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Synthesis of Mesoporous Silica Particles by Polymerization Induced Colloid Aggregation and Its Application for High-Performance Liquid Chromatography

B.B. ZHAO[1,*], F.Y. WANG[2] and T. LI[3]

[1]College of food science and technology, Henan University of Technology, Zhengzhou, P.R. China
[2]Chemical Engineering Institute, Nanjing University of Science and Technology, Nanjing, P.R. China
[3]Dalian Elite Analytical Instruments Co., Ltd., Dalian, P.R. China

*Corresponding author: Tel: +86 18236900558; E-mail: gentlebeibei@163.com

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Micrometer-sized mesoporous silica particles have been synthesized by a modified polymerization induced colloid aggregation method. Silica particles with particle size in the range of 2-5 μm could be obtained by controlling the reaction conditions. The obtained silica particles were characterized by scanning electron microscopy (SEM), N_2 physisorption technique and inductively coupled plasma emission spectroscopy (ICP-AES). The C18-modified silica particles were explored as stationary phase in reversed phase liquid chromatography and its chromatographic characteristics for separation of some small molecules have been studied.

Keywords: Polymerization induced colloid aggregation, Silica particles, High performance liquid chromatography, Stationary phase.

INTRODUCTION

Since the discovery of silica with organized mesoporous structures, many applications of these materials have been proposed and developed in the field of catalysis, separation, absorption, drug delivery, *etc.*[1,2]. Mesoporous silica particles prepared by polymerization induced colloid aggregation (PICA) method were featured by good monodispersity and regular morphology[3]. Titania spheres[4], zirconia spheres[5], carbon particles[6,7] and Fe_2O_3 spheres[8] could also be prepared by polymerization induced colloid aggregation method. Investigation revealed that both silica particles and bonded silica exhibited good chromatographic performance[9]. Separation of polypeptides and protein mixtures was performed in less than a minute using these 2 μm particles as packing materials[10]. Considerable progress has been made in the preparation of silica particles, whereas, synthesis of silica particles with both controllable particle size and pore size still remains a challenging task[1]. And tailing chromatographic peaks for bases caused by metal on the surface of silica-bonded phase was also a problem usually met in many application areas[11]. This work focused on synthesis of silica particles. By carefully controlling the reaction conditions, the particle size of silica particles could be tuned in the range of 2-5 μm. Also, total metal content in silica particles was lowered by an ion-exchange resin method. Silica particles with diameter of 5 μm were modified with methyloctadecylchlorosilane and explored as stationary phase in reversed-phase HPLC.

EXPERIMENTAL

Reagent grade urea, formaldehyde (37 %) and hydrochloric acid were used for the synthesis of the silica particles. Specification of four different kinds of silica sol are shown in Table-1. Success 262 ion-exchange resin were obtained from Jiangsu Success Resin Co., Ltd. Particle silylation reactions were carried out with dimethyloctadecylchlorosilane (Fluka) or trimethylchlorosilane (Fluka) in dry toluene with pyridine (Fluka) as the catalyst or acid acceptor. Mobile phase were prepared from HPLC grade methanol, acetonitrile and Milli-Q water.

The typical synthesis process of silica particles was as follows. Firstly, Success 262 ion-exchange resin was packed into a 25 × 500 mm column and washed with 4 mol/L HCl solution for about 4 h. Then the SW-25 silica sol flow through the resin column at a speed of 1.25 mL/min to remove part of the metal. And then, silica sol was coacervated into spheres by a urea-formaldehyde polymerization process. At a temperature of 25 °C, 56 mL purified SW-25 silica sol, 23 mL formaldehyde and 15 g of urea were added to a 1 L Nalgene beaker with 135 mL of distilled water inside it. As the pH of the solution was adjusted to 1.2 with hydrochloric acid, the coacervation reaction was initiated. Within a few minutes, the mixture had turned white and opaque, due to the polymerization induced collid aggregation. The solution was allowed to settle and the liquid was decanted. The resultant particles

TABLE-1
SPECIFICATIONS OF FOUR DIFFERENT SILICA SOL

Model	Diameter (nm)	Silica (wt. %)	Stabilizer	Manufacturers
JX-15	4-8	15-16	Na_2O	Qingdao Haiyang Chemical Co., Ltd
SW-25	10-20	25-26	Na_2O	Qingdao Haiyang Chemical Co., Ltd
P20-1	50	20	KOH	Shandong Peak-tech New Material Co., Ltd
P20-2	100	20	KOH	Shandong Peak-tech New Material Co., Ltd

were centrifuged, washed and dried at 110 °C under vacuum overnight. The polymer was burned out of the particles at 550 °C for at least 4 h. Finally, the particle was rehydroxylated by refluxing in 20 % HCl for 24 h to permit the desired silanization reaction to be performed.

