Titanium-Coated Silica Spheres Prepared by Self-Assembly Technique for Use as HPLC Packing

Jin Ge[1,2], Liang Zhao[1], Li-Ren Chen[1], and **Yan-Ping Shi[1,*]**

[1]Key Laboratory for Natural Medicine of Gansu Province, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou 730000, China; [2]Graduate School of Chinese Academy of Sciences, Beijing 100039, China

Abstract

Titania-coated silica spheres were prepared by a layer-by-layer self-assembly technique for use as a high-performance liquid chromatography packing. This packing has a high surface area of 202.1 m^2/g, a large pore volume of 0.36 cm^3/g, and a pore diameter of 7.0 nm. Furthermore, the packing particles exhibit narrow pore size distribution and good pore structure. The chromatographic behavior of the packing was studied under both normal and reversed-phase conditions. Low column pressures were observed. Aromatic isomeric compounds were well separated on the TiO_2/SiO_2 column under normal phase conditions; pyridine and aniline derivatives were separated on the octadecyl–bonded TiO_2/SiO_2 (ODT) column, respectively, under reversed-phase conditions, and highly symmetrical peaks were obtained for 15 basic compounds. The chemical stability of the stationary phase was tested with sodium phosphate solution (10 mmol/L, pH = 10) at 25°C and potassium phosphate solution (50 mmol/L, pH = 10) at 50°C.

Introduction

Since being introduced by Kawahara et al. (1) and Trudinger et al. (2), titanium dioxide (titania) has attracted increasing interest as an alternate support to silica for column packing in HPLC, due to its desirable mechanical and physical properties, which are as good as that of silica, and a chemical stability superior to that of silica (1–3), which enables the performance of separations at extreme pH values. Different methods, such as polymerization-induced colloid aggregation (PICA) (4) and sol-gel method (5) have been used to prepare titania particles, and the study of it as a sorbent in normal-phase, reversed-phase, ion-exchange, and affinity chromatography has been reported (4–15). Phosphorous compounds, including adenosine phosphates and sugar phosphates, could be selectively trapped by the titania column and typically eluted with a potassium phosphate buffer (16,17) phosphopeptides could also be selectively isolated from peptide mixtures containing nonphosphorylated peptides by the titania column (18,19). Furthermore, Collins and collaborators prepared titanized silica, modified it with various methods, and studied its properties in detail (20–24). Several overviews of titania-based stationary phase have been published (25–27).

Although the applications of titania in HPLC have been widely investigated, it has never became a competitive packing because of the lower specific surface area, pore volume, total porosity, and particle size of it compared with silica (28–30). The drawbacks of titania result in many disadvantages. For example, the carbon loading of titania (25) is about one-third of that of silica in the derivatizations of titania with octadecyltriethoxysilane.

The self-assembly technique has already been widely used in catalysis, sensing, film separation application. At first, it was used to construct multilayer films on flat templates. In recent years, fabrication of core-shell composites has attracted increasing interest. Several research groups have employed the self-assembly technique to fabricate particles consisting of a solid core covered with either a single layer of nanoparticles or a composite multilayer shell to produce nanostructured materials with functional properties (31–34). These "core–shell" particles often exhibit properties that are substantially different from these of the templated core (for example, increased stability, different surface chemical composition, higher surface area, and different magnetic and optical properties). Core–shell particles have been used for chromatographic supports by J.J. Kirkland since 1969 (35) and have been used for fast HPLC (36). In our laboratory, core–shell particles were prepared by the self-assembly technique to obtain a novel support material which can show excellent chemical stability, as well as hold some more favorable surface characteristics for HPLC applications. Another advantage of using self-assembly technique is that the size of the particles prepared by self-assembly technique is determined largely by the size of the core. The variable particle size can be achieved by changing the core to different size. In our experiment, we used silica as the core because the particle size of silica can be controlled easier, and silica is almost an ideal chromatographic support for HPLC.

