Analytical Methods

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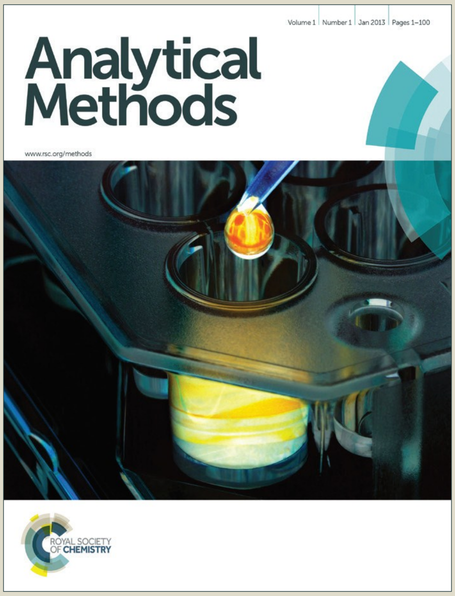

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ARTICLE

Synthesis and modification of monodisperse silica microspheres for UPLC separation of C_{60} and C_{70}†

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Monodisperse silica microspheres with average diameters from 1 μm to 2 μm were synthesized by a modified Stöber method, in which an ethanolic solution of tetraethyl orthosilicate (TEOS) was continuously supplied to the reaction mixture containing KCl electrolyte, water, ethanol and ammonia. The effects of reactant amounts, supply rate, and reaction temperature on the microsphere morphology were investigated. After surface modification with octadecyl trichlorosilane, the obtained monodisperse C_{18}-silica microspheres with an average diameter of 1.5 μm were applied to ultra performance liquid chromatography (UPLC) separation of fullerenes. For having smaller size, the 1.5 μm monodisperse C_{18}-silica UPLC stationary phase shows ultra high efficiency compared with the commercial C_{18}-silica HPLC stationary phase with average diameters from 5 to 50 μm.

Introduction

Silica particles with uniform size, shape and composition have a wide range of applications in the field of antireflective coating materials,[1,2] drug release,[3,4] catalysts,[5] stabilizers,[6,7] and chromatography.[8,9] The size and surface properties of colloidal spheres are highly important for their applications in these fields.[10,11] The demand for well-defined silica particles is constantly increasing.[12,13] Since the physical and optical properties of silica particles depend on their sizes, special attention has been paid to the controlled synthesis of silica particles with defined size.

The Stöber method is known as a classical method to produce monodisperse silica spheres.[14,15] In this method, silicon alkoxide such as tetraethyl orthosilicate (TEOS) is hydrolyzed in alcohol in the presence of a basic catalyst such as ammonia. The concentrations of TEOS and ammonia have been examined as parameters that control the size of silica particles. The size of silica spheres obtained in this method is usually less than 1 μm. Silica particles larger than 1 μm can be commonly prepared by a seeded-growth technique using monodisperse submicrometer-sized particles as seeds.[16] However, this technique involves multiple growth steps to get the targeted size of silica particles.[17] Besides, during the reaction, high TEOS concentration may cause seed particle aggregation and secondary particle generation, which result in polydisperse particles and affect the properties of the material.

Silica surfaces are rich in silanol groups, which are very important adsorption and reactive points.[18,19] By chemical modification of the silica surface with various types of organic reagents, the obtained silica microsphere could be used for various applications. An ideal separation system in liquid chromatography (LC) is one that is highly efficient, provides fast separations with low back pressure.[20-24] Monodisperse silica particles with smaller diameters have been used in ultra performance liquid chromatography (UPLC) which is concomitant with considerable back pressure.

