

Preparation and Characterization of Titania Microspheres and Their Application in a Liquid Chromatography Stationary Phase

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Abstract

Titania microspheres were synthesized using hydrothermal methods to exploit a new liquid chromatography stationary phase. The prepared titania microspheres were approximately 7 μm in diameter, and the particle size distribution was relatively narrow and uniform. Furthermore, the average specific surface area was $276.0 \text{ m}^2 \cdot \text{g}^{-1}$, the average pore volume was approximately $0.25 \text{ mL} \cdot \text{g}^{-1}$, and the pore diameter was approximately 35.9 nm for sintering titania microspheres. These parameters indicate that the titania microspheres prepared for this study have excellent surface properties for chromatography. Additionally, columns filled with the titania microspheres were able to separate basic compounds, including benzene, nitrobenzene and o-nitroanisole. It could be proposed that the titania microspheres prepared for this study would be a promising stationary phase for liquid chromatography.

Keywords

Hydrothermal Method, Porous Materials, Titania Microspheres, Sintering, Liquid Chromatography Stationary Phase, Surfaces

1. Introduction

High-performance liquid chromatography (HPLC) has been widely accepted as one of the main analytical techniques for organic compounds [1]. The stationary phase filled in the column is the main determinant for HPLC separations. Over the past few decades, the properties and performance of stationary phases have been significantly enhanced [2] [3], but silica is still the most popular packing material for HPLC columns because it can prolong organosilane chains. However, a silica stationary phase can only

be used over a narrow pH range (pH 3 - 10) because of its strong hydrolysis and unstable Si-O band; it can even irreversibly adsorb alkaline compounds, limiting its application in some special fields.

Recently, an increasing number of metal oxide materials have been tested for HPLC column preparation [4] [5]. Metal oxides can behave as amphoteric ion exchangers, meaning that they can act as cation and anion exchangers down to a pH of 1 and up to a pH of 14. Titania not only displays amphoteric ion-exchange properties but also has good chemical stability, mechanical strength and enough rigidity [5] [6] to make it an excellent stationary phase material for HPLC columns. A series of titania nanotubes or microspheres have been prepared using the sol-gel method [7], polymerization-induced colloid aggregation [8], and non-aqueous emulsions or co-precipitation. Furthermore, the titania microspheres have been used for HPLC chromatographic packing. However, the methods mentioned above are too complicated, and the material and reagents too expensive to be applied extensively. Conversely, hydrothermal processing is a promising approach due to its simplicity, fast reaction kinetics and low cost [9] and could create new possibilities for high surface area and large pore diameter titania morphologies.

This study was intended to test the hypothesis that titania microspheres synthesized by the hydrothermal method could display excellent surface parameters, an ideal mesopore structure, and the narrow size distribution needed for the HPLC stationary phase. Optimal experimental conditions for synthesizing titania microspheres were found. Furthermore, to test the chromatographic performance, the prepared titania microspheres were employed as the HPLC stationary phase to separate some basic compounds.

2. Experimental Section

2.1. Reagents

The titanium tetrachloride used as a precursor for the reaction was purchased from Sinopharm Chemical Reagent Company (Beijing, China); the ethanol and n-hexane used as the mobile phase were purchased from Tedia Company (USA). Ammonium sulfate, urea and other analytical grade reagents were obtained from Shanghai Chemical Reagent Company (Shanghai, China).

2.2. Synthesis of Titania Microspheres

To synthesize uniform titania microspheres, 0.72 g of ammonium sulfate, 8.5 g of urea, 8.0 mL of millipore water and 8.0 mL of ethanol were added into a 50-mL conical beaker. The mixture was homogeneously stirred, and an aliquot of 0.6 mL of titanium tetrachloride was added drop by drop while continually stirring for 5 h. A transparent solution with a pH value of approximately 2.0 was then obtained and transferred into 50-mL polyfluortetraethylene cups that were heated at 393 K for 5 h in an oven (DGF-30, Nanjing, China). Finally, the suspension was filtered, and the precipitate was washed twice with millipore water and ethanol, respectively. The prepared methods

need high temperature over 500 K, thus the microspheres obtained from the precipitate were finally ground, dried at 353 K and carbonized at 773 K in a muffle furnace for subsequent characterization.

2.3. Characterization of the Synthesized Titania Microspheres

The morphology of the titania microspheres was observed using a scanning electron microscope (SEM, JSM-5610LV, Japan). The crystal shape of the titania microspheres was determined using an X-ray diffractometer (D/max-2500 VL/PC, Japan). A micromeritics model ASAP-2010 surface analysis instrument (Micromeritics Corporation, USA) was employed to generate nitrogen adsorption/desorption isotherms. A laser particle size analyzer, model Master Sizer2000 (Malvern Company, U.K) was used to determine the particle size distribution (PSD).