In the previous study, we had successfully prepared ZrO_2 coated SiO_2 (37), which consisted of nanozirconia particles as the coating and micrometer-sized silica as the core, and chloroethylnaproxenate enantiomers had been well separated on the chiral polysaccharide polymer-coated ZrO_2/SiO_2 column. Titania-coated silica had also been prepared with the same method, and it shows high specific surface area and large pore volume than the conventional titania packing. In this paper, the suitability and advantages of the titania-coated silica as HPLC

*Author to whom correspondence should be addressed: email shiyp@lzb.ac.cn.

packing were investigated, and the core-shell structure of it was clearly observed by TEM for the first time. Then octadecyl–bonded TiO_2/SiO_2 (ODT) was prepared, and its stability was measured with fast and extreme methods that are often avoided in HPLC. Some basic compounds were also separated on the ODT column for the first time on this kind of packing prepared by the molecular self-assembly technique for HPLC.

Experimental

Chemicals and materials

Silica spheres were synthesized by the PICA method in our laboratory. The average particle size is 6 μm. The specific surface area, pore volume, and pore diameter are 110 m^2/g, 0.21 cm^3/g, and 13 nm, respectively. Tetrabutyl titanate was purchased from Shanghai Sanyou Reagent Factory (Shanghai, China). Acetic acid was purchased from Jiangsu Sanmu Group Chemical Factory (Jiangsu, China) and sodium dodecyl sulfate (SDS) from Xi'an Chemical Reagent Co. (Xi'an, China). Trichlorooctadecylsilane was purchased from Aldrich (Lancaster, UK). All were chemical-grade. Methanol, toluene, hexane, ethanol, and acetone were purchased from Tianjin No. 2 Chemical Reagent Plant (Tianjin, China) and were analytical-grade.

Instrumentation

The topography of the particles was displayed by JSE-5600LV scanning electron microscopy and JEM1200EX transmission electron microscopy. The existence and growth of titania on the support was proved by means of energy-dispersive X-ray analysis (EDX; Kevex). The crystal structure of titania coating was determined with a Rigaku-D/max-RB X-ray diffraction pattern instrument using Ni-filtered Cu Kα radiation and a rotating anode operating at 50 kV and 60 mA, and the size of the titania crystal on the surface was calculated by the Scherrer formulation. Specific surface area, pore volume, and pore size were measured by nitrogen adsorption and desorption isotherms at 77 K using an ASAP2010 surface analysis instrument (Micromeritics, Atlanta, GA). Elemental analysis was carried out with Vaivo Element analysis instrument.

Titania on micrometer-sized silica particles (TiO_2/SiO_2)

TiO_2/SiO_2 particles were prepared by the alternate adsorption of surfactant SDS and titania sol onto micrometer-scale silica spheres. In the self-assemble process, first SDS was fabricated onto silica surface through the chemical adsorption action between the solid and the liquid, then the electrostatic adsorption between the titania and SDS is the primary driving force (34,37). The preparative process includes three major steps. First, the surface of silica particles was treated before use (i.e., silica spheres were washed with acid, alcohol, and deionized water) followed by vacuum-drying. The dried silica particles were added to a solution containing 0.025–0.05 mol/L SDS for 30 min to form surfactant film, and then washed with water carefully before dried. Second, titania sol was prepared as follows: mixture A of tetrabutyl titanate, acetic acid, and ethanol (1:0.3:3.5, v/v/v) was stirred for several minutes. Then mixture B of ethanol and water (20:1, v/v) was added to mixture A slowly with vigorous

stirring. After tetrabutyl titanate was completely hydrolyzed, titania sol formed. Silica particles filmed with surfactant, SDS/SiO_2 was added into the titania sol and stirred for 3 h to form $TiO_2/SDS/SiO_2$ material, then it was washed with ethanol before dried. The multilayer $TiO_2/SDS/SiO_2$ was prepared by repeating the second and the third steps several times. Third, after being dried at 120°C for 12 h and sintered at 525°C for 5 h to burn off organic constitutes, multilayer $(TiO_2/SDS)/SiO_2$ became multilayer TiO_2 particles/core SiO_2 particles (TiO_2/SiO_2).