UPLC was first described as the use of nano-columns packed with none-porous 1.0-1.5 μm silica-based particles on a prototype system compatible with very high pressure by Jorgenson and co-workers.[25] The use of smaller particles could significantly reduce the height equivalent of a theoretical plate (HETP) generated in a separation. As particle diameter reduced, the analyte diffusion distance to reach the particle surface decreased, which facilitated the speeds of Eddy diffusion, longitudinal diffusion and mass transfer, and resulted in the high efficiency of separation.[26]

In this work, monodisperse silica particles with diameter of 1-2 μm were successfully synthesized by a modified Stöber method, in which an ethanolic solution of tetraethyl orthosilicate (TEOS) was continuously supplied to the reaction mixture containing KCl electrolyte, water, ethanol and ammonia. The effects of reaction conditions to microsphere morphology as well as the modification and application of the obtained microspheres to UPLC separation of fullerenes were studied and discussed.

Experimental

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Chemicals and reagents

Tetraethyl orthosilicate (TEOS, reagent grade) was obtained from Aladdin Chemical Reagent Company (Shanghai, China). Ammonia (analytical grade) and ethanol (analytical grade) were purchased from Yantai Sanhe Chemical Reagent Company (Yantai, China). Potassium chloride (KCl, analytical grade) was obtained from Tianjin Hengxing Chemical Reagent Company (Tianjin, China). Octadecyl trichlorosilane (reagent grade), trimethylsilyl chloride (reagent grade) and triethylamine (reagent grade) were obtained from Sinopharm Chemical Reagent Company (Shanghai, China). Dichloromethane (analytical grade) was purchased from Tianjin Fuyu Fine Chemical Company (Tianjing, China). Toluene (analytical grade) was obtained from Laiyang Fine Chemical Company (Yantai, China). Methanol (HPLC grade) was obtained from Tianjin Biaoshiqi Science and Technology Development Company (Tianjin, China). C_{60} and C_{70} were obtained from Funano New Material Company (Xiamen, China). Commercial C_{18} silica stationary phase with an average diameter of 50 μm was bought form Agela Technologies (S185006, Tianjing, China). Commercial C_{18} silica stationary phase with an average diameter of 5 μm was bought form Galak Technologies (18B150401, Wuxi, China). All chemicals were used as received without further purification. Water was distilled before use.

Preparation of SiO_2 particles

The synthesis of silica particles was conducted by a modified Stöber method in a 250 mL semibatch chemical reactor where an ethanolic solution of TEOS (solution I) was continuously supplied with a microfeeding pump to the reaction mixture (solution II) of water, ethanol, ammonia and KCl electrolyte. The amounts of ethanol, water and electrolyte in solution II were 65.0 mL, 6.75 mL and 0.017 g, respectively. The amount of ammonia ($V_{ammonia}$) in solution II was ranged from 6 mL to 9 mL. The supply rate of solution I (v) varied from 0.15 to 0.4 mL/min. The amount of TEOS (W_{TEOS}) in solution I was ranged from 2.00 g to 6.00 g. The amounts of ethanol in solution I was 33.3 mL. The reaction temperature (T) varied from 15 to 50 ^oC, and the stirring speed was 300 rpm. After supply of solution I, the obtained particles were purified by centrifugation and washed with ethanol for three times. Finally, the SiO_2 particles were dried under vacuum at ambient temperature.

Preparation of C_{18}-modified silica

2mL of octadecyl trichlorosilane, 5 g of as prepared silica, and 1 mL of triethylamine catalyst were added into 50 mL of toluene. The mixture was stirred at 85 ^oC. After reaction for 12 h, the solution was filtered. The obtained product was first washed with toluene, and then washed with methanol, and finally washed with dichloromethane for 3 times. After filtration, the product was dried in vacuum oven overnight. The dried product and 1 mL of trimethylsilyl chloride were added into a solvent of toluene to react under stirring at 85 ^oC. After reaction for 12 h, the solution was filtered. The obtained product was washed with dichloromethane for 3 times. After filtration, the product was dried under vacuum overnight to give C_{18}-modified silica.