Dimethylocatadecylchlorosilane was used to functionalize silica particles according to a previously reported method. Calcined silica with diameter of 5 µm was dried in vacuum at 120 °C for 5 h and dispersed in dried toluene (120 mL) under N_2. Subsequently, 2.9 mL dried pyridine and 9.7 mL dimethyloctadecylchlorosilane in 20 mL of dried toluene were added. The reaction mixture was refluxed for 12 h under N_2 and then filtered. The obtained C18-modified silica particles were washed with toluene, acetone, acetone/H_2O, acetone and diethyl ether, respectively. The resultant silica particles were dried at room temperature for 6 h and then in an oven at 80 °C overnight.

The morphology and size of silica particles were monitored by a scanning electron microscope (JSM-680LV, GEOL, Japan). The particle size and distribution of particles were measured using a digimatic caliper (CD-15C, Mitutoyo, Japan). Normally, two or three SEM images for each sample were used and about 50 particles at in each image were counted to calculate the average particle size based on particle population. Nitrogen adsorption-desorption isotherms were measured with surface area and pore size analyzer (V-Sorb 2800P, App-one, China) at 87 K after the samples were outgassed for 45 min at 423 K. The specific area was calculated using the BET theory from the adsorption branches in the relative pressure range of 0.05-0.25.

Chromatographic tests: The HPLC system (P230II, Dalian Elite, China) composed of a quaternary pump, a Valco injector with a 20 µL sample loop and an ultraviolet detector with variable wavelength was selected to carry out the test experiments. The original chromatographic column was substituted by a column packed with silica spheres by using the slurry technique with an HPLC slurry Packer.

The C18-modified silica particle was dispersed in isopropanol and packed into a stainless steel column (4.6 mm × 150 mm I.D.) under 40MPa. The standard mixture was separated in the column using a methanol/water mixture (85:15, v/v) as the mobile phase at 0.9 mL/min with UV detection at 254 nm. The component concentrations of the standard mixture injected were: uracil, nitrobenzene, naphthalene, fluorene. Also, 17 kinds of amino acids were separated in this column using a gradient mobile phase at 1 mL/min with the UV detection at 254 nm. The mobile phase gradient condition was shown as Table-2.

TABLE-2
GRADIENT SEPARATION CONDITION OF MOBILE PHASE FOR 17 KINDS OF AMINO ACIDS

Time (min)	0.05 mol/L CH_3COONa (%)	Acetonitrile/methol/H_2O = 60/20/20 (%)
0	95	5
39	55	45
40	0	100
50	0	100
51	95	5
65	95	5

RESULTS AND DISCUSSION

Physical characterization of silica particles: Typical SEM images of mesoporous silica particles obtained from four different kinds of silica sol were shown in Fig. 1. All silica particles exhibit regular spherical morphology with good monodispersity. The surface roughness of S4 was typical of particles made by this process from larger silica sols. As shown in Table-3, the average diameter of S1, S2, S3 and S4 was all about 5 µm. Fig. 2 showed that the silica particles ranged from 2 to 5 µm could be obtained by varying reaction conditions, such as water quantity, pH value, *etc*.

