***In-situ* Synthesis and Normal Chromatographic Properties of Nonporous SiO₂/ZrO₂ Core-shell Composite Microspheres**

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Sub-2 μm core-shell SiO₂/ZrO₂ composite microspheres with nonporous SiO₂ as the cores and ZrO₂ nanoparticles as the shells were synthesized by sol-gel method in the current work. The fabrication consisted of (1) preparing monodisperse silica spheres; (2) coating the spheres with zirconia nanoparticles *in-situ*; and (3) calcining the composite microspheres at 823 K. Then uniform, regular, and ordered arrays of nanometer-scale zirconia crystalline particles were formed and dispersed on the surfaces of nonporous silica microspheres. A potential deposition mechanism in the synthesis process was proposed according to isoelectric point theory and to observe microstructural characteristics. The result showed that this stationary phase also has an advantage in the separation of basic compounds in the normal phase.

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Introduction

Small nonporous silica packing materials of 1.5 μm used in HPLC were introduced in 1996.^{1,2} Such materials are generally proposed for separating macromolecular compounds that slowly diffuse in the mobile phase.³⁻⁵ However, silica does not withstand a high or low pH in the mobile phase or at high temperature.⁶⁻⁸ Alternative inorganic materials for chromatography is a research area of great interest. Modified zirconia stationary phases have been widely used in many applications,⁹ especially in bio-analysis for amino acids, peptides, proteins,¹⁰⁻¹³ and monoclonal antibodies.¹⁴ However it is difficult to obtain uniform and monodisperse zirconia microspheres of sub-2 μm diameter, because they easily aggregate during the preparation process.¹⁵ Since sub-2 μm monodisperse silica microspheres can be simply synthesized according to the modifying Stöber method,^{16,17} coating ZrO₂ nanoparticles on the surface of SiO₂ cores would be a good way to prepare monodisperse core-shell SiO₂/ZrO₂ composite microspheres.^{18,19}

To the best of our knowledge, no report has been devoted to the preparation of monodisperse SiO₂ microspheres coated with ZrO₂ crystalline nanoparticles in one step. The focus of this paper is the fabrication of SiO₂/ZrO₂ composite microspheres, which consist of micron-size nonporous silica spheres as cores and nanometer-sized zirconia crystalline particles as shells by a sol-gel method, followed by calcination to form crystals of ZrO₂ crystalline particles. The as-prepared materials were characterized by scanning electron microscopy (SEM), powder X-ray diffraction (XRD), X-ray photoelectron scanning microscopy (XPS), and Fourier transform infrared (FT-IR). The potential deposition mechanism in the synthesis process was

explained according to isoelectric point theory and observed microstructural characteristics. The resulting core-shell composite was used as a chromatographic support to separate neutral and basic compounds in the normal phase.

Experimental

Reagents and chemicals

Zirconyl chloride octahydrate (ZrOCl₂·8H₂O), tetraethyl orthosilicate (TEOS), sodium phosphate, hexane, isopropanol, ammonia and absolute ethanol, all of analytical grade, were purchased from Tianjing No. 2 Chemical Reagent Factory (Tianjing, China). Hydrogen peroxide (30%), analytical grade, was purchased from Beijing Chemical Reagent Co. (Beijing, China).

Preparation of monodisperse nonporous silica cores

Monodisperse SiO₂ microspheres were synthesized by a modified Stöber method.¹⁷ In a 250-ml baker, 21.2 mL of absolute alcohol and 82.0 mL of ammonia were mixed. Hydrolysis was accomplished by the addition of 29.4 mL of TEOS (within 1 min) with magnetic stirring (400 rpm) in an ice-water bath. After 5 min, turbidity started to appear, and the solution turned opaque white due to dense silica sol formation (Stöber particles). The sol was kept under stirring for 1.5 h. The resulting cake was filtered off using a G4 funnel with a fritted disc. The collected material was left in the oven for drying overnight at 373 K for 24 h. The average diameter of the silica particles was about 1.5 μm , as shown in Fig. 1.