Chromatography

The C_{18}-modified silica particles were packed into stainless columns (50 mm × 4.6 mm, I.D.) by the chromatographic column packing machine (GLK 2000, GALAK) using isopropanol/methanol (v/v = 1:1) as the mobile phase at a pressure of 30 MPa. The columns were then connected to the HPLC system with methanol passing through at a flow rate of 1 ml/min for about 2 h to equilibrate the column until a constant UV baseline was obtained. UPLC (Waters) was used to evaluate the separation performance of the C_{18}-modified silica stationary phase. The mobile phase is a mixture of hexane and isopropanol with a flow rate of 0.1-0.15 ml/min, and the UV detection wavelength is 254 nm.

Characterization

Surface morphology and structure of the SiO_2 spheres were investigated by scanning electron microscopy (SEM, JEOL JSM-6309LV). Thermogravimetric analysis (TGA) data were recorded using a Mettler Toledo TGA/DSC1/1600LF simultaneous thermal analyzer at a heating rate of 10 ^oC/min. Fourier transform infrared spectroscopy (FT-IR) was recorded on Tensor 27 produced by Bruker Corporation.

Results and discussion

Effect of TEOS amount on microsphere morphology

As shown in Table S1 in the ESI†, experiments are performed at a temperature of 30 ^oC with a stirring speed of 300 rpm and a solution I supply rate of 0.2 mL/min. The amount of ethanol in solution I is fixed at 33.3 mL. The amounts of ammonia, water, ethanol and KCl in solution II are fixed at 9 mL, 6.75 mL, 65 mL and 0.017 g, respectively. With the increase of TEOS amounts in the solution I, the diameter and polydispersity index (PDI) of the obtained microspheres increase (Figure 1). When the TEOS amounts are 2.00 g, 3.95 g and 6.00 g, the diameter of the obtained particles are 1.20 μm with a PDI of 2.36%, 1.75 μm with a PDI of 3.77% and 2.01 μm with a PDI of 4.86%, respectively. Although the increase of the TEOS concentration will result in more silica nuclei in the initial stage, it provides sufficient reactant for the growth of microspheres. In the semibatch chemical reaction conditions, the effect of TEOS amount on the growth is superior to that on nucleation. Additionally, as the diameter of the silica microspheres increases, the PDI of the microspheres becomes bigger.

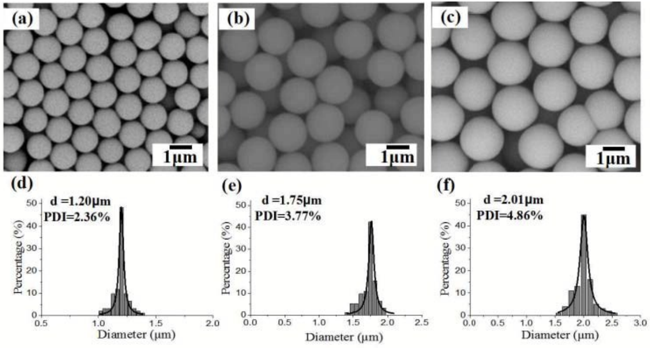

Fig. 1 SEM image (a-c) and PDI analysis (d-f) of silica particles obtained at different TEOS contents: (a)(d) 2.00g; (b)(e) 3.95 g; (c)(f) 6.00 g.

Effect of ammonia amount on microsphere morphology

As shown in Table S2 in the ESI†, experiments are performed at 30 ^{o}C with a stirring speed of 300 rpm and a solution I supply rate of 0.2 mL/min. The amounts of TEOS and ethanol in solution I are fixed at 3.95 g and 33.3 mL, respectively. The amounts of water, ethanol and KCl are fixed at 6.75 mL, 65 mL and 0.017 g, respectively. With the increase of ammonia amounts in the solution II, the diameter and PDI of the obtained microspheres decrease (Figure 2). When the ammonia amounts are 6 mL, 7 mL and 9 mL, the diameter of the obtained particles are 2.00 μm with a PDI of 4.63%, 1.84 μm with a PDI of 3.52% and 1.52 μm with a PDI of 3.21%, respectively. In the reaction system, ammonia acts as catalyst and source of water. With the increase of the ammonia concentration, the nucleation points of silica increase, which is the possible reason for the decrease of particle size. Additionally, as the diameter of the silica microspheres decreases, the PDI of the microspheres becomes smaller.

