

Studies on a poly(styrene-divinylbenzene)-encapsulated sub-micrometer sized organic–inorganic hybrid silica packing, its synthesis, some of its characteristics and applications

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Abstract

The hydrolyzed product of a mixture of tetraethoxysilane (TEOS) and vinyltriethoxysilane (VTEOS) was used to produce organic–inorganic hybrid silica by sol–gel processing. The product was a kind of composite with vinyl groups incorporated into the structure of the final particles through C–Si bonds. These hybrid silica particles were spherical and the average diameter was about 350 nm as measured by scanning electron microscopy (SEM). The particles were non-porous as proved by BET method.

The hybrid silica particles were subsequently encapsulated with a layer of PS–DVB, a polymeric mixture of styrene and divinylbenzene, with the vinyl groups on the particle surface as anchors of the polymer chains. The encapsulated packing was used as stationary phases in CEC, and the chromatographic behaviors were studied. Experimental results showed that, as expected, the smaller diameter stationary phase resulted in higher column efficiencies. In comparison with the columns packed with 5 μm diameter encapsulated-silica stationary phases of the same kind and other sub-micron stationary phases reported, it can be concluded that the EOF velocity is independent of the particle diameter when using 350 nm phase, but determined by the surface nature of the stationary phase.

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1. Introduction

Stöber and Fink [1] developed a method to produce monodisperse non-porous silica spheres by means of hydrolysis of tetraalkylsilicates in alcoholic solutions, ammonia was used as a morphological catalyst. This kind of sol–gel processing has been commonly adopted to produce organic–silica particles. Spherical particles have also been obtained from reagents with the general formula $\text{RSi}(\text{OR}')_3$, where $\text{R} = \text{Me}, \text{Ph}, \text{allyl}, \text{vinyl}, \text{aminopropyl}$, and $\text{R}' = \text{Me}$ or Et [2–4]. By co-hydrolysis of tetraalkoxysilane and alkyl-trialkoxysilane, organic–inorganic hybrid silica had also been manufactured [5–11]. The final products contained alkyl groups that were incorporated into their matrices, in which both silicious $[\text{Si}(\text{–O–})_4]$ and organically substituted groups $[\text{R–Si}(\text{–O–})_3]$ were mixed to form a hybrid.

Using such an approach, some groups reported their success in preparing column packings particularly for CEC [5,7,8,10]. Among them, Lüdtke et al. [12,13] synthesized 0.5 μm porous sub-micron silica beads. They subjected TEOS and octadecyltrimethoxysilane to hydrolysis to yield monodisperse silica beads, and subsequently calcinated the product at 550 °C to create pores by burning off the organic moieties. After silanization, the porous particles were used for the preparation of CEC column. The column efficiencies obtained ranged from 80,000 to 290,000 plates/m. While Reynolds and Colón [8] reported the production of 450 nm spherical organo-silica particles by hydrolysis and condensation of *n*-octyltriethoxysilane and TEOS under basic conditions, and the direct application of the material as the stationary phase in CEC. Their column efficiencies reportedly varied between 370,000 and 480,000 plates/m.

Following the above process, we produced sub-micron spherical organic–inorganic hybrid silica particles, by hydrolysis and condensation of a mixture of tetraethoxysilane (TEOS) and vinyltriethoxysilane

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(VTEOS) under basic conditions. The Si–C bonds, through which the vinyl groups were connected to the structure of the hydrolyzed product, were stable under normal hydrolysis conditions. The particles were characterized by scanning electron microscopy (SEM), BET method (low-temperature nitrogen adsorption) and elemental analysis.

On the basis of the vinyl groups on the surface of the packing, further modification of the packing can be realized. A styrene-divinylbenzene co-polymer layer was thereby encapsulated on the particles surface according to the procedure we applied previously [14], with the vinyl groups on the surface as anchors of the polymer chains. The optimal packing was obtained through investigations on different ratios between the two substituted silanes in particle production, as well as ratios between styrene and divinylbenzene in the encapsulation of particles. The encapsulated particles were characterized again by SEM and elemental analysis. From the difference of carbon content of particles before and after encapsulation, the thickness of the polymer layer can be calculated as ca. 3 nm.