Stationary phase octadecyl-bonded TiO_2/SiO_2 (ODT)

ODT stationary phase was synthesized by reaction between TiO_2/SiO_2 and trichlorooctadecylsilane. TiO_2/SiO_2 and trichlorooctadecylsilane were added into toluene, then continuously stirred and refluxed for 36 h. The products were carefully washed with toluene, methanol, and acetone, and then dried in a vacuum oven until constant weight. The resulting ODT were packed into a 150 × 4.6 mm i.d. stainless steel column at 37 MPa pressure.

Chromatographic conditions

The HPLC system consisted of a Waters 515 HPLC pump, 2487 absorbance detector, and a 7725i Rheodyne injection valve with a 10-μL loop (Milford, MA). Chromatograms were recorded with a SePu 3000 workstation. The pH of the mobile phase was adjusted with the corresponding hydroxide using a calibrated pH meter before addition of methanol. All of the mobile phases were degassed in an ultrasonic bath before use.

Results and Discussions

Characterization of TiO_2/SiO_2 particles

The topography of TiO_2/SiO_2 particle is characterized by transmission electron microscopy (TEM) at first. As shown in Figure 1A, there is a complete surface coating on the core particle with a thickness about 300–500 nm determined from Figure 1A. In our previous study, we found that a coating of 300 nm is thick
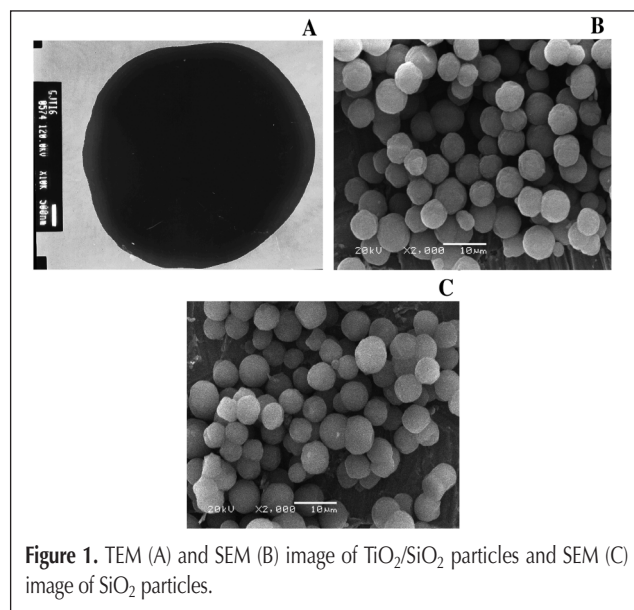

Figure 1. TEM (A) and SEM (B) image of TiO_2/SiO_2 particles and SEM (C) image of SiO_2 particles.

enough to protect the SiO_2 core (37), so six coating times were chosen. Figure 1B is the observation of several of the TiO_2/SiO_2 particles by scanning electron microscopy (SEM), compared with Figure 1C, which is the SEM image of silica particle before coating, in which we can see that the coating processes does not change the particle topography.

EDX (energy-dispersive X-ray analysis) has been carried out to ascertain the growth of titania on silica particles. Figure 2 is the titania percent on the surface of the support with different coating times. It can be seen that with increase in the number of coating steps, titania percent increases too.

XRD pattern of TiO_2/SiO_2 displays an anatase crystalline of the titania coating, and calculated by the Scherrer formulation TiO_2 particles on the surface are nanoparticles with a size of about 12 nm. Considering the coating times, the thickness of the titania coating, and the size of TiO_2 particles, it can be concluded that the titania nanoparticles for each coating should not be a monolayer but a multilayer.

In sum, multilayers of titania nanometer-scale particles are assembled onto micrometer-scale SiO_2 particles with every coating process, and the titania percent on the surface of the support increases with every coating process. After six coating steps, the thickness of the titania coating reaches about 300 nm, and the titania coating continues to maintain the spherical structure of the silica particles.