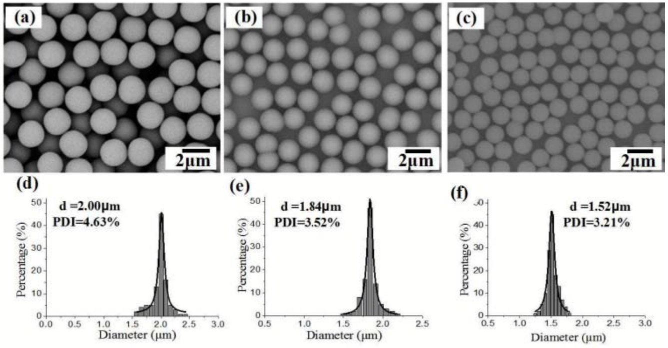

Fig. 2 SEM image (a-c) and PDI analysis (d-f) of silica particles obtained in different ammonia contents: (a)(d) 6 mL; (b)(e) 7 mL; (c)(f) 9 mL.

Effect of the temperature on microsphere morphology

As shown in Table S3 in the ESI†, experiments are performed at a stirring speed of 300 rpm with a solution I supply rate of 0.2 mL/min. The amount of TEOS and ethanol in solution I are fixed at 3.95 g and 33.3 mL, respectively. The amounts of ammonia, water, ethanol and KCl are fixed at 9 mL, 6.75 mL, 65 mL and 0.017 g, respectively. With the increase of reaction temperatures, the diameter and PDI of the obtained microspheres increase (Figure 3). When the reaction temperatures are 15 ^{o}C, 30 ^{o}C and 50 ^{o}C, the diameter of the obtained particles are 1.39 μm with a PDI of 2.98%, 1.60 μm with a PDI of 4.13% and 2.04 μm with a PDI of 4.98%, respectively. It is reasonable that the rise of temperature will accelerate the reaction kinetics, and thus contribute to the growth of particles. However, the PDI becomes bigger due to the high reaction rates at high temperatures.

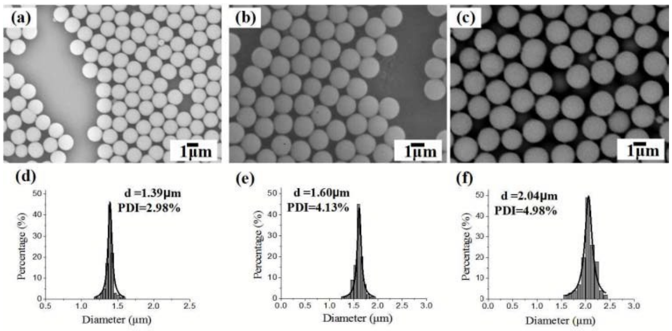

Fig. 3 SEM image (a-c) and PDI analysis (d-f) of silica particles obtained at different temperatures: (a)(d) 15 ^{o}C; (b)(e) 30 ^{o}C; (c)(f) 50 ^{o}C.

Effect of the supply rate of TEOS on microsphere morphology

As shown in Table S4 in the ESI†, experiments are performed at 30 ^{o}C with a stirring speed of 300 rpm. The amount of TEOS and ethanol in solution I are fixed at 3.95 g and 33.3 mL, respectively. The amounts of water, ethanol and KCl are fixed at 6.75 mL, 65 mL and 0.017g, respectively. With the increase of solution I supply rates, the diameter of the obtained microspheres decreases (Figure 4). When the solution I supply rates are 0.1 mL/min, 0.2 mL/min and 0.4 mL/min, the diameter of the obtained particles are 2.01 μm with a PDI of 4.10%, 1.48 μm with a PDI of 3.25% and 1.3 μm with a PDI of 3.67%, respectively. Therefore, the slower supply rate of TEOS helps to increase the final particle size. However, too fast or too slow supply rate of solution I leads bigger PDI.