The technique of CEC offers the selectivity of HPLC, whereas its driving force of the mobile phase is electroosmotic force (EOF) rather than the pressure generated by pumps as in HPLC. Thus, in CEC a column packed with very small diameter particles usually do not have the problem the same column inevitably would cause in HPLC, that is, the problem due to a high back pressure needed to drive the mobile phase through the column. In this paper, experimental results showed that the sub-micron packing material resulted in higher column efficiencies than obtained from a 5 μm phase.

2. Experimental

2.1. Instruments and reagents

2.1.1. Instruments

The CEC instruments were setup in this group with an Ultra Plus micro-pump (Micro-Tech Science, USA) and a CV⁴ UV-Vis detector (ISCO, USA). The high-voltage source (0–30 kV, 9323 type) was from Beijing Institute of New Technologies (Beijing, China), the capillaries (250 μm i.d.) were from Yongnian Optical Fiber Factory (Hebei, China).

2.1.2. Reagents

All reagents employed for the synthesis were of analytical reagent grade and obtained from Beijing Chemical Reagent Factory. HPLC-grade solvents for chromatographic separations were obtained from Fisher Chemicals, USA.

TEOS, ethanol ($\geq 99.7\%$) was filtered through 0.45 μm filtering film before use. Ammonium hydroxide with a concentration of 25–28% (w/w) was used as a catalyst. Double-distilled water was filtered through 0.22 μm

filtering film before use. Vinyltriethoxysilane, styrene and divinylbenzene were all of Chemical Purity Grade. Azoisobutyronitrile (AIBN) was recrystallized from alcohol. Tris(hydroxymethyl)amino-methane (Tris) and 2-(*N*-morpholino)ethanesulfonic acid (Mes) were from Sigma Chemical Co. (St. Louis, MO, USA). The test sample components, apart from the medicinal compounds such as emodin etc., were all of analytical reagent grade and were from Beijing Chemical Reagent Factory, Beijing, China. The medicinal compounds were reference compounds obtained from the Chinese National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China.

3. Synthesis of particles and their encapsulation

3.1. Synthesis of particles

The spherical organic–inorganic silica particles were obtained from hydrolysis of TEOS and VTEOS as the precursors using NH_4OH as catalyst.

Eighteen millilitres of distilled water, 1.5 ml aqueous ammonia and the appropriate amounts of ethanol were mixed with a magnetic stirrer in a 250 ml conical flask. After the solution was homogeneous, the mixture of TEOS (27 ml) and VTEOS (4.5 ml) was added dropwise into the solution while stirring. Stirring was kept on for 4 h after all the hydrolyte was added into the solution, and the solution was subsequently aged at room temperature for 12 h. The abundant amount of white precipitate obtained was filtered out through 0.45 μm filter membrane, washed with ethanol and acetone successively, each for several times, dried under vacuum at 80 °C for 48 h, and kept in desiccator before further treatment. The hybrid silica particles were characterized with SEM, BET method and elemental analysis.

3.2. Encapsulation of silicious particles

One gram of the organic–inorganic silica powder was placed in a three-necked flask equipped with a stirrer, 2 ml *n*-butanol and 10 ml acetonitrile were then added. The suspension was heated under reflux until no further water was liberated. A solution of 0.3 ml styrene, 0.3 ml divinylbenzene and 3.0 mg AIBN was subsequently added. Polymerization was carried out under reflux for 20 h. After cooling, the stationary phase was filtered out, washed with acetonitrile and acetone successively, each for several times, and dried under vacuum at 80 °C. The final product was characterized by means of SEM and elemental analysis.