Preparation of SiO₂/ZrO₂ composite microspheres

Zirconia sol: 0.2 mol (6.4464 g) zirconyl chloride octahydrate was dissolved in 200 mL of absolute ethanol by ultrasonication at room temperature to give a 0.1 mol/L zirconia sol precursor;

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Fig. 1 SEM images of SiO₂ microspheres. Fig. 2 Illustration of the synthesis process of SiO₂/ZrO₂ composite microspheres.

30% hydrogen peroxide (6 mL) was added to this solution dropwise under vigorous stirring. At first, the solution turned to opaque milky white; after 1.5 h, transparent ZrO₂ sol was finally obtained. The sol was finally transferred to a closed glass container and aged at 343 K for another 3 h.

An illustration of the synthesis process of core-shell SiO₂/ZrO₂ composite microspheres is shown in Fig. 2. Previously synthesized pure silica (3.0 g) was dispersed into a zirconia sol solution (0.1 mol/L, 25 mL) at room temperature by ultrasonication. Dilute aqueous ammonia (0.25 wt%, 25 mL) was added to the above suspension dropwise for 1 h under magnetic stirring. Then, the thus-obtained coagulated cake was collected and dried at 373 K for 24 h. Test samples were obtained by calcination of the dried powder in a muffle furnace at 823 K for 300 min at a heating rate of 10 K/min.

Apparatus

The resulting SiO₂/ZrO₂ microspheres were packed into a 150 × 4.6 mm i.d. stainless-steel column at 45 MPa pressure. Chromatographic tests were performed on an Agilent 1200 Series modular HPLC system with a binary pump, a 20-μL sample loop and a diode array detector (DAD).

The morphology of the samples was characterized by SEM (JSM-5600 (Jeol, Tokyo, Japan)) with an accelerating voltage of 20 kV. X-ray diffraction (XRD) was carried out using an X-ray diffractometer (Rigaku, Japan) D/Max-2400 system to investigate the internal array of zirconia. The patterns were run with nickel-filtered copper radiation ($\lambda = 1.541841 \text{ \AA}$) at 40 kV and 60 mA; the diffraction angle 2θ was scanned at a rate of 2° min^{-1} . Fourier-transform infrared (FT-IR) spectroscopy for the investigated samples was carried out using a Shimadzu FTIR-8101 Fourier-transform infrared spectrophotometer (Japan), in the range 4000 – 500 cm^{-1} ; the resolution was 2 cm^{-1} . The amount of zirconium was determined by X-ray photoelectron scanning microscopy (XPS) (VG ESCALAB 210), with Mg $K\alpha$ radiation as the exciting source.

Fig. 3 XPS pattern of SiO₂/ZrO₂ composite microspheres prepared at ZrO₂ sol concentration of 0.10 mol/L. Fig. 4 XRD patterns of SiO₂/ZrO₂ composite microspheres (a) and SiO₂ cores (b).

Results and Discussion

Characterizations of SiO₂/ZrO₂ particles

XPS was carried out to ascertain whether zirconia particles were present on the surface of the silica support. The XPS pattern of the SiO₂/ZrO₂ composite particles is given in Fig. 3. The contents of these elements on the surface of the prepared SiO₂/ZrO₂ composite microspheres were O, 43.653%; C, 31.601%; Si, 7.814%; Zr, 16.931%. From the contents of Zr

Fig. 5 SEM images of SiO₂/ZrO₂ composite microspheres.Table 1 Specific surface areas of ZrO₂/SiO₂ composite microspheres

Sample	$S_{\text{BET}}/\text{m}^2 \text{ g}^{-1}$	$S_{\text{BJH}}/\text{m}^2 \text{ g}^{-1}$	$V_{\text{BJH}}/\text{cm}^3 \text{ g}^{-1}$	Pore diameter/nm	
				Average BJH	
Pure SiO ₂	17.1228	17.3002	0.020422	3.6	4.7
ZrO ₂ /SiO ₂ (uncalcined)	22.6370	26.3233	0.021373	3.0	3.2
ZrO ₂ /SiO ₂ (calcined)	42.2722	47.1595	0.039833	2.9	3.4

S_{BET} is surface area from BET absorption mode; S_{BJH} is surface area from BJH absorption mode; V_{BJH} is pore volume from BJH absorption mode.

and Si, those of zirconia and silica were calculated to be 46.8 and 21.6%, respectively. The XPS pattern of the SiO₂/ZrO₂ particles obtained at a ZrO₂ sol concentration of 0.1 mol/L indicates a nearly complete shielding of SiO₂ spheres with ZrO₂ nanoparticle layers.