According to the experimental results, the optimized reaction conditions for the synthesis of monodisperse silica microspheres with a diameter of about 1.5 μm are: the amounts of TEOS and ethanol in solution I are fixed at 3.95 g and 33.3 mL, respectively. The amounts of ammonia, water, ethanol and KCl in solution II are fixed at 9 mL, 6.75 mL, 65 mL and 0.017 g, respectively. The temperature is fixed at 30 ^{o}C and the supply rate of solution I is fixed at 0.2 mL/min. After surface modification with octadecyl trichlorosilane, the microspheres can be used as column stationary phase for UPLC.

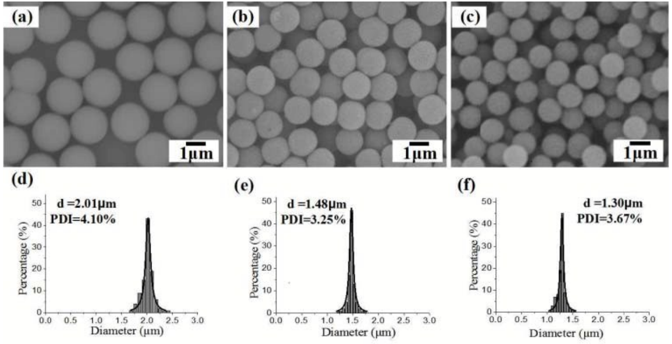

Fig. 4 SEM images of silica particles obtained at different supply rates of TEOS: (a) 0.1 mL/min; (b) 0.2 mL/min; (c) 0.4 mL/min.

IR spectra

Figure 5 shows the infrared spectra (IR) of the unmodified and C_{18}-modified SiO_2 microspheres with a diameter of 1.5 μm and PDI of 3.25%. Compared with bare SiO_2 microspheres, the appearance of $-CH_2-$ asymmetric stretching vibration peak at 2920 cm^{-1} and $-CH_2-$ symmetric stretching vibration peak at

2850 cm^{-1} in the modified microspheres indicates the success of surface modification with octadecyl trichlorosilane.

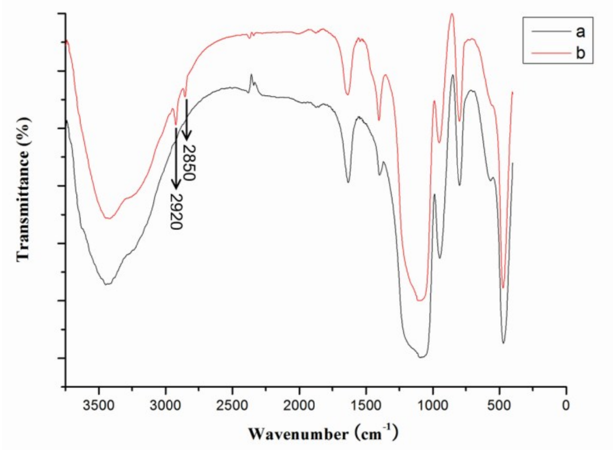

Fig. 5 Infrared spectra of unmodified SiO_2 microspheres (a) and C_{18}-modified SiO_2 microspheres (b) with a diameter of 1.5 µm and PDI of 3.25%.

TGA tests

As shown in Figure 6, thermogravimetic analysis (TGA) tests are performed on the bare and C_{18}-modified SiO_2 particles, respectively. Both the samples are losing weight at the beginning of the heating process due to the removal of physical absorbed water and degradation of TEOS residuals in the samples. However, the C_{18}-modified SiO_2 particles have more weight loss than the bare particles from 200 to 500 ^oC, because of the decomposition of octadecyl groups on the surface. According to the difference in weight loss of the bare and modified SiO_2 particles, the C_{18} content of modified SiO_2 particles are calculated to be 11.3 wt%.