2.4. Column Packing and Its Performance

The chromatographic column (250 × 4.6 mm i.d.) was filled with the prepared titania microspheres as the stationary phase at a pressure of 450 psi using a high-pressure pump (STV-150, USA). During the filling procedure, a mixture of isopropanol and methanol were used as the propulsion solvent. The performance of the columns was tested by separating special basic substances with an HPLC/MS (1290 - 6460, Agilent Corporation, American).

3. Results and Discussion

3.1. SEM Images and X-Ray Diffraction of Titania Microspheres

Titania microspheres were synthesized using the hydrothermal method. The morphology was characterized, as shown in **Figure 1**. The particle size distribution of the prepared titania microspheres was narrow, and the particle diameters were in the range of

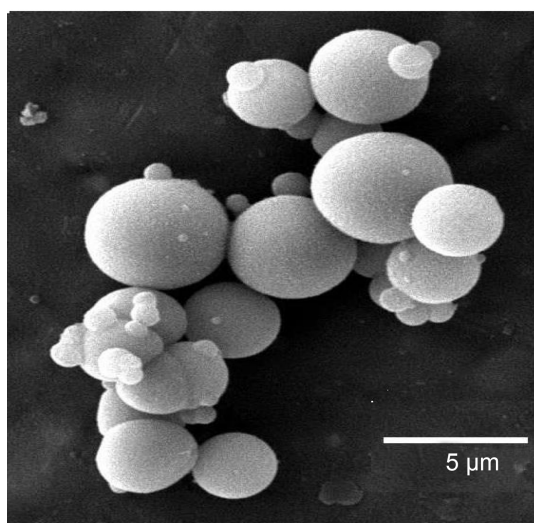


Figure 1. SEM image of the prepared titania microspheres.

6 - 10 μm . Further analysis of the particle size distribution showed that the average diameter of the titania microspheres was 7 μm .

Furthermore, the titania microspheres were characterized by X-ray diffraction (Figure 2). Apparently, the X-ray diffraction intensity of the titania microspheres that did not undergo heat treatment was very low, and the microspheres had an amorphous crystal structure. However, after being sintered at 500 °C, the diffraction intensity of the titania microspheres sharply increased, and diffraction peaks clearly appeared at 25.32°, 37.88°, 48.11°, 53.94°, and 62.51°, proving that the sintered material belonged to anatase-type of titania.

3.2. Surface Properties of the Titania Microspheres

It is critical for chromatographic columns to have enough free space available to provide a sufficient separation efficiency; this requires that the packing material have a large surface area to facilitate carrying large numbers of samples [4]. The sintered titania microspheres had smaller specific surface areas than the un-sintered microspheres. The specific surface area of the sintered titania microspheres was found to be 276.0 $\text{m}^2\cdot\text{g}^{-1}$, using the standard Brunauer Emmett Teller (BET) method, whereas the microspheres that were not heat treated had a surface area of 383.5 $\text{m}^2\cdot\text{g}^{-1}$. This could be attributed to the aqueous phase bound to the titania microspheres. At high temperatures, it is possible that the aqueous phase dissociated out of the compound and the adjacent Ti-OH bond condensed to form a Ti-O-Ti bond [10], thus leading to a decrease in the specific surface areas. However, the crystal surfaces that tended to be formed with the heat treatments were consistent with the surfaces of the elementary lattice cells. Thus,