The chromatographic behavior is also influenced by the crystalline structure of ZrO₂ particles. The XRD pattern of ZrO₂/SiO₂ given in Fig. 4, displays a pure tetragonal crystalline ZrO₂ nanometer particle; this type of crystal will be helpful to enhance the specific surface area of the support.²⁰ Further, XRD results indicated that no crystalline phases were formed when pure silica was calcined up to 823 K.

An SEM micrograph of a particle surface of prepared SiO₂/ZrO₂ is shown in Fig. 5; a uniform surface coating of ZrO₂ particles can be visualized. Almost no aggregation can be observed in Fig. 5. The average particles sizes of the SiO₂/ZrO₂ composite microspheres were approximately 1.6 μm , lightly bigger than the SiO₂ microspheres uncoated before. Uniform, regular, and ordered arrays of nanometer-scale zirconia crystalline particles were formed during heat treatment, and were dispersed on the surface of nonporous silica microspheres.

Table 1 gives the isotherm of ZrO₂/SiO₂ composite microspheres calcined at 823 K, which were first predried at 353 K for 24 h. It can be clearly seen that the dispersion of zirconia particles increased the surface area of the silica cores. Apparently, the specific surface areas and the pore volumes are relatively small compared to the normal supports used in HPLC applications. These materials, when using as a chromatogram support, could minimize the pore diffusion and the mass-transfer resistance effects. Therefore, nonporous ZrO₂/SiO₂ columns can work in a much wider flow-rate range. Nonporous

Fig. 6 Topical FT-IR of SiO₂/ZrO₂ composite microspheres and SiO₂ microspheres (inset figure: FT-IR spectrum of SiO₂/ZrO₂ composite microspheres).

ZrO₂/SiO₂ supports are also more resistant, and remain stable at high temperatures and pressures. However, due to the low surface areas, nonporous supports exhibit lower retention times and loading capacity than porous columns. Such materials are proposed for separating macromolecular compounds that generally diffuse slowly in the mobile phase.

Potential deposition mechanisms

The deposition mechanisms may be explained according to the electrostatic attractive force between SiO₂ and ZrO₂. The pH (6.0) of the mixed suspension is higher than the isoelectric point of SiO₂ (reported values of the isoelectric point of SiO₂ range from 1.8²¹ to 2.7).²² Therefore, SiO₂ particles under these conditions will have a negative surface charge density,²³ and will be expected to experience a significant electrostatic attraction to ZrO₂, which have a positive surface charge density in the system (reported values of the isoelectric point of ZrO₂ colloid particles were 6.4).²⁴ This means that, at pH 6.0, ZrO₂ colloid particles could be deposited on the surface of SiO₂ microspheres due to the electrostatic attraction between ZrO₂ and SiO₂; and these surface charges could be removed. Besides, a surface condensation reaction between the silanol group of SiO₂ and the zirconol group of ZrO₂ might occur. The association between the surface hydroxyls of the silica cores and zirconia shell by hydrogen binding could also result in enhanced deposition.

Whether chemical bonds existed in the interface of SiO₂ and ZrO₂ needs more proof. Figure 6 is the FT-IR spectra of

Fig. 7 Chromatogram of the separation of neutral compounds on 150×4.6 mm i.d. $\text{SiO}_2/\text{ZrO}_2$. Mobile phase, *n*-hexane:isopropanol = 85:15 (v/v); flow rate, 0.5 mL/min; temperature, 25°C; detection, UV at 254 nm. 1, Benzene; 2, toluene; 3, xylene; 4, biphenyl; 5, naphthalene.