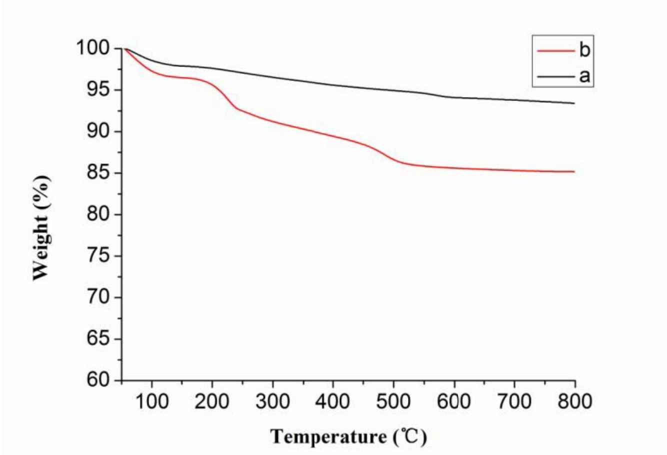

Fig. 6 TGA curves of unmodified SiO_2 microspheres (a) and C_{18}-modified SiO_2 microspheres (b) with a diameter of 1.5 µm and PDI of 3.25%.

C_{18}-modified silica particles for UPLC separation of fullerenes

Since discovery in 1985, fullerenes have been considered as one of the most promising materials in nanotechnology due to their unique structures and properties.[27] Fullerenes have been applied in many fields such as optics, electronics, cosmetics and medical research. Liquid chromatography is an ideal method for the separation of the main component of fullerenes. For example, Saito *et al.* isolated C_{60} and C_{70} by high performance liquid chromatography (HPLC) equipped with alkyldiphenyl (C_{18}Diph) bonded stationary phase.[28] However, the column they used is very long (200 mm), and the separation efficiency is not very high due to the big size of the silica stationary phase (~5 µm) in the column. Thereby, there's still room for further improvement by adopting the newly developed UPLC technique with smaller silica stationary phase.

In our experiment, a self-made column as short as 50 mm filled with the 1.5 µm C_{18}-modified SiO_2 microspheres is applied to the UPLC separation of fullerenes. The effect of mixed mobile phase on the separation results is investigated. As shown in Figure 7, we can see that when the volume ratio between hexane and isopropanol is 1:3, the C_{18} column works best.

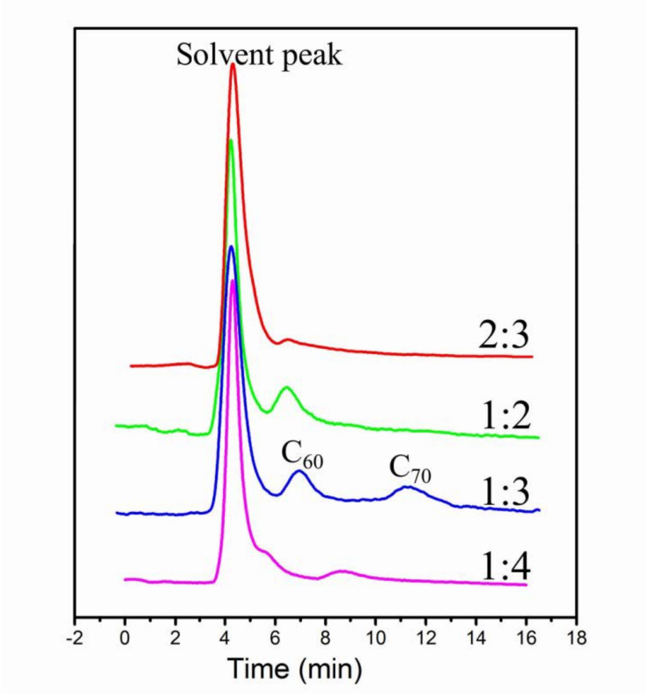

Fig. 7 Effects of volume ratio of hexane and isopropanol on the separation performance of the 1.5 µm C_{18}-modified silica stationary phase. (Flow rate, 0.1 mL/min; back pressure, 1300 psi; injection size, 2 µL; detection wavelength, 254 nm; column, 50 mm × 4.6 mm, I.D.)