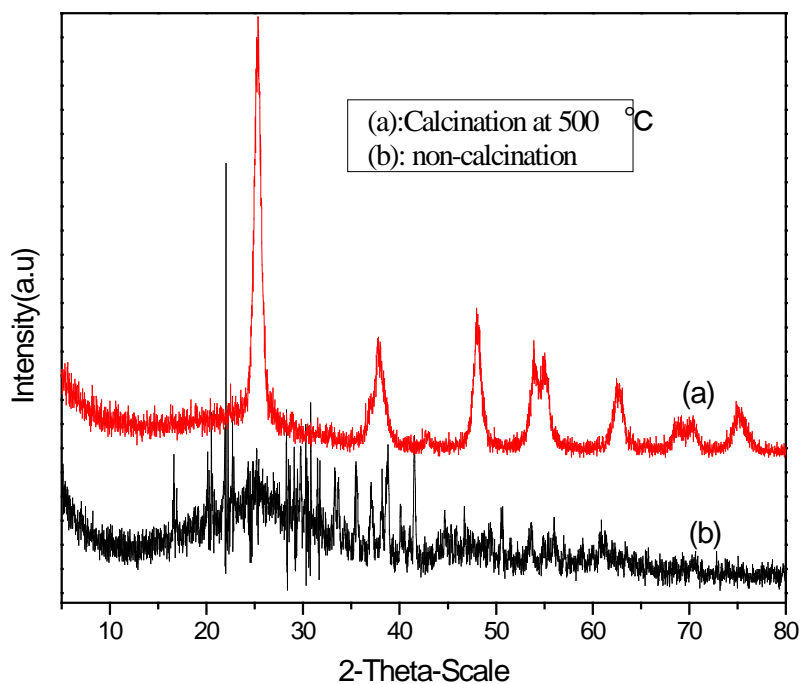


Figure 2. XRD patterns of the titania microspheres.

the sintered titania microspheres surfaces were more homogeneous, which is one of the physical requirements for the HPLC stationary phase.

As suggested by Thekkudan *et al.* [11], ideal packing should be in accordance with suitable physical and chemical parameters. The packing can be described by the porosity of the material, which is generally determined by nitrogen adsorption and desorption from the microspheres after heat treatment. As shown in **Figure 3**, the adsorption branch was a IV-like isotherm and displayed an H type hysteresis loop, indicating the presence of a well-developed mesoporosity in the titania microspheres. Furthermore, according to the desorption isotherm, the average pore volume of the titania microspheres was approximately $0.25 \text{ mL}\cdot\text{g}^{-1}$, and the average pore diameter was approximately 35.9 nm based on the Barret-Joyner-Halenda (BJH) models. These results correlate well with the report that the pore diameter of microspheres must be in the range of 2 to 50 nm [12].

3.3. Chromatographic Condition

Normal-phase separation of the aromatic compounds was performed on the packed titania column. It was found that there was $10 \mu\text{L}\cdot\text{mL}^{-1}$ benzene, $0.4 \mu\text{L}\cdot\text{mL}^{-1}$ nitrobenzene and $0.2 \mu\text{L}\cdot\text{mL}^{-1}$ o-nitroanisole. These compounds were detected at 254 nm, and the mobile phase was a combination of n-hexane and ethanol with a gradient elution containing 10% ethanol at 7 min with a rate of $0.6 \text{ mL}\cdot\text{min}^{-1}$. The column temperature was controlled to be 35°C , and the initial pressure of the column was approximately 280 bar. As shown in **Figure 4**, all of the substances were well separated on the titania column. Benzene and nitrobenzene were retained on the stationary phase as a Lewis base.

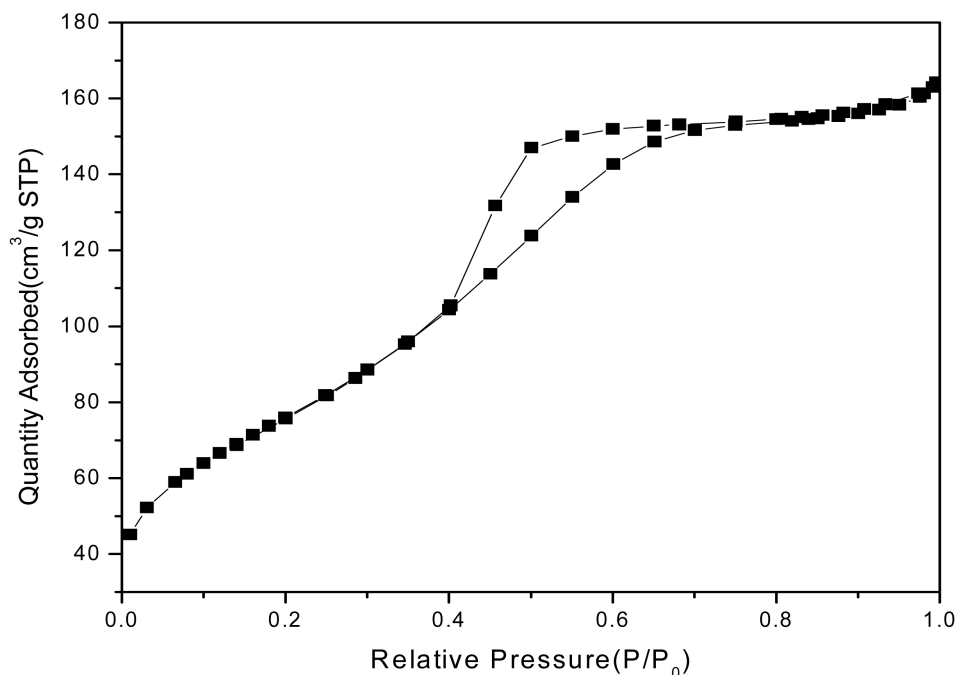


Figure 3. Nitrogen adsorption and desorption isotherms of the titania microspheres.