$\text{SiO}_2/\text{ZrO}_2$ composite microspheres and pure silica (both calcined). The FT-IR spectra of the composite materials show bands at 3464–3365 and 1635–1628 cm^{-1} due to –OH stretching and bending vibrations of water in the samples. Water is easily adsorbed onto the surfaces of the composite materials, because the silanols and zirconols on surface easily form hydrogen bonds with atmospheric water. A broad peak at 960 cm^{-1} indicates the presence of the Si–O–Zr bond in the composite materials.²⁵ In addition to an increase in this band, reductions in the intensity of the structural Si–O bands at 800 and 454–439 cm^{-1} also reflect an increase of the Zr in the SiO_2 network as a result of forming heterolinkages of Zr–O–Si. Due to possible hydrolysis with ambient water, the Si–OH groups are expected to be located on the surface, while bulk silicon atoms are present in the forms of both Si–O–Si linkages. When Zr–OH of zirconia reacts with Si–OH, Zr–O–Si linkages are formed. Not all of the Zr atoms were chemically bonded to Si atoms; a majority of ZrO_2 was deposited on the surfaces of SiO_2 owing to the electrostatic attraction and hydrogen bonding. Thus, the linkages between silica and zirconia are not only physical deposition (electrostatic attraction), but also form the chemical linkages of Zr–O–Si. It should be emphasized that FT-IR does not give a quantitative result. It does show, however, that heterolinkages of the Zr–O–Si bond have taken place.

Chromatographic evaluation

The chromatographic behavior was studied under the normal-phase conditions. Some neutral compounds and basic isomers were separated. Figure 7 is a chromatogram of the separation of aromatic compounds on $\text{SiO}_2/\text{ZrO}_2$ (150×4.6 mm i.d.). The retention times are benzene < toluene < xylene < biphenyl < naphthalene. The efficiency is ~5000/m. Figure 8 is a chromatogram of the separation of nitroaniline using hexane-isopropanol (85:15, v/v) as the mobile phase. Both neutral and basic compounds could be well separated on $\text{SiO}_2/\text{ZrO}_2$ with short retention times and symmetrical peaks. This indicated that the $\text{SiO}_2/\text{ZrO}_2$ stationary phase could be used directly to separate some neutral and basic compounds.

A stability test was carried out by passing a fresh sodium phosphate solution (10 mmol L^{-1} , pH 10) through the column at 0.7 mL min^{-1} to a total volume of 3000 mL. Chromatograms of a mixture of solutes (benzene, toluene, xylene, biphenyl, naphthalene) were obtained periodically with every 500 mL

Fig. 8 Chromatogram of the separation of basic compounds on 150×4.6 mm i.d. $\text{SiO}_2/\text{ZrO}_2$. Mobile phase: *n*-hexane:isopropanol = 85:15 (v/v); flow rate, 1.0 mL/min; temperature, 25°C; detection, UV at 254 nm. 1, *o*-Nitroaniline; 2, *m*-nitroaniline; 3, *p*-nitroaniline.

mobile-phase flushing with methanol–water (70:30, v/v) after being conditioned for 30 min. The results showed that the $\text{SiO}_2/\text{ZrO}_2$ stationary phase is stable under this condition without any noticeable effect on the retention factor or peak width of the test solutes.

Conclusions

Core-shell materials take advantage of highly dense silica particles (Stöber particles) of great mechanical stability, and overcome their disadvantage of inert surface *via* dispersion of the active zirconia phase. Important features of this method are as follows: (1) the morphology of the coating layer can be readily controlled by varying the concentration of ZrO_2 sol; (2) the size and shapes of the spheres produced are mainly determined by the sizes of the silica core employed; (3) the method is generally applicable to a wide variety of charged inorganic particles, thereby making possible the production of various inorganic and composite supports. Using this new stationary phase, common basic compounds have been successfully separated with good peak shape and high column efficiency. The prepared composite microspheres could be potentially used in the separation of proteins, peptides and bio-macromolecules, as well as catalysts, supporting materials and adsorptive coatings.

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