Surface parameters of TiO_2/SiO_2 particles

Trudiager et al. (2) investigated the change in surface area during the thermal treatment of titania and found that the specific surface area of titania decreased with increase in the calcination temperature. Calcinations above 500°C generally result in a specific surface area of less than 50 m^2/g for titania. In fact, porous titania supports for HPLC were usually prepared by the polymerization-induced colloid aggregation (PICA) method, the sol-gel method and the oil emulsion (OEM) method (Table I), in which a temperature higher than 700°C is needed to remove the large amount of organic materials in the aggregated spheres. So, the titania packing prepared by conventional methods usually has a specific surface area of less than 50 m^2/g. This leads to the low loading capacity of these stationary phases, as well as a decrease in selectivity and resolution for many chromatographic separations. In contrast, TiO_2/SiO_2 core-shell composites synthesized by the self-assembly approach results in a support with a higher specific surface area and pore volume compared with the titania packing prepared by conventional methods (shown in Table I). The specific surface area, total pore volume, and average pore diameter of TiO_2/SiO_2 are 202.1 m^2/g, 0.36 cm^3/g, and 7.0 nm, respectively. The high surface area mainly comes from its shell material, which consists of multilays nanometer-scale titania crystalline particles formed in the self-assembly process. According to the pore size of SiO_2 and TiO_2/SiO_2, it was thought that some smaller titania nanoparticles were entered into the larger silica pores, so the pore size of ultimate packing decreased. Calculated by the Scherrer formulation, TiO_2 particles on the surface are nanoparticles with size of ~ 12 nm. The average pore size of silica is 13 nm. Thus, some smaller titania particles could enter into the larger silica pores and the pore size of ultimate packing decreased. The layer of titania in pores seems to be much

thinner because the pore's diameter decrease slightly and the surface area and the pore volume increased. Speculation on the increase in surface area and pore volume as well as the decrease of pore size, the external layer of titania must be more porous than the silica pore and the external layer of titania must have smaller pores than the silica core.

The isotherm for TiO_2/SiO_2 (Figure 3A) is a type IV adsorption isotherm with a hysteresis loop H1 according to the IUPAC recommendations (37,38), denoting a good mesoporous structure of cylindrical shape with a narrow pore-size distribution (39,40). The pore-size distribution of TiO_2/SiO_2 displayed in Figure 3B centers between 6.0 and 9.0 nm in diameter, with a maximum at 7.0 nm (Figure 3B), this is a relatively narrow range compared with most titania supports.

The apparent density of titania is ~ 2–3 times higher than that of silica. This makes it difficult to classify the particles into a narrow distribution. So, uniform titania supports are not easy to obtain. The particle size and the particle size distribution of TiO_2/SiO_2 supports is determined mainly by the dimensions of the core material employed. The more uniform the silica cores are, the narrower the particle size distribution of the TiO_2/SiO_2 spheres. This is also an advantage of TiO_2/SiO_2 over other titania supports.

Pressure of column

In HPLC, pressure of column is always another important factor. At low pressure we can use a longer chromatographic column as well as a wider polarity range for mobile phase constituents freely. In our experiments, we found that the pressure of self-assembled TiO_2/SiO_2 stationary phase column was low. Using n-hexane as the mobile phase, the column pressure was
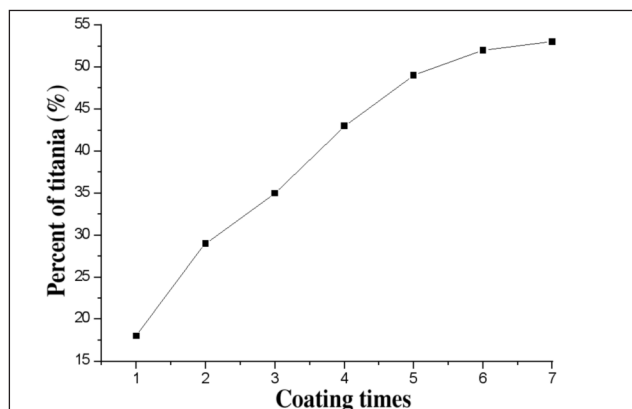
Figure 2. Percent of titania in the coating after each deposition step by EDX.

