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Synthesis, Characterization, and Evaluation of Divinylbenzene-Coated Spherical Nonporous Silica[†]

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Monodisperse nonporous silica particles were coated with different amounts of a divinylbenzene-based polymer resulting in a new class of reversed-phase high-performance liquid chromatographic material. An optimization of the polymer layer, with respect to the thickness, was performed to obtain optimal surface properties of the chromatographic sorbent. The materials were characterized using solid-state ^{13}C CP/MAS NMR spectroscopy, thermogravimetry analysis, BET surface area measurements, scanning electron microscopy, and transmission electron microscopy. The application of the new materials in reversed-phase HPLC revealed extremely short separation times while maintaining a high chromatographic resolution.

1. Introduction

Reversed-phase high-performance liquid chromatography (RPHPLC) is a frequently used method to separate, as well as identify, a great diversity of organic compounds.[1] The sorbent used in a RPHPLC column is composed of a hydrophobic stationary phase, such as C-8 or C-18, which is immobilized on a spherical support, such as silica. Both nonporous and porous silica are often used as supports in chromatography because of their good mechanical stability.[2,3] The use of nonporous silica will give less band broadening compared to porous silica and in combination with the low surface area a faster separation will be achieved, therefore nonporous silica with a hydrophobic surface modification can be useful in protein separations.[4−6] Besides the chemical functionalization of the silanol groups, always present on the silica surface, it is also possible to coat silica particles by means of a physically adsorbed polymer.[7−9] Hence, a reduction of unwanted analyte−silanol interactions is expected.[10,11]

Polymer resins with a low degree of cross-linking suffer from mass transfer limitations as well as swelling problems which can lead to poor chromatographic performance.[12] On the other hand, highly cross-linked styrene−divinylbenzene (DVB) copolymers are in general not as permeable for organic solvents and will therefore not swell to the same extent.[13,14] Therefore a thermal-initiated radical polymerization of pure DVB using dibenzoylperoxide as an initiator was used to coat nonporous spherical silica particles.[15,16]

The different coated silica materials were evaluated using solid-state ^{13}C CP/MAS NMR spectroscopy, thermogravimetry analysis (TGA), Brunauer−Emmett−Teller surface area measurement (BET), scanning electron microscopy (SEM), and transmission electron microscopy (TEM). The coated silica materials were also packed into stainless steel tubes and evaluated as stationary phases under RPHPLC conditions. It was shown that in order to obtain good chromatographic behavior as well as a uniform coating, the thickness of the polymer layer had to be optimized. The best compromise, with respect to chromatographic properties, was obtained when 200 mg of DVB per g of silica gel (200 mg DVB/g silica) was used as starting material during the coating reaction. This paper describes a complete synthesis and characterization of a potentially useful chromatographic material.

[†] In Memory of Prof. Dr. Ernst Bayer.

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2. Experimental Section

2.1. Coating of the Nonporous Silica. Nonporous silica (2 g) (Micra NPS Silica 1.5 μm) was suspended in mineral spirits (60 mL; Aldrich, alkane mixture, CAS 69551-17-7) in a three-necked 250-mL round-bottomed flask. To avoid grinding of the silica the reaction mixture was stirred with a mechanical stirrer at such a high spinning rate that the particles did not stick together. The suspension was heated to 100 °C and divinylbenzene (80 mg; Aldrich, techn., 80%, mixture of isomers) was added. Thereafter dibenzoyl peroxide (29.76 mg; Fluka, purum > 97%, based on dry substance, moistened with ~25% water) was added to initiate the polymerization reaction. The reaction mixture was stirred for 18 h and then cooled to room temperature and filtered. The sorbent was washed with mineral spirits (2 × 60 mL), ethanol (2 × 60 mL), and diethyl ether (2 × 60 mL), and thereafter dried for 2 h at 100 °C under reduced pressure. The experiment was repeated using 20, 60, 80, 100, 200, 300, 400, and 500 mg of divinylbenzene/g of silica, respectively. The molar ratio divinylbenzene/dibenzoyl peroxide (moistened with 25% water) was 10:1 in all experiments.