3.3. Preparation and test of the packed columns with the prepared phases

The inlet and outlet frits, as well as the detection window, were prepared as in [14]. The stationary phase bed was typi-

cally packed by sucking into a capillary a slurry made of 10% (w/v) stationary phase in acetonitrile from the open end, after the inlet frit was in place. The stationary phase bed was then compressed, and finally the outlet frit was sintered on top of it.

Upon test of the packed columns, the mobile phases were composed of the respective buffer solutions (see the legends of the figures) mixed with acetonitrile. They were filtered through 0.45 μm filtering membrane and ultrasonically degassed for 5 min. The columns tested were equilibrated with the mobile phase before testing. The column was subsequently running at a low voltage of 3 kV, until the current was stable. The samples were injected electrokinetically at 3 kV, for 5 s. All the experiments were done at room temperature, the inlet and outlet were all kept at atmospheric pressure. On-column detection was used with a variable wavelength UV detector, with the wavelength set at 254 nm.

4. Results and discusses

4.1. The characterizations of the organic–inorganic hybrid silica and the polymer-encapsulated particles

The particles were characterized by scanning electron microscopy, BET measurement and elemental analysis.

4.1.1. SEM

Particle size, and morphology of the particles were determined by SEM (Joel JEC-5600LV). The sample was coated with a thin conducting layer of gold by sputter coating. From the SEM photograph of organic–inorganic hybrid silica particles (Fig. 1), it can be seen that the particles were monodisperse and spherical. An average diameter $d_p = 350$ nm was obtained from measurements for three times; 30 particles were taken as samples each time. A SEM picture

of polymer-encapsulated hybrid silica particles is shown in Fig. 2, in which no agglomeration of the particles, as well as a significant change in the average diameter of particles can be observed.

4.1.2. BET measurement

The specific surface area of hybrid silica before encapsulation was measured using the BET method, on the basis of the isotherms of nitrogen adsorption at 77 K, with a BC-1 model surface area meter (Lüshun Instruments, China). The sample was purged in a stream of dry nitrogen at 373 K for 2 h prior to measurement.

The results showed that the sample had a specific surface area A_{sp} (BET) of 12.27 m^2/g , a specific pore volume V_{sp} (BET) of 0.31 mm^3/g , and a mean pore diameter D_p ($=4V_{sp}/A_{sp}$ [15]) of 0.10 \AA . Particles with such characteristics are considered to be non-porous in nature.

4.1.3. Elemental analysis

The elemental analysis showed that the carbon content of hybrid silica is 2.24%, after encapsulation the carbon content is 5.78%. The thickness of the polymer layer was calculated with the following equation:

$$d_c = \frac{1000x/C_{\text{polym}}}{A_{\text{sp}}d_{\text{polym}}} (\text{nm}) \quad (1)$$

where d_c is the thickness of the polymer layer, x is the carbon content increment after encapsulation (%), by elemental analysis, C_{polym} is the percentage of carbon in the polymer layer, in our case it was 90% (assuming that the styrene–divinylbenzene ratio remained to be 1:1 in the polymer), A_{sp} is the specific surface area (m^2/g), and the d_{polym} the density of the polymer (g/m^3 , the density of polystyrene is 1.05–1.08 g/m^3 , in our case, a figure of 1.08 is adopted). Thus the thickness of the polymer layer was ca. 3.0 nm by calculation.