**Table I. Surface Parameters of Titania Particles Synthesized by Different Methods***

Method	p_d/μm	a_s/m^2/g	v_p/cm^3/g	d_p/nm	C/%	Ref.
LBL	6.4	202.1	0.36	7.0	11.51	TM
PICA	3.5 ± 0.5	36.7	0.30	3.2	2.87	(4,7)
Sol-gel	4.5	34	0.22	2.6	3.3	(11)
OEM	4-7	78	0.23	8.0	2.02–2.36	(2)

* p_d = particle diameter; a_s = surface area; v_p = pore volume; d_p = pore diameter; C = carbon amount; TM = this method.

only 98 psi (0.675 MPa) at 1 mL/min for a 150 mm TiO_2/SiO_2 column and 210 psi (1.45 MPa) for a 250 mm TiO_2/SiO_2 column. When using 2-propanol and *n*-hexane (3/7, v/v) as the mobile phase at 1 mL/min, the column pressure was 253 psi (1.74 MPa) for a 150 mm TiO_2/SiO_2 column. After changing the flow rate to 1.5 mL/min, the column pressure of the column only reached 365 psi (2.52 MPa). Under reversed-phase conditions, the column pressure was 426 psi (2.94 MPa) for a 150 mm ODT column using 100% methanol as the mobile phase at 1 mL/min; it was 900 psi (6.20 MPa) using 60% methanol–water as the mobile phase at 1 mL/min. It is thought that the good permeability of this column packing comes from the porous titania shell and the cylindrical structure of the pore, which results in good pore connectivity, and allows rapid and unhindered equilibration of both solute and mobile-phase molecules.

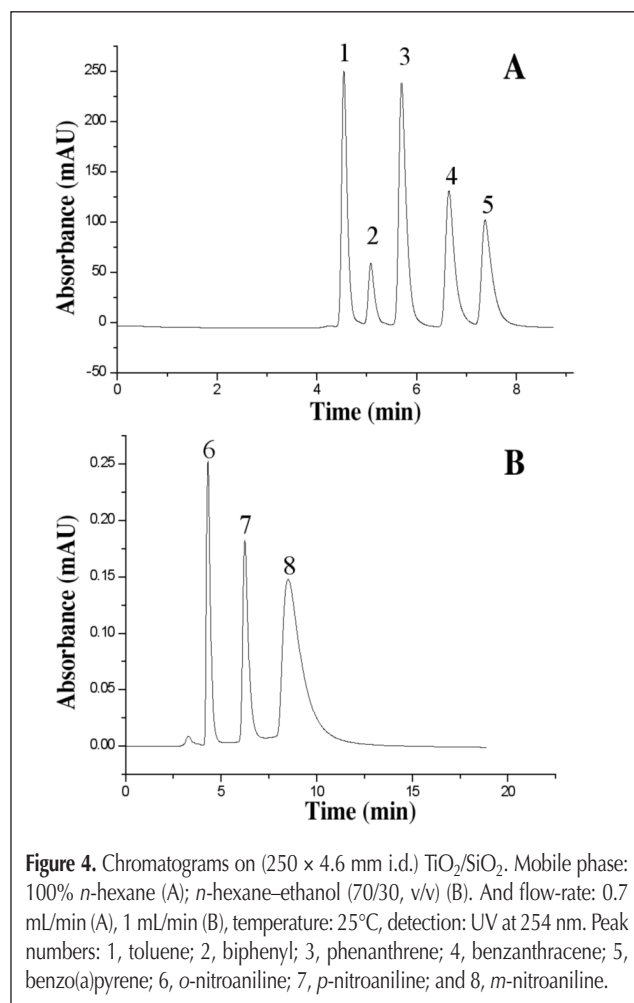### Carbon load of octadecyl-bonded TiO_2/SiO_2.

Elemental analysis shows that the carbon load of octadecyl-bonded TiO_2/SiO_2 is 11.51%. In contrast, several teams also reported the preparation of octadecyl-bonded TiO_2 in which low carbon loads were obtained (show in Table I). We can see that the carbon load of octadecyl-bonded TiO_2 is much lower than that of octadecyl-bonded TiO_2/SiO_2. This may be due to the low surface area of TiO_2 prepared by conventional methods, because the low surface area will result in a low number of hydroxyl groups. When the total hydroxyls on the surface is low, the hydroxyls could available to react with trichlorooctadecylsilane will be few and the carbon load of the column will be low. Thus, the higher carbon load of octadecyl-bonded TiO_2/SiO_2 is a result of the larger surface area of TiO_2/SiO_2.