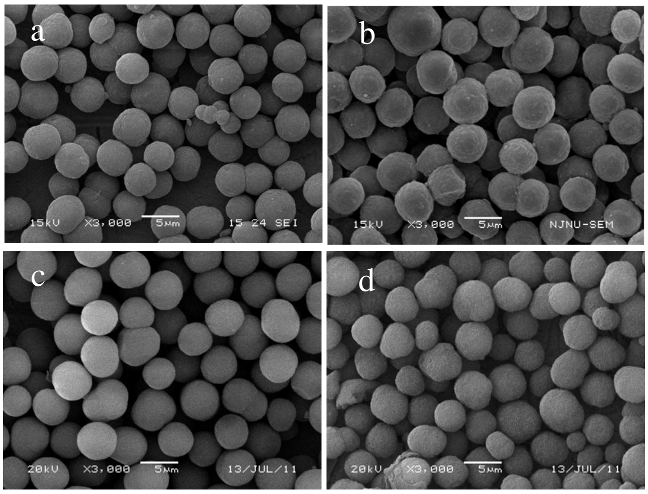

Fig. 1. SEM images of silica particles: (a) S1, (b) S2, (c) S3, (d) S4

Generally, the pore structure of mesoporous silica particles was resulted from the accumulation of the silica sol, thus we could adjust it *via* employing silica sol with different sizes. N_2 adsorption-desorption isotherms and the corresponding BJH pore size distribution curves of the mesoporous silica particles were shown in Fig. 3. As shown in Fig. 3a, all adsorption-desorption isotherms exhibit representative IV isotherms with H_1 hysteresis loop at 87 K. BET specific surface area, pore volume and pore diameter of silica particles were summarized in Table-3. From Table-3, a decrease of both surface area and pore volume of silica particles could be observed with the silica sol size increasing. With the silica sol size increased from 5-8 to 100 nm, the average pore diameter of silica particles increased from 8.7 to 62 nm. That also could be seen in the pore diameter

TABLE-3
PHYSICAL PROPERTIES OF SILICA PARTICLES

Sample	Silica sol	Silica particles			
		Particle size (µm)	Specific surface area ($m^2 g^{-1}$)	Pore volume ($m^3 g^{-1}$)	Average pore diameter (nm)
S1	JX-15	4.8	253	0.55	8.7
S2	SW-25	5.0	188	0.47	10.0
S3	P20-1	5.0	46	0.40	34.8
S4	P20-2	4.6	33	0.18	62.0

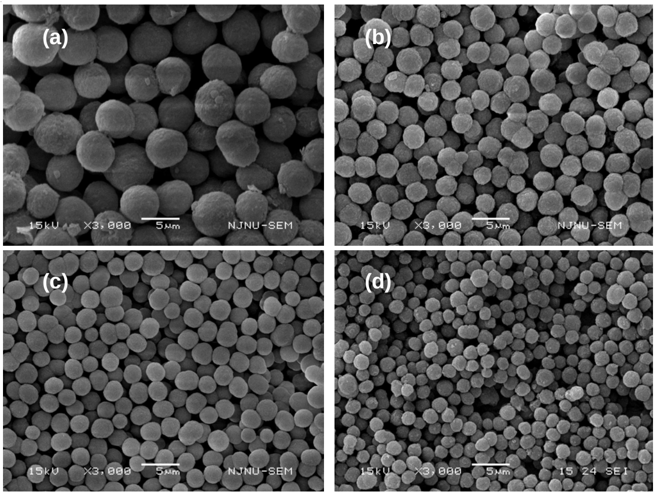

Fig. 2. SEM images of silica particles with different particle size: (a) 5 µm, (b) 3.5 µm, (c) 2.6 µm, (d) 2 µm

distribution curves of silica particles in Fig. 3b. It has been documented that the pore size of mesoporous silica obtained by polymerization induced colloid aggregation method became larger with larger silica sol.

As Table-4 shown, metals in silica particles prepared with commodity silica sol without purification were determined by ICP-AES. Total metal of S3 and S4 was higher than that of S1 and S2. As the stabilizer of SW-25 and JX-15 silica sol was Na_2O, the content of Na in S1 and S2 was obviously higher than other metals. So as to P20-1 and P20-2, the content of K in S3 and S4 was higher than other metals. As Table-4 shown, total metal of S2 was 1461 ppm and refluxing S2 in HCl solution (S5) resulted in a reduction of total metal to 946 ppm, about 64.8 % of S2. Metals could also be removed more effectively by adopting resin treated sol as raw material. As for sample S6, total metal further reduced to 677 ppm, about 45.5 % of S2. Although more than half of the total metal could be removed by this modified polymerization induced colloid aggregation method, ultra purity silica particles could not be obtained.

Effect of preparation conditions: During the polymerization induced colloid aggregation process, acid-catalyzed polymerization took place, the oligomer so formed adsorbed onto the surface of the colloid particles causing them to aggregates and then mesoporous particles were obtained after a high temperature burning process[12]. The morphology and

TABLE-4
ICP-AES DETERMINATION OF TRACE METALS IN SILICAS (ppm)

Sample	Ca	Fe	Al	Na	Mg	K	Zn	Ti	Total metal	SiO_2 (%)
S1	16	91	781	702	11	ND	6	84	1692	99.831
S2	ND	56	607	731	2	ND	ND	65	1461	99.854
S3	22	ND	40	58	ND[a]	6500	ND	ND	6621	99.338
S4	31	10	31	68	3	7100	ND	7	7250	99.275
S5	9	ND	483	359	8	34	2	51	946	99.905
S6	7	46	376	192	3	ND	1	52	677	99.932

