Use of alumina with anchored polymer coating as packing material for reversed-phase high-performance liquid chromatography

Ying Mao, B.M. Fung*

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK 73019-0370, USA

Abstract

A new material with anchored polymer coating on alumina can be used as a packing material for the reversed-stationary phase of HPLC columns. The coatings are formed by in situ polymerization of maleic acid adsorbed on alumina with 1-octadecene, with cross-linking by 1,4-divinylbenzene. The chromatographic performance of this material was studied and compared with that of 20–30 μm octadecyl-bonded silica particles and a commercial column packed with 5 μm octadecyl-bonded silica. The results indicate that this new material is a promising alternative to silica-based reversed-phase packings due to its competitive performance and stability in acidic and basic media. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, LC; Alumina stationary phases; Polymer coating; Coating

1. Introduction

Silica-based packing materials have dominated as reversed-phase (RP) high-performance liquid chromatographic (HPLC) packings for decades due to the availability of various kinds of silica. Silica surface and organosilanization chemistry are well understood, and many types of bonded-silica phases can be synthesized using organosilanes [1–4]. However, silica-based reversed-phases have an important limitation [5,6] in their operating pH range because the silica back-bone, Si–O–Si, hydrolyzes at pH>8, and the siloxane bond is unstable at pH<2.

Alumina is an attractive alternative to silica owing to its inherent tolerance towards wider pH variation ranging from 1–13 [7]. Conventionally, alumina is widely used as a polar adsorbent in column chromatography. Recently, efforts have been made to seek

suitable alumina-based reversed-phase packings to compensate the drawback of silica-based ones [6]. The first attempt to modify an alumina surface was made to employ organosilanization chemistry [8,9], using a silanization reagent to react with the hydroxyl groups on alumina to form Al–O–Si linkage. However, the simple Al–O–Si linkage is unstable and easily hydrolyzed under acidic conditions [5], preventing it from being used as efficient packing material for RP-HPLC. The stability of the Al–O–Si linkage can be increased by first treating alumina with trialkoxysilane, followed by reacting the silyl hydride groups with 1-alkene to cover the surface with long alkyl chains [10–12]. Chromatographic testing demonstrated that the material exhibited reversed-phase behavior, and no significant degradation was observed in a phosphate solution over an extended period. However, because the synthesis involves a two-step reaction and requires a Pt catalyst, the cost of the material is of practical

*Corresponding author.

concern. In addition to organosilanes, organic phosphonic acid was also used to form a linkage of Al–O–P–R on the surface [11,13]. This kind of material can be used in basic solvents with basic solutes, but the linkage is not stable in phosphate buffer solutions at pH 7. In another approach, alumina was first chlorinated with thionyl chloride, followed by reaction with an organolithium compound [5], resulting in an Al–C linkage, which may have a better stability than the Al–O–Si–R or Al–O–P–R linkage. The material is reported to be stable in nonpolar, polar and aqueous media with pH 1.0, 7.0 and 12.0. However, the procedure requires the use of an organolithium reagent, making it uneconomical.

Because of the problems related to the formation of an organic coating on alumina via chemical bonding, polymer-coated alumina seems to be a more practical alternative [14–22]. In this approach, a polymer such as polybutadiene is dissolved in a proper solvent and mixed with alumina particles for a period of time. Then the solvent is evaporated, leaving the polymer on the alumina surface [17]. If the polymers (or prepolymers) still have a reactive functionality, they can be crosslinked by using radiation or initiator to improve its stability [18]. In this process, the coverage of the alumina surface is greatly increased and the polymer thickness can be easily controlled by simply varying the concentration of prepolymers. However, the polymers are physically coated on alumina, and the interaction with the support is weak even though crosslinking improves the stability to some extent.