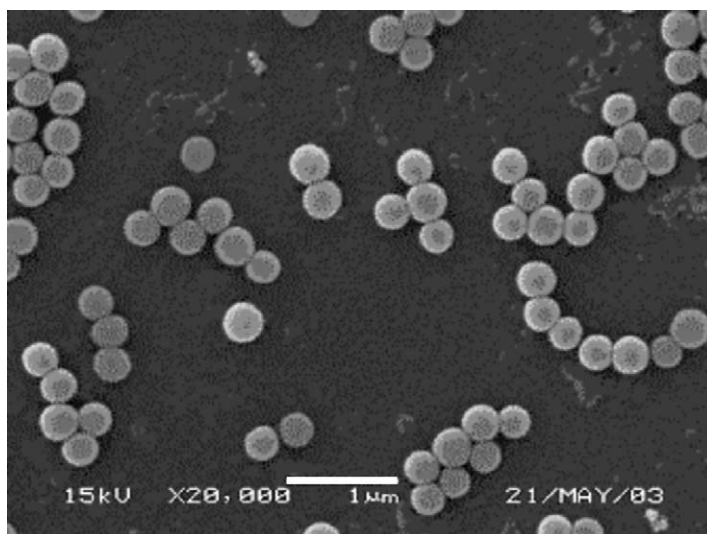


Fig. 1. SEM picture of the organic–inorganic hybrid silica particles.

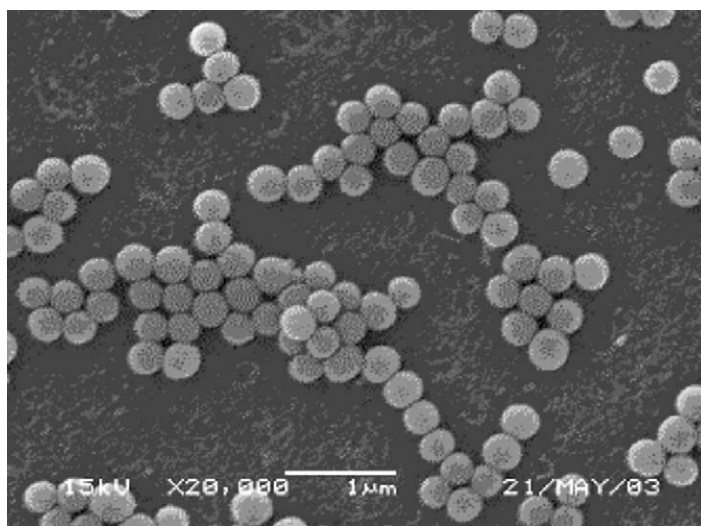


Fig. 2. SEM picture of the polymer-encapsulated packing materials.

From Figs. 1 and 2, no significant difference between the surface smoothness is discernable, it might suggest that an even and intact layer of polymer is encapsulated on the particle surface. Considering the bond-length of the atoms (C–C: 0.154 nm; C–Ph: 0.151 nm; the bond between two neighboring carbon atoms in a phenyl group: 0.139 nm) involved in the polymer, a thickness of 3 nm suggests that there should be more than one layer of polymer molecules on the surface. Experimental results on this kind of stationary phases, however, showed that these more-than-one polymer layers resulted in acceptable chromatographic effects. If a thicker polymer layer than this was formed on the particle surface by changing the conditions in preparing the particles and/or in encapsulation, that would result in tailing peaks and bad column efficiencies.

A polymer layer of 3.0 nm is at the high end of the range of polymer layer thickness reported by the authors studying polymer encapsulation of the stationary phases [15–18]. In fact, also considering the relatively low carbon content of the phase, this polymer thickness is much thicker than other authors' reports. A probable explanation to this discrepancy could be attributed to the non-porous nature of our particles.

In the three-step procedure [14], it is necessary to vinyl-silanize the silanol groups on the surface first. The procedure is time consuming and demanding, because a perfect silanization of the surface silanol groups needs the reactions of three of the latter groups to vinyl-triethoxysilane, if not simultaneously. Besides, the silanization reaction demands strictly on dryness of the reacting system. The procedure used in this paper is free of these problems. The hybrid silica surface was clear and homogeneous. Our results showed that its products were suitable for further modification to produce the stationary phases with good chromatographic characteristics.

However, only after all the factors involved in the mixture hydrolysis procedure are fully elucidated and understood, will this procedure be used to produce high-efficient and reproducible stationary phases. The process is still being investigated in the present author's group. Following are some of the results from the investigations on the characteristics of the phases produced.