Figure 3. Adsorption isotherm (A) and pore size distribution (B) of TiO_2/SiO_2.
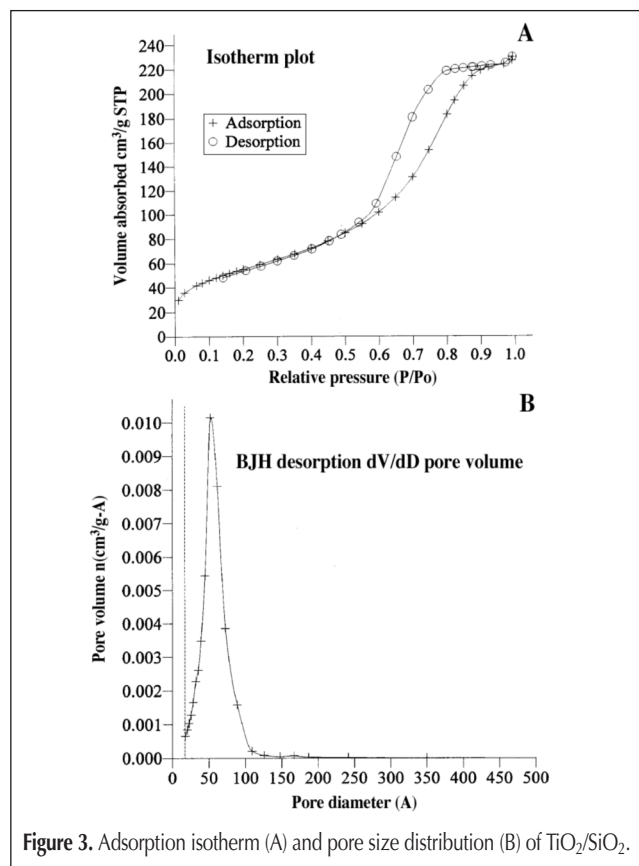
Chromatographic evaluation

The chromatographic behavior was performed under normal phase conditions at first. Some neutral compounds and basic isomers were separated. Figure 4A is a chromatogram of the separation of aromatic compounds on TiO_2/SiO_2 (250 × 4.6 mm i.d.). The retention times are toluene < biphenyl < phenanthrene < benzanthracene < benzo(a)pyrene. The efficiency is ~ 4000/m. Therefore the support can be used to separate aromatic isomeric directly. Figure 4B is a chromatogram of the separation of nitroaniline using hexane–ethanol (70/30, v/v) as the mobile phase. All of the compounds are well separated. This indicated that TiO_2/SiO_2 supports could be used directly to separate some aromatic and basic isomers.

The separation of basic compounds under normal phase conditions is not as favorable as reversed phase. So, we also studied the chromatographic behavior using octadecyl-bonded TiO_2/SiO_2 under reversed phase conditions. In our work, 15 basic compounds were studied on an ODT column. The resulting asymmetry factors on the ODT column are between 1.0 and 1.2. In addition, nine aniline derivatives were separated on the ODT column. These compounds were well separated and good peak symmetry was obtained (Figure 5A). For strongly alkaline compounds, we separated pyridine derivatives on the ODT column as shown in Figure 5B. Good separation and symmetrical peaks were obtained for these compounds too.

Figure 4. Chromatograms on (250 × 4.6 mm i.d.) TiO_2/SiO_2. Mobile phase: 100% *n*-hexane (A); *n*-hexane–ethanol (70/30, v/v) (B). And flow-rate: 0.7 mL/min (A), 1 mL/min (B), temperature: 25°C, detection: UV at 254 nm. Peak numbers: 1, toluene; 2, biphenyl; 3, phenanthrene; 4, benzanthracene; 5, benzo(a)pyrene; 6, *o*-nitroaniline; 7, *p*-nitroaniline; and 8, *m*-nitroaniline.