The sorbents were slurry-packed into stainless steel tubes with the dimensions 4.6 × 33 mm, using standard procedures.

2.2. Chromatography. Analytical liquid chromatography was performed with a Merck-Hitachi 6200A solvent delivery pump and a Merck-Hitachi L-4000A variable wavelength UV detector.

Samples were introduced via a Rheodyne injector equipped with a 20-μL loop. Double-distilled water and HPLC grade acetonitrile (Merck, Darmstadt) were used. The void volume was determined from the position of acetone or uracil. The mobile phases used during the separations were mixtures of water/acetonitrile, and all analytes were dissolved in acetonitrile.

2.3. NMR Studies. All ^{13}C−CP/MAS NMR spectra have been recorded on a Bruker ASX 300 Spectrometer (7.05 T) at a spinning rate of 10 000 Hz with a 4-mm double bearing probehead. The proton 90° pulse length was 3.5 μs and the temperature was 295 K. The spectra were obtained with a cross-polarization contact time of 2 ms. The pulse intervals were 1 s. Glycine was used as reference and to adjust the Hartmann−Hahn condition. The number of transients used in the experiments was between 40 000 and 80 000 depending on the amount of surface loading.

2.4. Sample Preparation and Electron Microscopy. For scanning electron microscopy (SEM), the sample powder was placed on a commercial specimen stub covered with a conductive adhesive tab. Subsequently, the sample was provided with a sputtered 15-nm gold coating which prevents charging during the investigations but is not thick enough to obscure sample structures on the scale of SEM. Scanning electron micrographs were recorded on a Philips XL 30 scanning electron microscope with a primary beam energy of 15 keV at normal beam incidence detecting secondary electrons.

Specimens for transmission electron microscopy (TEM) were prepared by ultramicrotomy.[17] The sample powder was embedded into EMbed 812 epoxy resin purchased from Science Services, München. The resin was allowed to infiltrate the specimen for 24 h and subsequently polymerized at 60 °C for 48 h. Ultrathin sectioning was performed with freshly prepared glass knives on an Ultrotome III 8800A ultramicrotome by LKB. Because of the extreme hardness of the silica core the use of a diamond knife was abandoned.

The sample particles are nonporous and their silica core is extremely hard and brittle for the purposes of ultramicrotomy. For this reason, the connection between the resin and the particles is insufficient. A considerable amount of the particles fracture, and additionally the blade of the knife pushes the fractured parts out of the resin. This results in TEM samples of only moderate quality and with a thickness of about 60 nm to be estimated from the color of the ultrathin sections. These were transferred to commercial copper TEM grids provided with a 10-nm amorphous perforated carbon foil as specimen support. It has to be noted that only nearly equatorial sections of the particles are found to remain within the resin. This means that forces perpendicular to the plane of knife movement balance each other when the blade hits the spherical particles almost rectangularly, i.e., near their equator.

Energy-filtering transmission electron microscopy (EFTEM) was performed on a LEO 912 Ω transmission electron microscope. The instrument is equipped with a Köhler illumination system, a microdose focusing system, an Ω-type imaging electron energy filter,[18] and a cooled high-speed slow scan charge coupled device (CCD) camera and was operated at a primary beam energy of 120 keV. Element spectroscopic imaging[18] (ESI) was performed using the three window technique with an energy window of about 12 eV.

3. Results and Discussion

To synthesize a reversed-phase chromatographic sorbent the silica particles were coated with different amounts of DVB-polymer. Nine coatings using different amounts of starting material varying between 20 and 500 mg of DVB per g of silica gel (mg DVB/g silica) were made.

Scanning electron microscopy (SEM) was performed on the coated particles with 100−400 mg DVB/g silica to detect differences in the surface coverage. These results show, at least when 400 mg DVB/g silica was used (Figure 1), that both the thickness and the surface coverage of the DVB-polymer were increased when the amount of starting material used in the polymerization reaction was increased.