[a]Nondetected

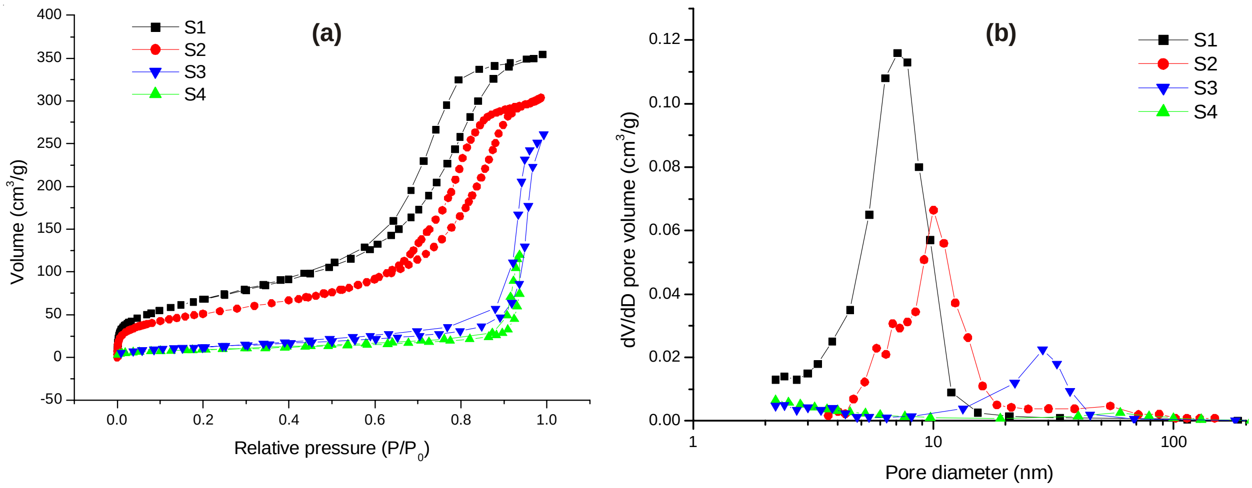

Fig. 3. N_2 adsorption-desorption isotherm (a) and pore diameter distribution curve from the desorption (b) of silica particles

size of particles depend on a variety of parameters, such as molar ratio of reactants, pH, temperature, agitation, *etc*.

Effect of molar ratio of reactants: As Fig. 4 shown, formaldehyde/urea (F/U) molar ratio has great effect on the particle size. With a fixed amount of urea, a higher F/U molar ratio means more amount of formaldehyde. Polymerization rate of formaldehyde and urea increased with the F/U molar ratio rising, leading to higher nucleation amount. Therefore, more nuclei with smaller particle size were obtained at a higher F/U ratio. The experimental results shown that regularly spherical particles with good monodispersity could be achieved at ratio of 1:1-1:1.25, while regular particles could hardly be obtained at either higher or lower ratio.

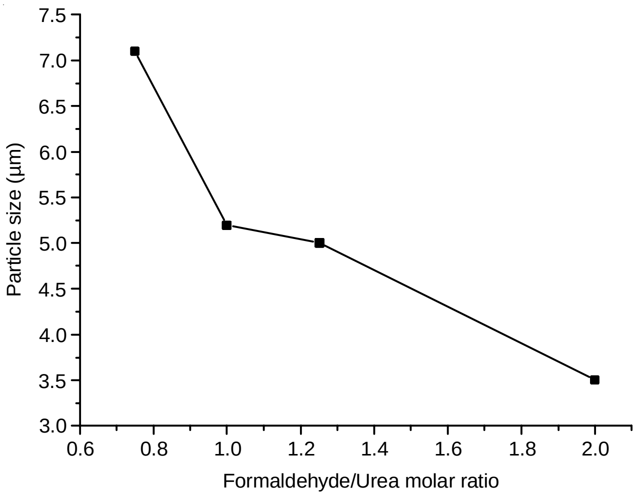

Fig. 4. Effect of formaldehyde/urea molar ratio

The effect of organic precursors/silica sol molar ratio was also examined. As shown in Fig. 5, the particle size increased as the ratio up to 2.4 and regular spherical particles with monodispersity could be achieved at a ratio of 2:1, while monodisperse particles could not be obtained at either higher or lower ratio. It was assumed that increasing this ratio raised the amount of polymer available in solution to assist in particle growth, hence the particle size increased[13].