Chemical stability

The basic stability of ODT phases was performed with the more aggressive mobile phase of phosphate buffer (27) under two conditions. First, the stability test was carried out by passing fresh sodium phosphate solution (10 mmol/L, pH = 10) through the column at 0.7 mL/min at 25°C to a total volume of 3000 mL. Chromatograms of a mixture of solutes (anthracene, phenanthrene, *N,N*-dimethylaniline, and *p*-methylaniline) were obtained periodically with every 500 mL mobile phase flushing with methanol–water (70:30, v/v) as mobile phase after conditioned for 30 min. The results showed that the ODT phase is stable under this condition without any noticeable effect on the retention factor or peak width of test solutes (Figure 6A).

Alkaline mobile phases containing phosphate or carbonate, and especially the use of elevated temperatures, diminish the column lifetime of silica-based phases due to the greater solubility of the silica support under these conditions (22). In routine use, these conditions should be avoided. However, they were chosen to gain more information about the stability of the ODT phases. In the second stability test, namely life test, fresh methanol–0.05 mol/L K_2HPO_4 solution at pH 10.0 (70:30, v/v) was used as mobile phase and the temperature was adjusted to 50°C. These conditions are so aggressive that both Hypersil ODS and NovaPak C_{18} degraded after ~ 250 mL of this aggressive mobile phase at 50°C (41); however, Hypersil ODS can resist the passage of 33,000 column volumes (82,200 mL) of pH 9 mobile phase at room temperature. NovaPak C_{18} can resist the passage of 23,000 column volumes (57,300 mL) without significant change. In routine use, this kind of aggressive conditions should be avoided. In our experiments, they were chosen to gain more information about the stability of the ODT phases and to give a fast evaluation of the column.

In the second stability test, the column was continuously purged at 1 mL/min, and then it was conditioned with water and methanol–water (70:30, v/v), respectively. The retention factor for acenaphthene and naphthalene were monitored as functions of the volumes of the mobile phase that had passed through the column (shown in Figure 6B). The k values for acenaphthene decreased from 6.3 to 4.5 after 1000 mL, and then it did not change significantly until 2300 mL. The peak shape also changed significantly at 2300 mL. Thus, we concluded that degradation of the stationary phase occurred at 2300 mL. Collins and collaborators reported that C_{18} bonded titanized silica (SiTiC$_{18}$) phase and C_{18} bonded doubly-titanized silica (SiTi2C$_{18}$) phase showed stationary phase collapse around 3700 column volumes (corresponding to 2650 mL) under the same conditions at 0.6 mL/min (22). Trudinger et al. (2) also studied the basic stability of octadecyl-bonded titania. They reported that the reversed-phase packing they prepared is stable up to pH 11 without report about how the basic test was performed. Compared with the Hypersil ODS and NovaPak C_{18}, the SiTi2C$_{18}$ and SiTiC$_{18}$, we concluded that TiO_2/SiO_2 exhibits good basic stability. It is thought that the
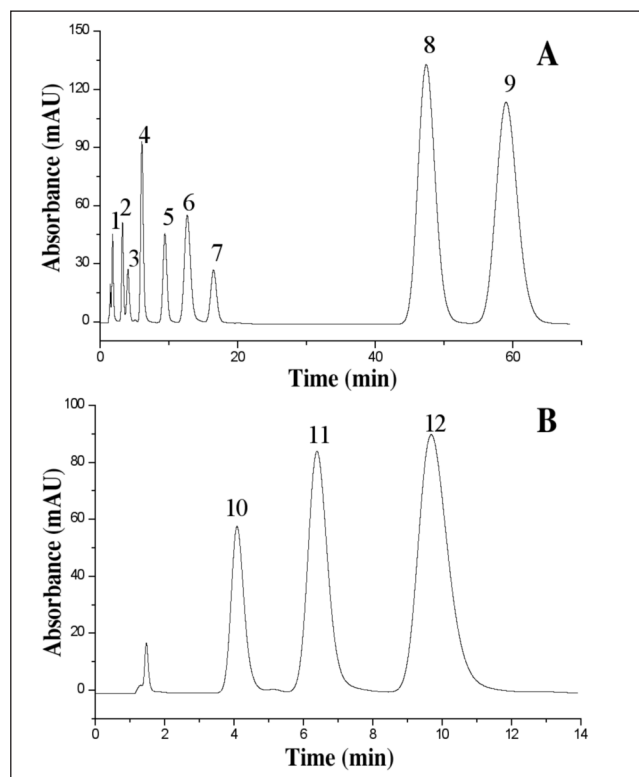