4.2. Chromatographic behavior of the columns packed with polymer-encapsulated organic–inorganic hybrid silica materials

4.2.1. Effect of variables on EOF

According to Knox and Grant [19] the u_{EOF} is independent of the average particle diameter, d_p , of the packing material, as long as d_p is greater than 40 times the thickness of the electrical double layer δ . The thickness of the electrical double layer δ is defined by Eq. (2):

$$\delta = \left(\frac{\epsilon_0 \epsilon_r RT}{2cF} \right)^{1/2} \quad (2)$$

where c is the concentration of the buffer solution and F the Faraday constant. It is obvious that δ is affected by the concentration of the electrolyte, which usually varies between 1 and 100 mM. Hence, when the buffer concentration is 5 mM, the thickness of the electrical double layer δ is 4.5 nm and the calculated minimum particle diameter is 180 nm ($=40 \times 4.5$ nm).

In comparison with 5 μm polymer-encapsulated particles, the 350 nm particles of the same kind prepared in this work still resulted in comparable EOF velocity. This appears to conform to the above theory. The general studies on the effect of variables on EOF on the phase prepared in this paper, such as the acetonitrile content or pH of the mobile phase, the results showed the same tendency as in the case of 5 μm phases we prepared previously [14].

However, this EOF velocity was much lower than that obtained from 0.5 μm C₈-stationary phase by Lüdtkke [12,13,20] used in CEC. It seems that the EOF is related to surface properties of the stationary phases.

4.2.2. An investigation on the resulting column efficiencies

Table 1 shows the theoretical plate numbers measured from the columns packed with both the stationary phase prepared in this work and in our previous work. In comparison with the latter, the smaller particle size did result in higher column efficiencies. The reduced plate height, however, is higher compared with others' reports on sub-micron stationary phases [8,12,13]. It should be pointed out though that many factors can be causative of the higher reduced plate

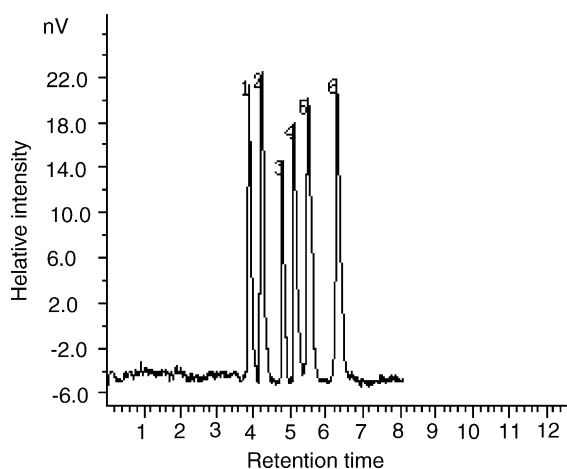


Fig. 3. Electrochromatogram of a mixture of aromatic compounds. Stationary phase: Poly(styrene-divinylbenzene)-encapsulated hybrid silica packing (350 nm). Column: 250 μm \times 16 cm, 49 cm total. Mobile phase: ACN:5 mM Tris-HCl (pH 7.5) = 70:30. Applied voltage: 15 kV. Detection: 254 nm. Peak identification: (1) thiourea, (2) aniline, (3) benzene, (4) toluene, (5) ethylbenzene, and (6) naphthalene.