Recently, we have developed a new material with anchored polymer coatings on alumina [23,24], which is abbreviated as APCA in the following discussion. The material is prepared in a two-step process. Acid-washed alumina particles are allowed to equilibrate with an aqueous solution of 0.1 M maleic acid at pH 2.0. Then the maleic acid molecules adsorbed on the surface of the alumina are copolymerized with a 1-alkene, such as 1-octadecene, at 80°C in the presence of a free-radical initiator. The adsorption of carboxylic acids on alumina is very strong with adsorption energies much larger than those of other organic compounds [25]. When the adsorbed maleic acid molecules form a 1–1 alternating copolymer with a 1-alkene through

in situ polymerization, the resulting polymer film has one out of every two segments strongly adsorbed on alumina, and can be considered to be anchored on the surface. The anchoring mechanism is attributed to the formation of two strong hydrogen bonds by each carboxylic group with the alumina surface [24]. A cross-linking agent such as 1,4-divinylbenzene (DVB) is added during the polymerization process to increase the molecular mass and the stability of the copolymer to further extent. The product formed is a monolayer of polymer coating, which is stable under washing for 24 h with water, 0.1 M HCl, 0.1 M NaOH, methanol, water–methanol and water–acetonitrile mixtures. Procedures for making the APCA material and characterization of the polymer films are described in detail elsewhere [23,24]. In this report, we describe the potential utilization of the APCA product as a packing material in RP-HPLC applications.

2. Experimental

2.1. Materials

All chemicals were reagent, analytical-reagent or HPLC grade and were used as received unless otherwise specified. Acid alumina is thin-layer chromatography (TLC) grade from Alltech, the particle size was 10–15 mm. Vydac 218TP, an octadecyl-bound silica with particle size of 20–30 μm and pore diameter of 30 nm, was purchased from Sigma. An Econosphere C_{18} 5 mm HPLC column (15 cm×4.9 mm) was purchased from Alltech, and is designated as EcSi–C_{18}.

2.2. Preparation of DVB-crosslinked-poly(maleic acid-1-octadecene)-coated alumina

A 60.0 ml volume of 0.1 M maleic acid solution in water was added to 5.0 g of alumina. The mixture was left in a water-bath shaker at room temperature for 48 h. The alumina particles were then filtered and air-dried. To a 50 ml 3-neck round bottom flask, 4.0 g of alumina with adsorbed maleic acid, 0.10 g of azobisisobutyronitrile (AIBN) and 12 ml of 1-octadecene were added. After the reaction proceeded for 1 h at 80°C under stirring, 0.24 ml of DVB (2%

Y. Mao, B.M. Fung / J. Chromatogr. A 790 (1997) 9–15

11

of the total monomers in volume) was added from an addition funnel placed in one neck of the reaction flask, and the reaction was allowed to continue for two more hours. Afterwards, the mixture was filtered and the alumina particles were washed with acetone to remove the unreacted monomers. The polymer-coated alumina products were dried in air.

2.3. Column packing

The modified alumina was used to pack a 15 cm×4.6 mm stainless-steel HPLC column using the

Fig. 1. Chromatograms using APCA–C_{18} (top), VySi–C_{18} (middle), and EcSi–C_{18} (bottom) columns. A mixture of p-nitroaniline (1), methyl benzoate (2) and xylene (3) in water–acetonitrile (50:50, v/v) was passed through each column at the flow-rate of 1.0 ml/min.

slurry packing technique. A 3.1 g amount of the packing material was mixed with 22 ml of acetone and sonicated to form a slurry right before being packed into the column. The packing pressure was 7320 p.s.i. (1 p.s.i.=6894.76 Pa). The column is designated as APCA–C_{18}. Immediately after packing, the column was conditioned with water–acetonitrile (50:50) at 1.0 ml/min until a smooth and stable baseline was obtained. This was achieved after about 250 ml of the mobile phase was pumped through the column. Vydac 218TP silica was packed into another column of the same dimension under the same conditions for the purpose of evaluating the packing technique, and the column was designated as VySi–C_{18}.

2.4. Chromatography

The equipment consists of a Scientific Systems Model 520C HPLC system connected to a Showdex RI-71 refractive index detector. HPLC grade acetonitrile and deionized distilled water were used to prepare the mobile phase (water–acetonitrile, 1:1) and the mobile phase was degassed and filtered through a 0.45 mm nylon 66 membrane with a in-line filter de-gasser from Alltech. A mixture of p-nitroaniline, methyl benzoate and o,m,p-xylene with a concentration of 0.7 mg/ml each was used for testing the reversed-phase characteristics of the material. A mixture of acetone, nitrobenzene and toluene with the concentration of 0.1 ml/ml for each component was used for the selectivity study. Both mixtures use the mobile phase as the solvent. 20 ml of each sample were injected and the flow-rate was operated at 0.5 or 1.0 ml/min.