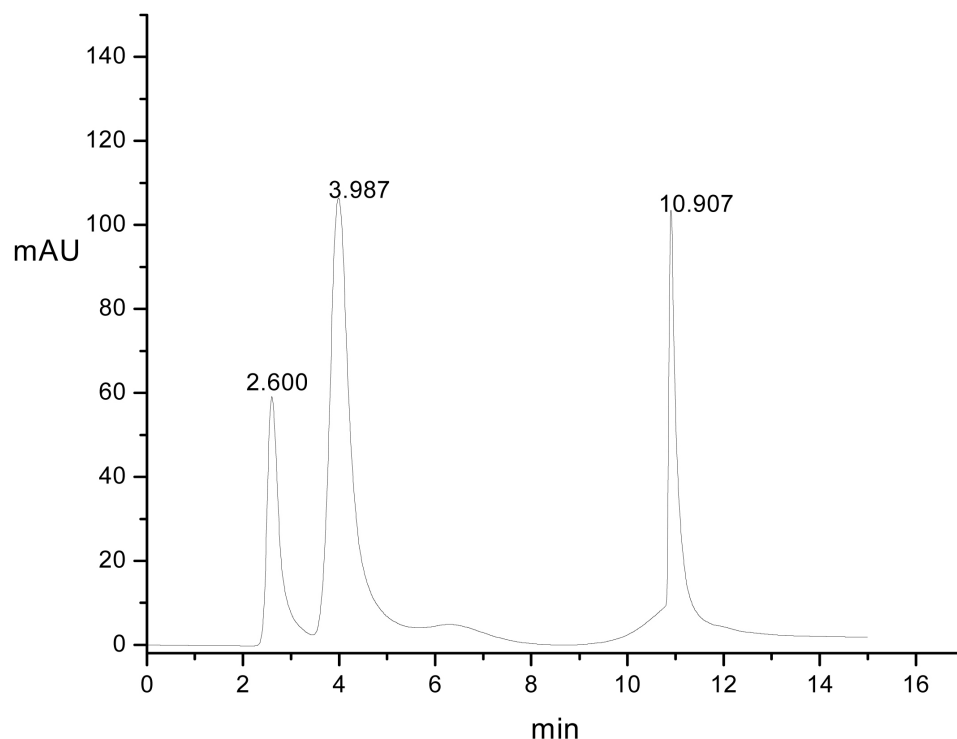


Figure 4. Chromatogram of the separation of the aromatic compounds with the titania microspheres as the stationary phase (250 × 4.6 mm i.d.). Solute: benzene (eluting at 2.6 min), nitrobenzene (eluting at 3.9 min), o-nitroanisole (eluting at 10.9 min). Eluent: n-hexane and ethanol. Flow: 0.6 mL·min⁻¹. Detection: UV at 254 nm. Temperature: 35°C. Pressure: 280 bar.

The π electron of these molecules can react with the weakly acidic surface of titania microspheres [13]. However, due to the strong polarity, the o-nitroanisole was retained much longer in the chromatographic column. Furthermore, the filled chromatographic column could be reused for separating these basic compounds. Thus, the titania that was synthesized by the hydro-thermal method was considered to be a promising material for the HPLC stationary phase.

4. Conclusion

Traditional silica columns would lead to a larger solubility in the basic mobile phases at higher temperatures. Simultaneously, tailed peaks appeared when alkaline substances were separated on a silica column. Thus, our work focused on exploring a new liquid chromatography stationary phase. The titania microspheres synthesized using hydro-thermal treatments were approximately 7 μm in diameter and had a relatively narrow and uniform particle size distribution. The pore size of the titania microspheres was in the range of mesopores; the average specific surface area was 276.02 $\text{m}^2\cdot\text{g}^{-1}$, and the average pore volume was 0.25 $\text{cm}^3\cdot\text{g}^{-1}$. Furthermore, benzene, nitrobenzene and o-nitroanisole all separated easily in the titania column. All of the parameters highlighted above indicate that titania microspheres prepared using a hydrothermal method would provide an excellent normal phase for liquid chromatography.

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