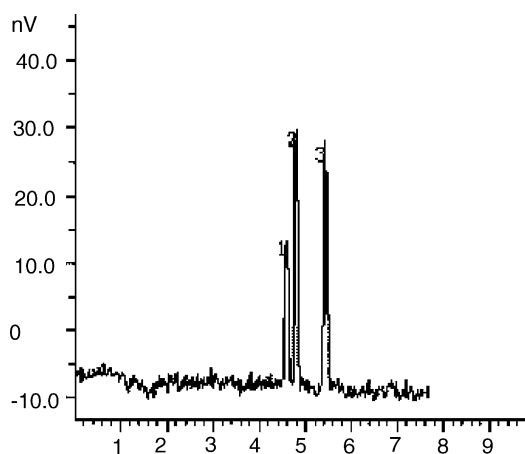


Fig. 4. Separation of acid compounds on a PS-DES column. Stationary phase: poly(styrene-divinylbenzene)encapsulated hybrid silica packing (350 nm). Column: 250 μm \times 15.8 cm, 46 cm total. Mobile phase: ACN:5 mM phosphate buffer (pH 7.5) = 65:35. Applied voltage: 14 kV. Detection: 254 nm. Peak identification: (1) thiourea, (2) acetanilide, and (3) β -naphthol.

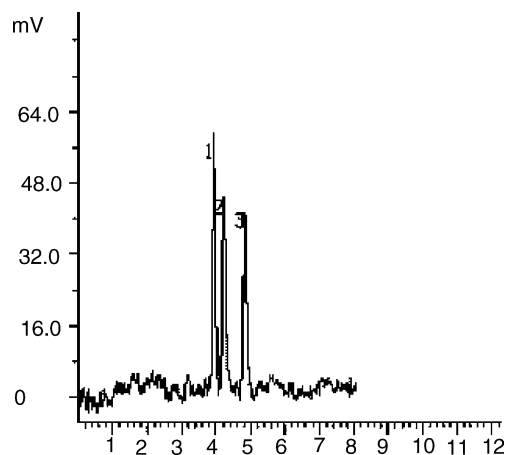


Fig. 5. Separation of basic compounds on a PS-DES column. Stationary phase: poly(styrene-divinylbenzene)-encapsulated hybrid silica packing (350 nm). Column: 250 μm \times 15.8 cm, 46 cm total. Mobile phase: ACN:5 mM phosphate buffer (pH 7.5) = 65:35. Applied voltage: 14 kV. Detection: 254 nm. Peak identification: (1) thiourea, (2) aniline, and (3) 1-naphthylamine.

height: (1) The effect of overlapping of the electrical double layer; (2) The inhomogeneity of the packed bed; (3) The effect of mass transfer in the stationary phase, due to its multilayer structure for example; and (4) The effect of non-optimal mobile phase velocity.

4.2.3. Applications of 350 nm PS-DES phase: CEC analysis of polar compounds and pharmaceuticals

Figs. 3–5 show the separation of some of neutral- and acidic or alkaline aromatic compounds. Good peak shapes were obtained from the separation with the stationary phase prepared. Fig. 6 shows the application of the 350 nm phase

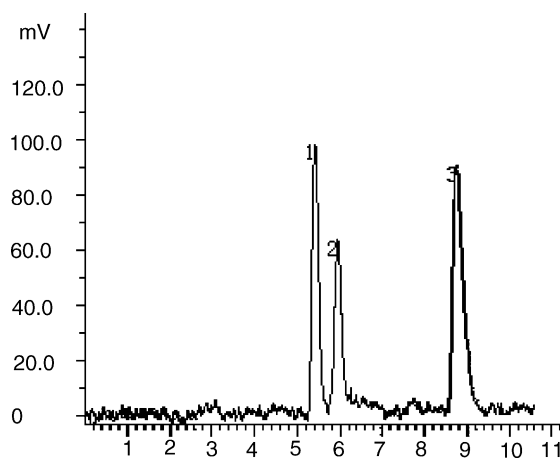


Fig. 6. Separation of some acidic pharmaceutical compounds. Stationary phase: poly(styrene-divinylbenzene)-encapsulated hybrid silica packing (350 nm). Column: 250 μm \times 16.5 cm, 48 cm total. Mobile phase: ACN:buffer(1 mM HClO₄ + 5 mM Mes, pH 4.06) = 70:30 (v/v). Applied voltage: 15 kV. Detection: 254 nm. Peak identification: (1) aloë-emodin, (2) emodin, and (3) chrysophanol.