2.5. Surface area measurement

The experiments were carried out on Accelerated Surface Area and Porosimetry System ASAP 2010. A 0.5 (±0.0001) g amount of sample was loaded into a sample holder which was then connected to the de-gas port. De-gassing was accomplished at 60°C until the vacuum pressure reached below 1 mmHg (1 mmHg=133.322 Pa). Then the sample holder with the de-gassed sample was transferred to the analysis port. The nitrogen adsorption and desorption isotherms were performed at 77 K. The
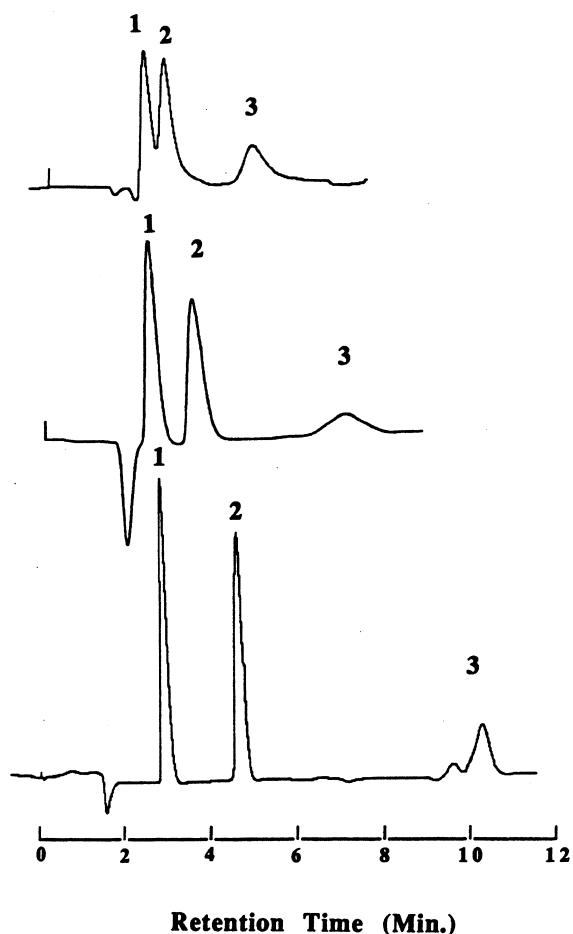
Table 1
Retention times for the APCA–C_{18} column

Solute	t_R (min)
p-Nitroaniline	2.3
Methyl benzoate	2.9
Xylene	4.9
Mixture	2.4, 2.9, 5.0

analysis and data processing were automated by computer control.

2.6. Scanning electron microscopy

The experiments were performed on a JEOL JSM 880 system with LaB6 filament at 15 keV accelerating voltage. The samples were prepared by means of sputter coating with Au–Pd (60–40) using the Technique Hummer VI apparatus.

3. Results and discussion

The mixture of *p*-nitroaniline, methyl benzoate and xylene is a commonly used standard for RP-HPLC testing [16,19]. Therefore it was used in this study to test if the APCA material possesses the packing characteristics of reversed-phase (Fig. 1). The retention times for individual solutes and for

each component in the mixture were obtained and are summarized in Table 1. Because the retention times are such that *p*-nitroaniline<methyl benzoate< xylene, the elution order clearly shows that polar solutes are eluted from C_{18}–Al column faster than less polar ones, demonstrating the reversed-phase characteristic of the APCA material.

For comparison, the same mixture was also injected into VySi–C_{18} and EcSi–C_{18} columns to obtain corresponding chromatograms (Fig. 1). The characteristics of the stationary phases used in the study for comparison are summarized in Table 2, and values of the retention factor, selectivity and theoretical plate number calculated from the elution curves (Fig. 1) are listed in Table 3.

The results in Fig. 1 and Table 3 show that the performance of the APCA material is acceptable, but not as good as the octadecyl C_{18} silica material, especially when the particle size of the silica is smaller, as in the commercial column EcSi–C_{18}. An exception to this is that the values of *k* and *N* for nitroaniline for the APCA material are higher than those for the VySi–C_{18} material (Table 3).

To investigate the origin of the difference in the performance of the HPLC columns, we have examined and compared the scanning electron micrographs of our APCA–C_{18} material and the VySi–C_{18} particles. From the scanning electron microg-

Table 2
Chracteristics of stationary phases

	APCA–C_{18}	VySi–C_{18} [a]	EcSi–C_{18} [a]	Unisphere Al–C_{18} [b]
Surface area (m^2/g)	80	120–125	200	47
Particle size (nm)	10–15	20–30	5	8
Pore diameter (nm)	8	30	8	24.5
Carbon loading (%)	4	9	10	

[a] Information regarding VySi–C_{18} and EcSi–C_{18} was provided by the manufacturers.
[b] Information regarding unisphere Al–C_{18} is given in Refs. [16,20].