If an insufficient amount of starting material was used in the polymerization it was possible to detect areas that were not covered totally with DVB-polymer, as shown in Figure 1a. However, uncovered surface areas were still present in the 200 and 300 mg DVB/g silica materials (see Figure 1b and c); still the total surface load increased as confirmed from the TGA measurements. On the other hand, if too much starting material was used during the polymerization reaction irregularly coated silica particles were obtained (Figure 1d). Unfortunately, the silica particles were clogged together and formed a polymer/silica mixture when an excess of starting material was used, this is also shown in Figure 1d. This loss of monodispersity of the sorbents can lead to insufficient chromatographic behavior and must therefore be avoided. Another problem associated with a thick polymer layer is a bad mass transfer which leads to a reduction of the efficiency of the separations. These assumptions were confirmed from the chromatographic experiments.

The ^{13}C−CP/MAS NMR investigations show a similar tendency from the particles coated with small amounts of pDVB to those with relatively higher amounts of pDVB.

Five ^{13}C−CP/MAS NMR spectra with different amounts of polymer coating were recorded, see Figure 2. The number of transients was increased, to obtain similar signal-to-noise ratio, when sorbents synthesized with a low amount of starting material were analyzed.

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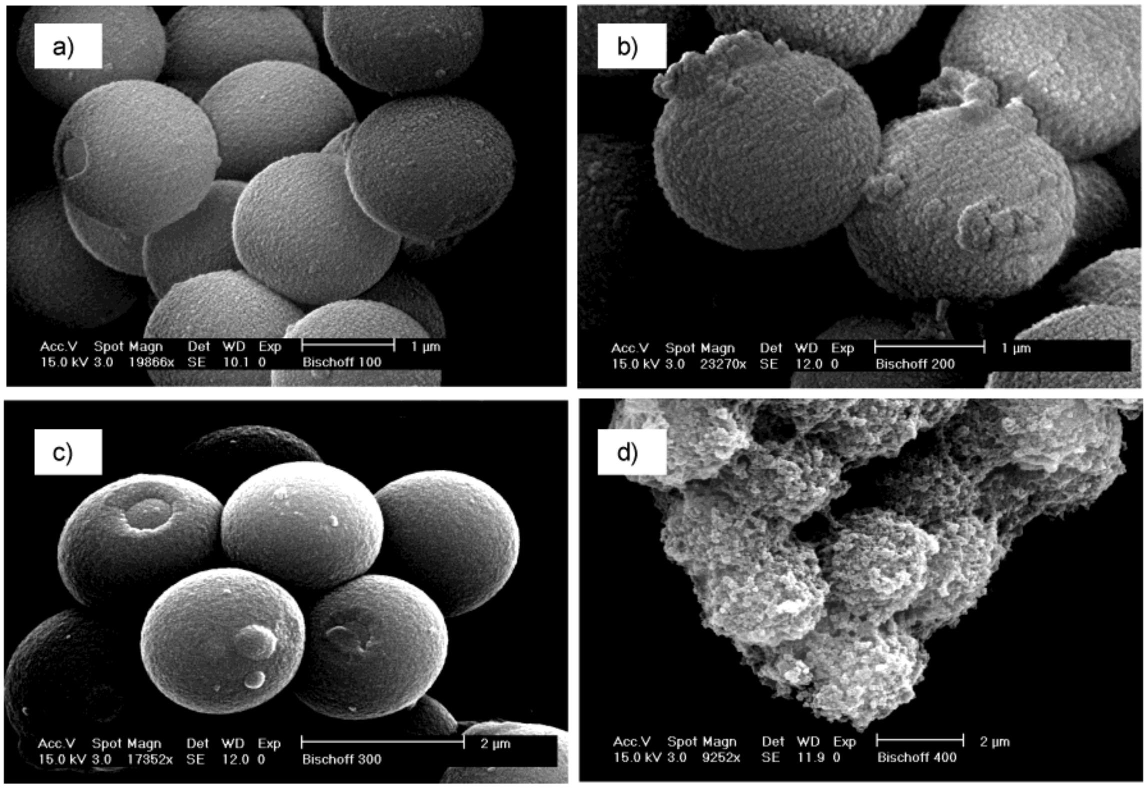

Figure 1. SEM pictures showing DVB-coated nonporous silica with different concentrations of starting material: (a) 100 mg DVB/g silica; (b) 200 mg DVB/g silica; (c) 300 mg DVB/g silica; and (d) 400 mg DVB/g silica.