Table 1

A comparison of retention factors and theoretical plate numbers as obtained from the CEC columns packed with PS-DES 5 μm and 350 nm of diameters

Component	PS-DES (5 μm) ^a			PS-DES (350 nm)		
	k'	$N (\times 10^4 \text{ m}^{-1})$	Reduced plate height	k'	$N (\times 10^5 \text{ m}^{-1})$	Reduced plate height
Thiourea		5.5	3.7		2.2	13
Benzene	0.344	4.8	4.2	0.290	1.9	15
Toluene	0.453	3.3	6.0	0.399	1.5	19
Ethylbenzene	0.595	5.1	3.9	0.540	1.2	24
Naphthalene	0.792	3.0	6.7	0.798	1.1	26
Aniline		5.6	3.6	0.098	2.2	13
1-Naphthylamine		7.8	2.6	0.311	1.8	16
Acetanilide		9.9	2.0	0.045	1.8	16
β -Naphthol		13	1.5	0.173	1.7	17

^a Data cited from the Master Thesis, Zhang (2000) [21].

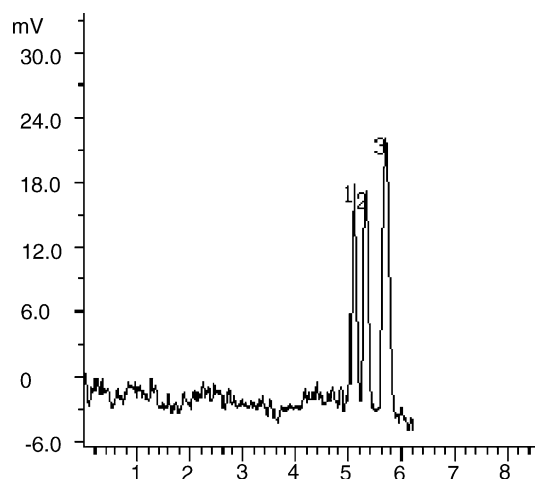


Fig. 7. Separation of some alkaline pharmaceutical compounds. Stationary phase: poly(styrene-divinylbenzene)encapsulated hybrid silica packing (350 nm). Column: 250 μm \times 16.5 cm, 48 cm total. Mobile phase: ACN:1 mM Mes (pH 4.72) = 70:30 (v/v). Applied voltage: 15 kV. Detection: 275 nm. Peak identification: (1) isocaffeine, (2) theine, and (3) caffeine.

in the analysis of some acid pharmaceuticals: three rhubarb anthraquinones. From the chromatogram, it can be seen that better peak shapes could be obtained when pH of the mobile phase was lower. In this case, faster analysis can be realized making use of the characteristics of the phase used, i.e., EOF did not change much when mobile phase pH was low. Excellent separation can also be obtained for alkaline drugs using this stationary phase, see Fig. 7.

5. Conclusions

Tetraethoxysilane and vinyltriethoxysilane were used as the precursors to produce 350 nm spherical, non-porous organic–inorganic hybrid silica by sol–gel processing, on the basis of the vinyl groups incorporated in the material, a polystyrene–divinylbenzene layer was subsequently encapsulated on the surface. The chromatographic charac-

teristics of the packing prepared were evaluated by testing the columns packed with it. Experimental results showed that, the retention characteristics of the 350 nm phase were generally very similar to the 5 μm PS-DES phase we prepared previously. However, due to the smaller diameter of the latter, same good separation could be realized by using shorter effective length at low running voltage. The easily controllable procedure used in this paper could be used to obtain stationary phases of different diameters, which have homogenous encapsulated layer, having reasonably good separation ability. It might be anticipated that, by encapsulating the particles with copolymerization of divinylbenzene and styrene with a substitutional group of different polarities, etc., stationary phases of different selectivity could be produced.

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