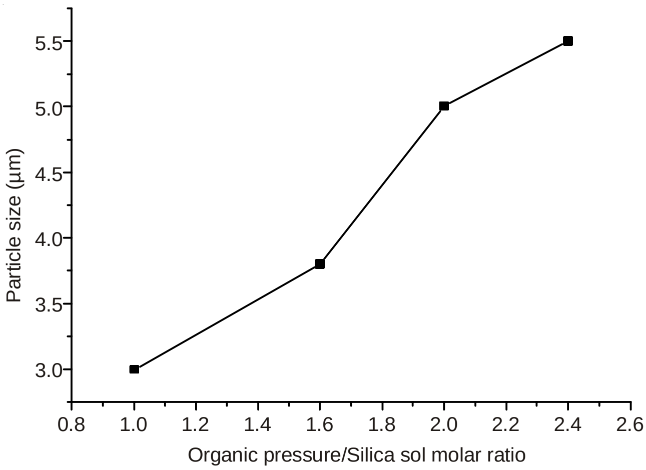

Fig. 5. Effect of organic precursors/silica sol molar ratio

Effect of reaction pH: The reaction pH has also been shown to be important in affecting particle size. Fig. 6 shows that over a wide range of pH (0.5-1.8), particles with monodispersity could be obtained, while both particle size and nucleation time increased with pH up to 1.8. The polymerization of urea-formaldehyde could be catalyzed by acid and thereby, a higher degree of acidity would lead to a higher polymerization rate. As a result, with increasing acidity, the number of hybrid microspheres increased, but their size decreased. However, an excessively high acidity would lead to the separation of silica sol from polymers in the product because of the extremely fast polymerization rate, whereas too low of an acidity would cause the incomplete polymerization of urea-formaldehyde[14]. For instance, monodisperse particles could not be prepared at a pH value of lower than 0.5 because of particle aggregation. Besides, at a pH value of higher than 1.8, the lowered polymerization rate of urea-formaldehyde caused secondary particles formation, thus resulting in a broad particle size distribution.

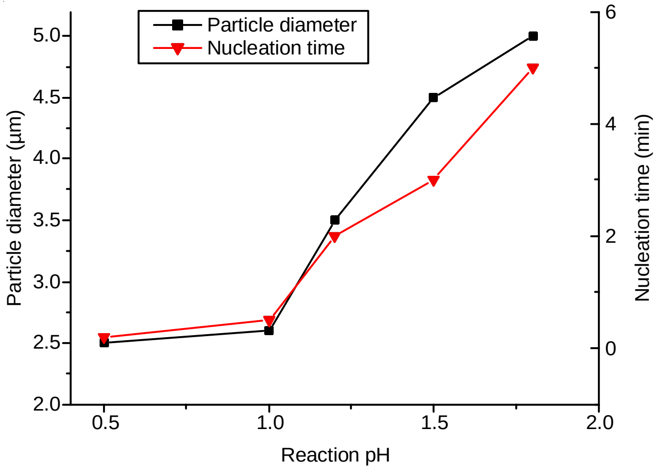

Fig. 6. Effect of reaction pH

Effect of water quantity: Fig. 7 shows the effect of water quantity on the particle size and nucleation time. Along with the increased water quantity, the particle size was decreased, but the nucleation time was much longer. As the water quantity increased, the concentration of reactants decreased, decreasing the reaction and nucleation rates, producing more nuclei and decreasing the particle size. Over a wide range of water/ silica sol molar ratio (35-600), particles with monodispersity