This implies, at least qualitatively, that there are different amounts of polymer deposited on the silica surface.

Because of the cross polarization technique it is not possible to compare quantitatively even “similar groups” within one spectrum. This is because the carbon atom types are not accounted for equally and hence have a difference in the cross polarization constants.[19,20] Still it is possible to compare identical groups between different spectra if the NMR conditions are the same.

In Figure 2 the signals between 40 and 50 ppm correspond to the aliphatic backbone of the DVB-polymer ($-CH_2-$ and $-CH-$ groups), this is in accordance with other studies.[21−24] The resonances between 30 and 15 ppm represent the methylene and methyl carbon impurities from the ethyl groups attached to the aromatic residues.

The signal at 125 ppm, indicated **a**, corresponds to the aromatic part of the DVB-polymer, and the signal at 146 ppm, indicated **b**, corresponds to the two quaternary carbon atoms in the aromatic ring.

Nonpolymerized DVB side chains (unreacted vinyl groups) included in the polymer are expected to result in signals at 136 and 113 ppm, here indicated **c** and **d**.

By using CP/MAS NMR only direct comparisons of the same atoms between the different spectra in Figure 2a−e were possible. Obviously the **c** and **d** peaks were more pronounced when a low level of DVB (spectrum e) was used in the synthesis than when more DVB was used (spectrum a). This implies that the polymerization process becomes less efficient when a decreasing amount of DVB monomer is used.

Also, the signals of the aliphatic carbon atoms ($-CH_2-$ and $-CH-$ groups) between 40 and 50 ppm were broadened. This result also implies that the polymerization process becomes less efficient on the way from spectra a to e. The remaining signals at 60−80 ppm and 160−170 ppm result from the reaction between the initiator and DVB. From a comparison of spectra a to e an increase of these signals was noticed. Because the initiator was situated at the ends of the polymer network a less efficient polymerization of the polymer from spectra a to e was again indicated.

The amount of coating was further investigated with TGA (see Table 1). The results showed an increased loss of weight from the sorbents synthesized with higher amounts of DVB. This indicates that the DVB-polymer layer on the silica particles was thicker when a higher amount of starting materials was used in the synthesis. Two samples of each material were measured to control a good reproducibility of the measurements. The results from the TGA are in agreement with the NMR and the SEM measurements.

For more detailed information about the homogeneity of the thickness of the coating layer transmission electron microscopy was performed, see Figure 3. Because of the well-known difficulties of treating the brittle 1.5-μm coated silica, a model compound with a