Table 3
Comparison of column performance[a]

	APCA–C_{18}			VySi–C_{18}			EcSi–C_{18}		
	k	α	*N*	*k*	α	*N*	*k*	α	*N*
p-Nitroaniline	0.44		352	0.28		257	0.88		1528
Methyl benzoate	0.81	1.8	269	0.94	3.4	330	2.0	2.3	2070
Xylene	2.1	2.6	381	3.5	3.7	347	5.4	2.7	3576

[a] *k*=retention factor; α=selectivity; *N*=column efficiency.

raphs (Fig. 2), it can be seen that the particles of APCA–C_{18} have irregular shapes and a diverse size distribution, whereas the VySi–C_{18} particles, although larger on the average, have rather regular sizes and shapes. At a higher magnification (Fig. 3), it can be seen that the silica particles have much better surface porosity than the alumina particles. Therefore, the difference in the HPLC performance is due to the inferior nature of the alumina support

rather than differences in the organic coatings. The importance of the inorganic support is more obvious when the performance of the two kinds of silica particles are compared (Fig. 1 and Table 3). The superior performance of the EcSi–C_{18} column can be attributed to the small size (5 μm) of the particles which were packed under a higher pressure (25 000 p.s.i.), whereas the column packed with VySi–C_{18} has a much lower efficiency due to its larger particle
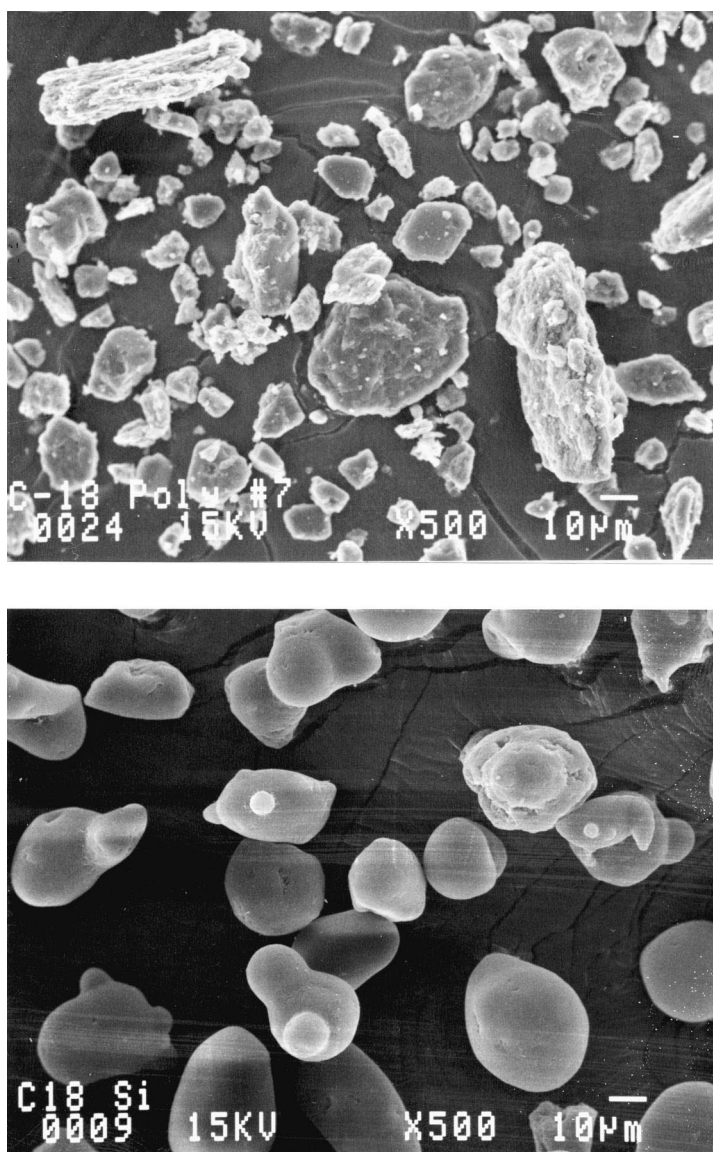