Figure 8 shows the effect of different column stationary phase on the separation of C_{60} and C_{70}. Compared with the 1.5 µm C_{18}-modified silica stationary phase (Figure 8d), the unmodified silica stationary phase with a diameter of 1.5 µm (Figure 8a), the commercial C_{18}-modified silica stationary phase with an average diameter of 50 µm (Figure 8b) and 5 µm (Figure 8c) have no separation effect. Thereby, the smaller particle size and surface modification contribute to the ultra high efficiency of the 1.5 µm C_{18}-modified silica stationary phase. Additionally, the separation time for the 1.5µm C_{18}-modified silica stationary phase is less than 7 min, which is 4-5 times faster than the traditional 5 µm C_{18}Diph-modified silica stationary phase.[28]

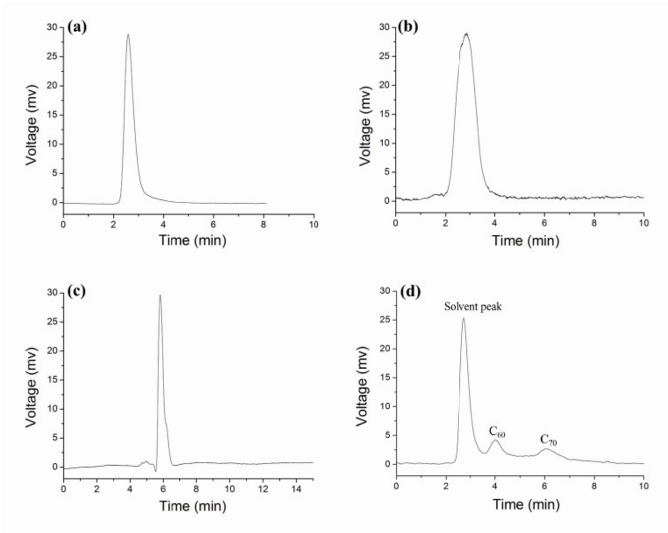

Fig. 8 Chromatograms for the separation of C_{60} and C_{70} fullerenes by different stationary phase: (a) monodisperse bare silica (1.5 µm) stationary phase with a column length of 50 mm; (b) commercial C_{18}-modified silica stationary phase (50 µm) with a column length of 50 mm; (c) commercial C_{18}-modified silica stationary phase (5 µm) with a column length of 150 mm; (d) monodisperse C_{18}-modified silica (1.5 µm) stationary phase with a column length of 50 mm. (Mobile phase, hexane/isopropanol, 1:3 v/v; flow rate, 0.15 mL/min; injection size, 2 µL; detection wavelength, 254 nm; column I.D., 4.6 mm)

Conclusions

Monodisperse silica microspheres with diameters from 1 µm to 2 µm were synthesized successfully by a modified Stöber method, in which an ethanolic solution of TEOS was continuously supplied to the reaction mixture containing KCl electrolyte, water, ethanol and ammonia. The optimized reaction conditions for the synthesis of monodisperse silica microspheres with a diameter of about 1.5 µm are: the amounts of TEOS and ethanol in solution I are fixed at 3.95 g and 33.3 mL, respectively. The amounts of ammonia, water, ethanol and KCl in solution II are fixed at 9 mL, 6.75 mL, 65 mL and 0.017 g, respectively. The reaction temperature is fixed at 30 °C and the supply rate of solution I is fixed at 0.2 mL/min. The microspheres were modified with octadecyl trichlorosilane and applied in UPLC separation of fullerenes. With a column as short as 50 mm, the 1.5 µm C_{18}-modified silica stationary phase can separate C_{60} and C_{70} in 7 minutes with ultra high efficiency.

Acknowledgements

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Synthesis and modification of monodisperse silica microspheres for UPLC separation of C_{60} and C_{70}†

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Using a modified Stöber method, monodisperse silica microspheres with average diameters from 1 µm to 2 µm are synthesized as UPLC column fillers. With a column as short as 50 mm, the 1.5 µm C_{18}-modified silica stationary phase can separate C_{60} and C_{70} in 7 minutes with ultra high efficiency.