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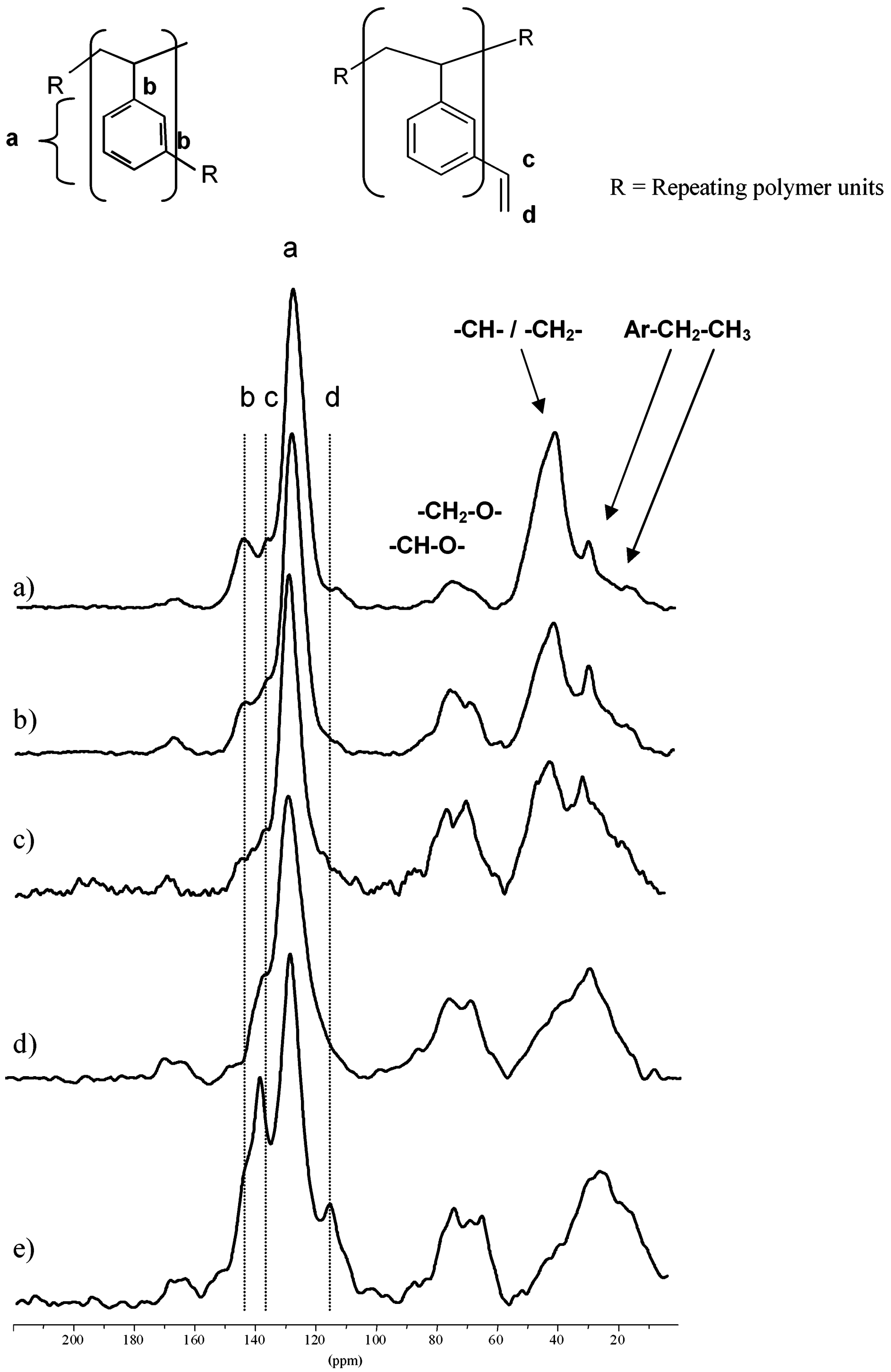

Figure 2. 13-C CP/MAS-MNR spectra of coated nonporous silica from synthesis using (a) 500, (b) 300, (c) 200, (d) 80, and (e) 20 mg DVB/g silica as starting material.

Table 1. TGA Measurements (Weight Loss) of DVB-Coated Silica Particles in Concerns of Reproducibility

	amount of DVB used for synthesis				
	100 mg	200 mg	300 mg	400 mg	500 mg
weight loss A	8%	13%	20%	26%	29%
weight loss B	10%	14%	18%	27%	32%

diameter of 550 nm was synthesized using the Stöber process as described elsewhere.[25] This material was coated with 200 mg DVB/g silica and subsequently investigated by EFTEM. The size of this model compound can be found within the size distribution of the 1.5-μm material.

An example zero-loss filtered transmission electron micrograph of silica particles coated with 200 mg DVB/g silica is displayed in Figure 3a. An intact cross section of a silica sphere with a diameter of about 550 nm embedded in the resin can be seen (position 1) and also a void occurs where the cross section of a silica sphere was removed during the sectioning process (position 2).