Fig. 2. Scanning electron micrographs at ×500 magnification. Top: alumina coated with poly(maleic acid-1-octadecene); bottom: VySi–C_{18}.
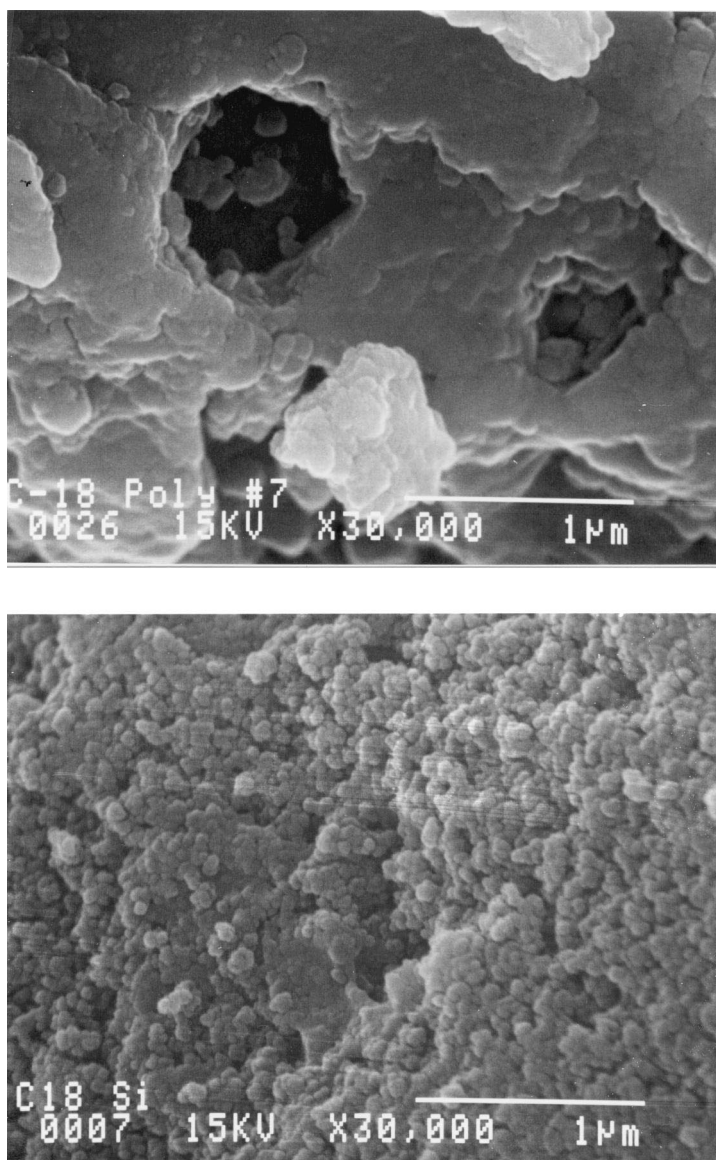
Fig. 3. Scanning electron micrographs at ×30 000 magnification. Top: alumina coated with poly(maleic acid-1-octadecene); bottom: VySi–C_{18}.

size (20–30 μm). Usually, larger particles are used in preparative columns and have a smaller resolution.

An attempt was also made to compare the APCA–C_{18} column with a commercial polymer–coated alumina column, Unisphere Al–C_{18}, using the same solutes and mobile phase. The selectivity of toluene relative to nitrobenzene, calculated using the retention data obtained from Fig. 4 was 2.1, and that for the Unisphere was obtained from Ref. [14] was 2.7. These data show that the APCA–C_{18} column is comparable with Unisphere Al–C_{18} column in terms of selectivity. The Unisphere alumina particles have a very uniform diameter of 8 μm and regular pore structure [14,18] and is a very good support for polymer-coated particles. Unfortunately, they are not available to us in acid-washed form; commercial
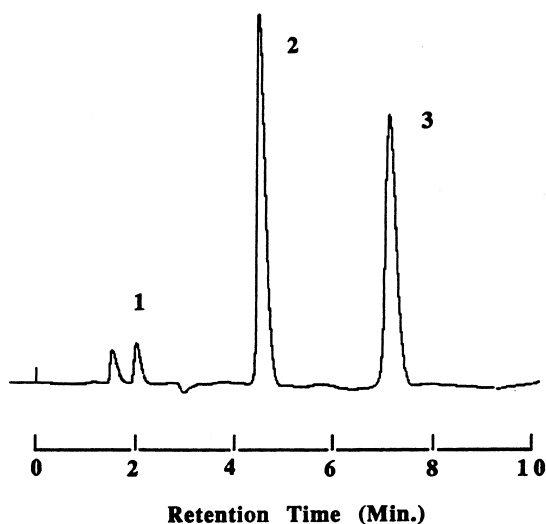
Fig. 4. Chromatograms using an APCA–C$_{18}$ column with water–acetonitrile (50:50, v/v) at the flow-rate of 1.0 ml/min. 1: Acetone; 2: nitrobenzene; 3: toluene.