Figure 5. Chromatograms of the separation of basic compounds on (150 × 4.6 mm i.d.) ODT. Mobile phase: methanol/sodium phosphate solution 40 mmol/L (40/60, v/v) flow-rate: 1 mL/min, temperature: 25°C, detection: UV at 254 nm. Peak numbers: m-phenylenediamine, 1; aniline, 2; pyridine, 3; *N*-methylaniline, 4; *N*-ethylaniline, 5; 1-naphthylamine, 6; *N,N*-dimethylaniline, 7; diphenylamine, 8; *N,N*-diethylaniline, 9; pyridine, 10; 2-methyl pyridine, 11; 2,4-dimethylpyridine, 12.
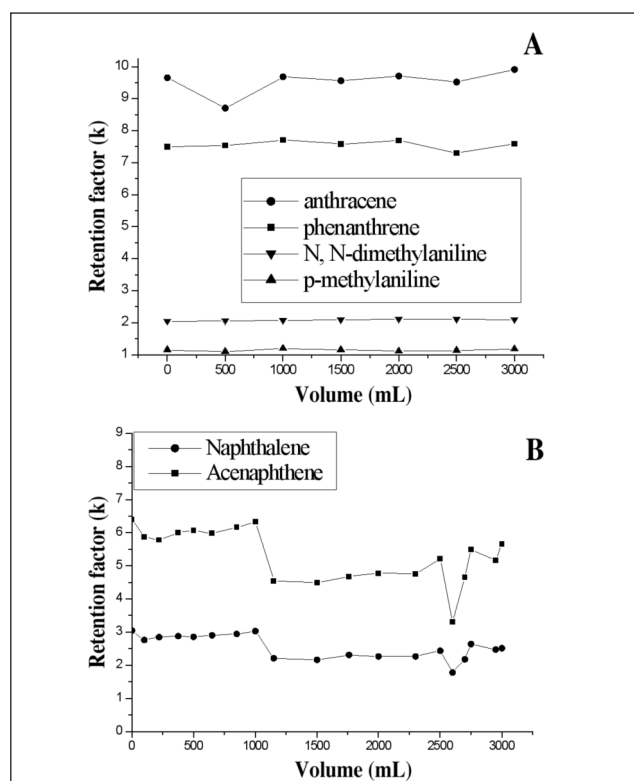

Figure 6. Life test of the column: chromatographic parameters versus the volume of basic solution flushed through the column. Mobile phase: 10 mmol/L sodium phosphate solution at pH = 10, flow rate: 0.7mL/min, temperature: 25°C, detection: UV at 254 nm (A). Mobile phase: methanol–0.05 mol/L K_2HPO_4 solution at pH 10.0 (70:30, v/v), flow rate: 1 mL/min, temperature: 50°C, detection: UV at 254 nm (B).

presence of titania film and a crosslinked alkylpolysiloxane layer on the TiO$_2$/SiO$_2$ particle largely improves the chemical stability.

Conclusions

It has been demonstrated that the self-assembly technique, applied to prepare TiO$_2$ nanoparticle multilayers on SiO$_2$ core, is a valid way to fabricate inorganic supports with more favorable character for HPLC use. TiO$_2$/SiO$_2$ prepared by the self-assembly technique has a higher specific surface, more controllable particle size distribution, and larger pore volume than the titania particles made by conventional methods; also, it has good pore structure, narrow pore size distribution, and good permeability. Furthermore, the octadecyl-bonded TiO$_2$/SiO$_2$ showed symmetrical of peaks for basic compounds and exhibited good stability in aggressive mobile phases. These results indicate that TiO$_2$/SiO$_2$ has the good properties of conventional titania. In sum, the titania nanoparticle coated silica not only has more favorable characteristics for HPLC applications than conventional titania but also has the desirable propertities of titania made by conventional methods.

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