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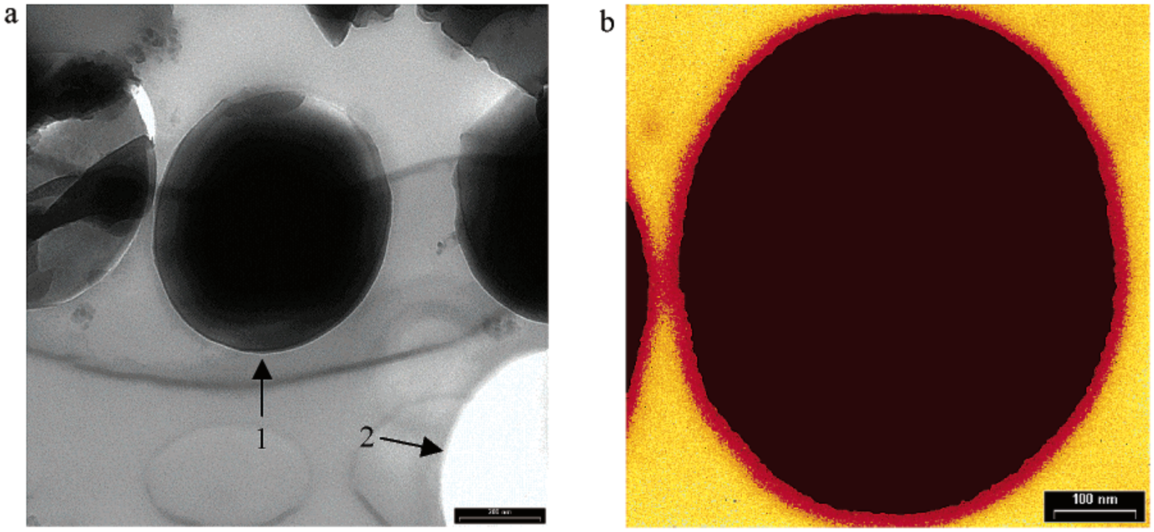

Figure 3. TEM pictures showing 2 different coatings. (a) Zero-loss filtered TEM cross-sectional view of 0.7-μm nonporous silica particles coated with 200 mg DVB/g silica prepared by ultramicrotomy. An intact cross-section of a silica sphere with a diameter of about 550 nm embedded in the resin can be seen (position 1). Also, a void occurs where a silica sphere was located before its removal during the sectioning process (position 2). A large part of the specimen is obscured by particles from fractured silica spheres (scale bar = 200 nm). (b) Carbon distribution across a 1.5-μm nonporous silica particle coated with 200 mg DVB/g silica (position 1 in Figure 3a), mapped by electron spectroscopic imaging (ESI). Around the silica core (dark area) a nearly uniform layer of lower carbon content or density than the surrounding epoxy resin with an approximate thickness of 30 nm is detected. This is attributed to the coating of the particle (scale bar = 100 nm).

Table 2. Structural Properties of Unmodified and Modified Silica with DVB

sample	BET (m^2/g)	total pore volume (cm^3/g)
bare silica	2.9	0.004
100 mg DVB	42.9	0.031
200 mg DVB	69.2	0.050
400 mg DVB	160.1	0.165
500 mg DVB	134.6	0.099

A large part of the specimen is obscured by particles from fractured silica spheres.

The elemental distribution of carbon across the silica sphere situated at position 1 in Figure 3a was mapped by element spectroscopic imaging (ESI) at the carbon K edge (energy loss $\Delta E = 284$ eV) to provide an estimate of the coating thickness.

The specimen had to be stabilized by preirradiation at low electron doses prior to performing ESI. During this process, electron-induced mass loss is completed. As different polymers exhibit different mass losses,[26-29] the uniform layer of lower carbon content as shown in Figure 3b represents the DVB coating with an approximate thickness of 30 nm. Because of the curvature of the particle, an overlap region exists where the electron beam probes resin and coating. However, from geometric considerations, this overlap is only about 2 nm at a sphere diameter of 550 nm and a specimen thickness of about 60 nm and therefore can be neglected within the limits of error. The TEM shows that it is possible to use this coating procedure to obtain uniform DVB coating of the silica spheres.

The BET measurements (Table 2) confirm that the surface area increases with increasing amounts of DVB-polymer up to 400 mg DVB/g silica, and thereafter decreased when more DVB was used in the synthesis.

