosterone propionate (3), and phenanthrene (4) obtained by using (a) IPISi-A, (b) CPISi, (c) IPISi-A, and (d) IPISi-B as a stationary phase.

for estrone was separated from that of testosterone with the IPISI-A (Figure 7a), but those two peaks overlapped with the CPISi (Figure 7b). To be interesting, when phenylurea groups were introduced into the cavity, the IPISi-B showed the highest affinity for phenanthrene, which is a tricyclic aromatic compound without a hydrogen-bonding site. We can see the elution sequence inversion of the estrone and phenanthrene when stationary phase is changed from IPISi-A to IPISi-B (Figure 7c,d). Likewise, the retention factors with IPISi-B of anthracene and naphthalene are larger while those of estrone, testosterone, and testosterone propionate are smaller than the retention factors with IPISi-A. This trend is likely due to the $\pi-\pi$ interaction between the phenyl group inside the cavity and the aromatic compounds. These results show that the specific recognition ability of the IPISi for the template molecule and its structural analogues could be tailored by controlling the nature of the functional group inside a cavity.

Conclusions

We fabricated core−shell type imprinted spherical particles by using a conventional polymer solution coating method. A poly(amic acid), a precursor of a polyimide, was soluble in polar organic solvents and used for the coating of a silica sphere. The poly(amic acid) layer was converted to the polyimide layer, which has a rigid structure suitable for molecular imprinting. Compared to the grafting method for the preparation of a core−shell type particle, this approach has advantages such that various spherical inorganic particles can be used as core materials and the thickness of the shell can be controlled easily. We utilized thermally reversible urethane bond in the template−monomer complexation, which allowed us to readily introduce various functional groups into the cavities during the removal of the template. The imprinted polyimide-coated silica particles were used as a stationary phase in HPLC mode. The analysis results showed the possibility that the nature of the functional group inside a cavity could be controlled with the approach described in this paper and thereby the affinity of the imprinted particle to a target molecule tailored.

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