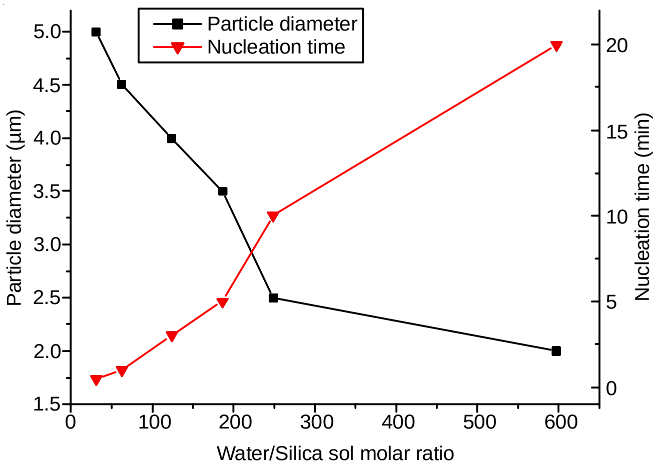

Fig. 7. Effect of water quantity

TABLE-5
SEPARATION PARAMETERS OF C18-MODIFIED S2 SILICA COLUMN

No.	Solutes	*t* (min)	k	A_s	*N* (× 10^4 N/m)	R_s
1	Uracil	1.84	0.00	1.58	3.0	-
2	Nitrobenzene	2.52	0.37	1.37	4.9	3.17
3	Naphthalene	3.75	1.04	1.25	6.4	6.08
4	Fluorene	5.56	2.02	1.17	7.3	2.81

could be obtained. Smallest silica particles of 2 μm were obtained at a ratio of 600 (Fig. 2d).

Chromatographic testing: As Fig. 8 shown, separation of standard mixture was completed in 6 min, with separation factors for all the solutes larger than 3. The separation parameters of silica particles were listed in Table-5. The C18-modified silica column prepared in this work shows high separation efficiency and narrow peaks. Separation of 17 kinds of amino acids was almost not possible under isocratic conditions (Fig. 9). Baseline separation of this mixture was obtained by adopting a gradient mobile phase, as Fig. 9 shown. The above results demonstrate that the C18-modified silica can be used as packing materials for HPLC.

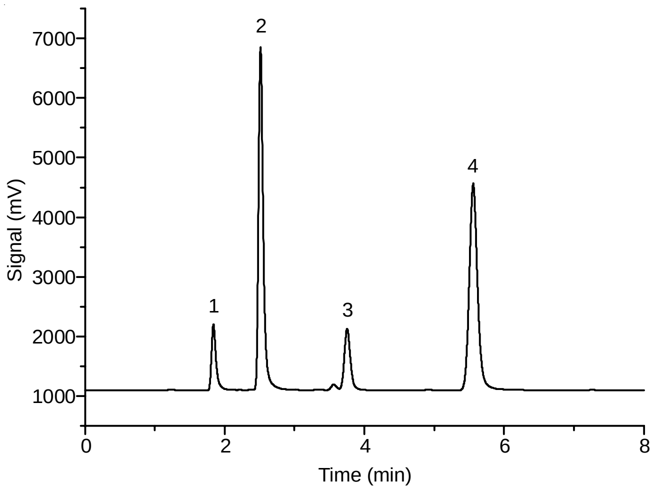

Fig. 8. Chromatographic testing of C18-modified S2 silica column

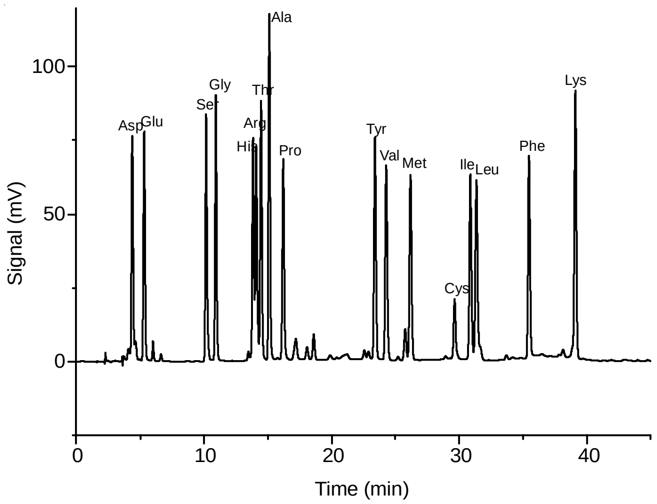

Fig. 9. Seperation of 17 kinds of amino acids; 1. Asp; 2. Glu; 3. Ser; 4. Gly; 5. His; 6. Arg; 7. Thr; 8. Ala; 9. Pro; 10. Tyr; 11. Val; 12. Met; 13. Cys; 14. Ile; 15. Leu; 16. Phe; 17. Lys

Conclusion

In conclusion, micrometer-sized mesoporous silica particles have been synthesized by a modified polymerization induced colloid aggregation method. Silica particles with particle size in the range of 2-5 μm could be facilely obtained by controlling the reaction conditions. The C18-modified silica particles were explored as stationary phase in reversed phase liquid chromatography and baseline separation of 17 kinds of amino acids was obtained.

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