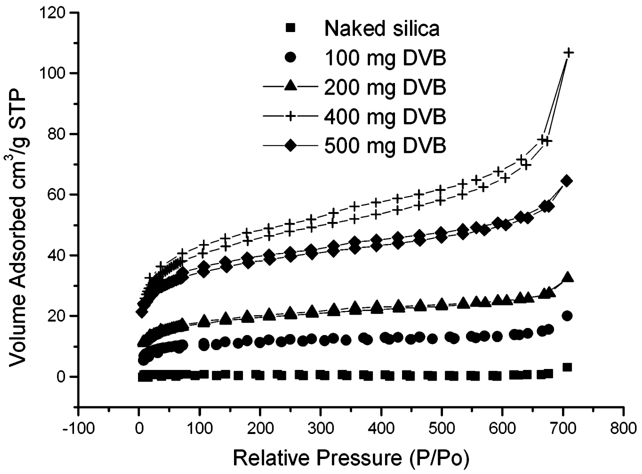

Figure 4. Adsorption and desorption isotherms for the different DVB-coated silica.

The adsorption and desorption isotherms of the sorbents are shown in Figure 4; these results show that the isotherms are of type II which is the normal one obtained from nonporous adsorbents.

To evaluate the chromatographic properties of the coated silica gels five micro-columns were packed with the sorbents obtained from the synthesis using 100–500 mg DVB/g silica gel as a starting material. Eight analytes (Scheme 1) were eluted from the five different columns using a solvent mixture of acetonitrile/water. The t_0 value was estimated from the nonretarded analytes uracil or acetone.

From the chromatograms (Figure 5) it is obvious that the k' value for a given analyte is increased when the DVB-polymer layer is increased. This is in agreement with eq 1 where β is the phase ratio ($V_{\text{stationary phase}}/V_{\text{mobile phase}}$).

$$k' = K \times \beta \qquad (1)$$

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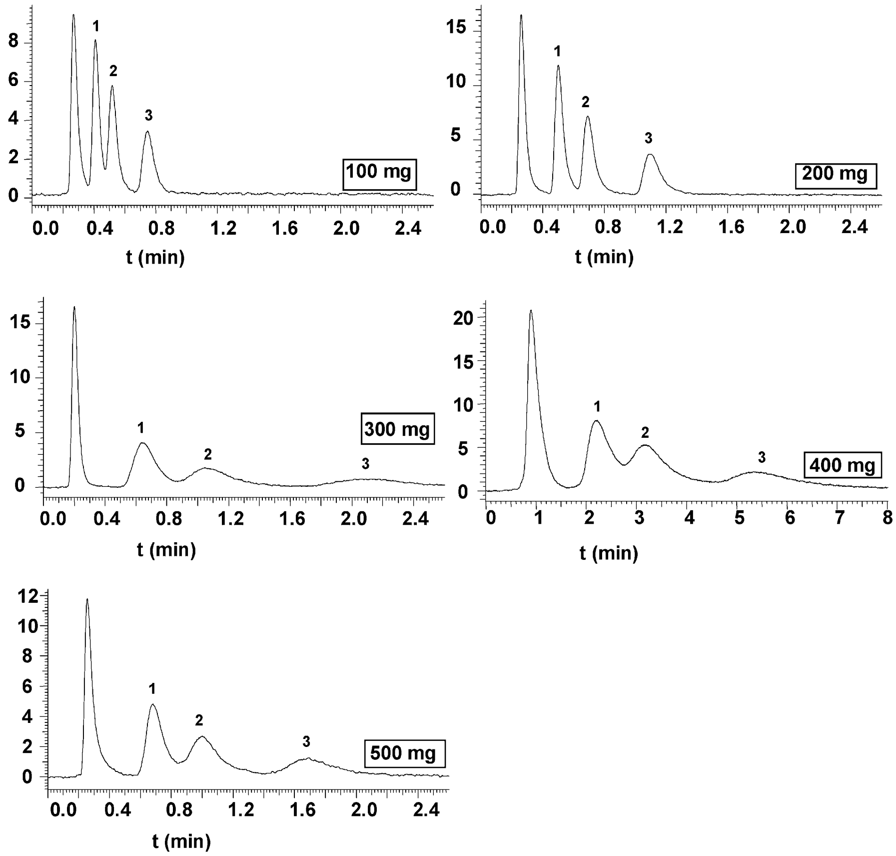
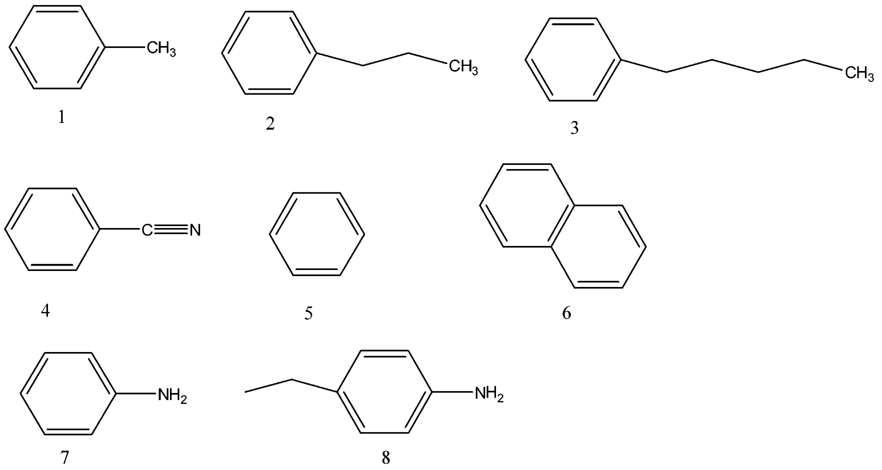