HPLC quality alumina particles that were acid-washed in our laboratory did not adsorb maleic acid very well, so that we could not prepare good APCA material from them. This is likely due to the fact that the surface properties of alumina are largely determined by the production process rather than post-production treatment.

In conclusion, we have found that alumina particles coated with DVB-crosslinked-poly(maleic acid-1-octadecene) demonstrate reversed-phase properties and have a promising chromatographic performance in terms of selectivity. The column efficiency would be greatly improved if spherical particles with smaller size and narrower size distribution are employed as the support, and the column is packed under higher pressure. Because the anchored polymer coating can be easily derivatized when allyl compounds instead of 1-alkene are used to copolymerize with the adsorbed maleic acid [23], future studies should focus on specific applications using various types of APCA material as the stationary phase in RP-HPLC.

Acknowledgements

References

[1] H. Engelhardt, W. Low, M.M. Eberhardt, Chromatographia 27 (1989) 535.

[2] K.K. Unger (Editor), Packings and Stationary Phases in Chromatographic Techniques, (Chromatographic science series, Vol. 47), Marcel Dekker, New York, 1990.

[3] R.P.W. Scott, Silica Gel and Bonded Phases, Wiley, New York, 1993.

[4] M.J. Worth, R.W.P. Fairbank, H.O. Fatumbi, Science 275 (1997) 44.

[5] J. Pesek, H.-D. Lin, Chromatographia 28 (1989) 565.

[6] R. Kaliszan, J. Petrusewicz, R.W. Blain, R.A. Hartwick, J. Chromatogr. 458 (1989) 395.

[7] C. Laurent, H.A.H. Billiet, L. de Galan, Chromatographia 17 (1983) 253.

[8] J.H. Knox, A. Pryde, J. Chromatogr. 112 (1975) 171.

[9] S.V. Slavov, K.Y. Chuang, A.R. Sanger, J. Phys. Chem. 99 (1995) 17019.

[10] C.-H. Chu, E. Jonsson, M. Auvinen, J.J. Pesek, J.E. Sandoval, Anal. Chem. 65 (1993) 808.

[11] J.J. Pesek, J.E. Sandoval, M. Su, J. Chromatogr. 630 (1993) 95.

[12] J.J. Pesek, V.H. Tang, Chromatographia 39 (1994) 649.

[13] J.E. Haky, S. Vemulapalli, L.F. Wieserman, J. Chromatogr. 505 (1990) 307.

[14] V. Arenas, Analyst 119 (1994) 1303.

[15] P.T. Jedrzejewski, L.T. Taylor, J. Chromatogr. Sci. 33 (1995) 438.

[16] G. Schomburg, LC·GC 6 (1988) 36.

[17] J.R. Garbow, J. Asrar, C.J. Hardiman, Chem. Mater. 5 (1993) 869.

[18] G. Schomburg, Trends Anal. Chem. 10 (1991) 163.

[19] D.A. Hanggi, N.R. Marks, LC·GC 11 (1993) 128.

[20] A. Kurganov, U. Trudinger, T. Isaeva, K. Unger, Chromatographia 42 (1996) 217.

[21] M. Petro, D. Berek, Chromatographia 37 (1993) 549.

[22] A. Kurganov, V. Davankov, T. Isajeva, K. Unger, F. Eisenbeiss, J. Chromatogr. A 660 (1994) 97.

[23] Y. Mao, Ph.D. dissertation, University of Oklahoma, Norman, OK, 1996.

[24] Y. Mao, B.M. Fung, J. Colloid Interface Sci. 191 (1997) 216.

[25] L.R. Snyder, J. Chromatogr. 23 (1966) 388.