Figure 5. Chromatograms of compounds 1−3 from the columns 1−5 with a mobile phase mixture of acetonitrile/water (85:15).

Scheme 1. Analytes 1−8.



It can further be seen that the peaks are broadened when the DVB-polymer layer is increased; this is most certain an effect of an unfavorable mass transfer. However, when the analytes 1−6 were eluted from the columns packed with 400 and 500 mg DVB/g silica sorbents the capacity factors decreased significantly compared to that of the 300 mg DVB/g silica. This is due to a reduction of theoretical plate numbers (N) caused by the clogging of the silica particles.

The best chromatographic properties, with respect to resolution, were obtained when the material from the synthesis using 200 mg DVB/g silica gel as starting material was used. A baseline separation of analytes 1 and 4−6 was obtained within 2 min (Figure 6). The

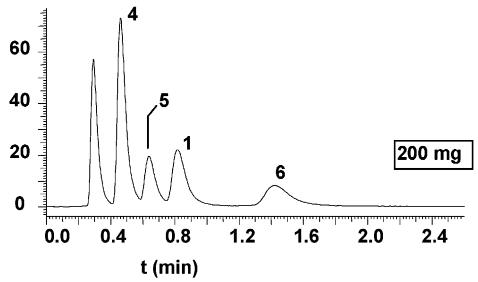


Figure 6. Chromatogram of compounds 1 and 4−6 from column 2 with a mobile phase mixture of acetonitrile/water (90:10).

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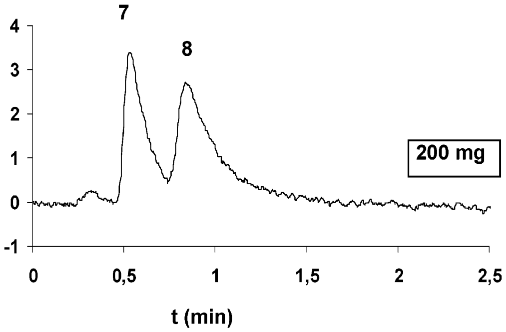

Figure 7. Chromatogram of compunds 7 and 8 from column with a mobile phase mixture of acetonitrile/water (40:60).

separation of the basic analytes 7 and 8, without significant tailing, implies that the silanol groups at the silica surface are satisfactorily coated, see Figure 7. These results show the high potential of this new class of stationary phases for efficient rapid separations which might be useful in the field of proteomics and metabolomics.[2,30]

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