



Short communication

A simple cladding process to apply monolithic silica rods in high performance liquid chromatography

Joseph Chamieh*, Yvan Zimmermann, Anne Boos, Agnès Hagège

Laboratoire de Chimie Analytique et Sciences Séparatives, UMR 7178 IPHC-DSA, ECPM 25 rue Becquerel, 67087 Strasbourg Cedex, France

ARTICLE INFO

Article history:

Received 1 July 2010

Received in revised form 1 September 2010

Accepted 7 September 2010

Available online 16 September 2010

Keywords:

Silica monolith

Bimodal porous material

Cladding

Liquid chromatography

ABSTRACT

A simple cladding procedure was developed to apply monolithic silica rods in chromatography. This was used to evaluate the performances of new monolithic silica phases synthesised using a triblock copolymer as a phase separation inducer. The cladding procedure is found efficient after several tests, including scanning electron microscopy and mercury porosimetry, and the obtained columns present a relatively good efficiency in adsorption chromatography (H_{min} is about 50 μm) as compared to other lab-scale developed columns.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

During the last 10 years, much attention has been paid on monolithic silica columns in separation field. Their high permeability associated to their high performances under fast operating conditions compared to particulate columns is one of the main reasons for the development of these columns. Moreover, the silica chemistry is well known and can lead to a wide range of phases with different selectivities. However, in contrast to organic monoliths, silica monolithic columns of classical diameters cannot be prepared in situ due to the shrinkage accompanying the drying stage.

This difficulty led most of the research to the preparation of monolithic columns inside capillaries. Their small dimension presents an advantage since it was reported that up to a diameter of 100 μm , the shrinkage was insignificant. Thus a silica capillary can be used directly as both the preparation mould and the chromatographic column [1]. Moreover, a previous alkali treatment of silica capillaries to activate the silanol functions, allows the monolith to react with those silanols and bind covalently to the capillary inner wall [2] avoiding the formation of a preferential path for mobile-phase solvent between the capillary and the monolith.

In the case of larger monoliths, minimizing the space in-between the silica rod and the column tube remains challenging. Commercially available columns Chromolith covered with a PEEK (polyetheretherketone) resin by a Merck proprietary process, shows quite good performance [3] but the cladding method is

unfortunately not described in the literature and well patent-protected [4–7]. As a consequence, only little research was conducted to propose solutions for the cladding process. Some authors used heat shrink PTFE (polytetrafluoroethylene) to cover the silica rods [8–13]. Two methods were essentially proposed, both using PTFE heat shrinkable tubing. Tanaka et al. [13] wrapped monolithic silica with heat shrinkable PTFE tubing and equipped it with PTFE ferules. The obtained columns were used with a Waters Z-module system, which can apply a radial external pressure of 100 kg/m^2 to guarantee a leakproof system. This method is simple to use but does not allow high operating pressures. Another alternative is to glue the whole heat shrink PTFE-monolith inside a stainless steel tubing using an epoxy resin [12]. This method presents the disadvantage to restrict the range of solvents that can be used since the epoxy resin used is attacked by some organic solvents, e.g. n-heptane.

Recently Morisato et al. [14] proposed an alternative cladding inside glass tubes: the monolithic silica gels were put in silicate glass tubes and the glass wall was adhered by heating while evacuating the inside of the tube.

In this paper we describe a cladding process using PEEK material which can be used on a laboratory scale to test monolithic columns. It was used to encase new silica monoliths synthesised using F68 triblock copolymer $((\text{EO})_{80}-(\text{PO})_{30}-(\text{EO})_{80})$.

2. Materials and methods

2.1. Chemicals

Tetramethoxysilane (TMOS, from Sigma–Aldrich, Saint-Quentin, France) was used as the silica source. Poly(ethylene

* Corresponding author.

E-mail address: joseph.chamieh@univ-lyon1.fr (J. Chamieh).

glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) F68: (EO)₈₀-(PO)₃₀-(EO)₈₀ with an average molecular weight of 8400 (hydrophilic/lipophilic balance, HLB > 24) was purchased from Sigma–Aldrich (Saint-Quentin, France). Acetic acid (Carlo Erba – SDS, Paris, France) was used as a catalyst for hydrolysis. Aqueous ammonia (Carlo Erba, Torino, Italy) was used for the generation of mesopores. Nitric acid (Fluka, Paris, France) and ethanol (Carlo Erba, Torino, Italy) were used for washing the monoliths before the drying step.

For the chromatographic measurements a mixture of *n*-heptane (Carlo Erba, Torino, Italy) and 1,4-dioxane (Sigma–Aldrich, Saint-Quentin, France) 95:5 (v:v) was used as the mobile phase and a test mixture of 15 µg mL⁻¹ toluene, 1 µg mL⁻¹ 2,3-dimethylanthraquinone and 1 µg mL⁻¹ 2-nitroacetanilide (all purchased from Sigma–Aldrich, Saint-Quentin, France) in solution in the mobile phase was used for the separations.

2.2. Preparation of silica rods

Silica monoliths were prepared according to the procedure described elsewhere [15]. Briefly, TMOS was added to a 0.01 M acetic acid solution in the presence of F68 and the mixture was magnetically stirred in an ice bath. The quantity of the organic polymer was varied in order to obtain silica monoliths with different physical characteristics. The resulting mixture was then poured into cylindrical moulds and kept for gelation and aging for 48 h at 40 °C using a thermostated water bath. Wet silica rods thus formed were washed with ultra pure water and treated at 120 °C with ammonium hydroxide to generate mesopores, the concentration of ammonium hydroxide and the duration of the treatment were adapted to the desired mesopore size. Afterwards, the gels were immersed in 0.1 M nitric acid then in a 25:75 ethanol:water (v:v) mixture and dried at 50 °C for 3 days. Finally, they were heat treated, with a temperature ramp of 1 °C min⁻¹ and kept for 2 h at 600 °C to remove the organic polymer and stabilize the silica surface.

2.3. Characterisation of both silica rods and columns

The resulting silica rods were characterised by mercury porosimetry with a Micromeritics Pore sizer 9320 instrument (GA, USA) for total pore volume and macropore size determinations. A JEOL JSM 6700F scanning electron microscope (SEM) (Tokyo, Japan) was used for both observations and estimations of the skeletal size by taking the average thickness of at least 50 narrow portions between branching points [9]. The domain size was calculated by summing the skeletal size and the macropore size. Nitrogen adsorption/desorption isotherms were recorded at 77 K using a Sorptomatic 1990 (Thermoquest, CE instruments, Milan, Italy). The adsorption branch was used to estimate the average pore size and the pore size distribution using the Barrett–Joyner–Halenda (BJH) method [16].

2.4. Cladding process

The silica monolith was placed in a PEEK tube having an inner diameter slightly larger than the monolith's diameter, the whole being surrounded by a heat shrinkable PTFE tube.

The whole assembly was then placed in an oven and heated at 315 °C for 60 min and finally raised and maintained at 345 °C for another 60 min. After cooling down to room temperature, the PTFE layer was removed and both ends of the PEEK–monolith rod were reshaped in order to insert chromatographic fittings. This method was used throughout the whole study.

2.5. Chromatographic tests

Separations in adsorption chromatography mode were realised to test the columns. HPLC separations were carried out using a Waters Alliance Separations Module 2965 pump connected to a photodiode array detector (PDA, Waters 2996) (all from Waters, Milford, USA) used at 254 nm to monitor the chromatograms.

A commercial monolithic column, Chromolith-Si (4.6 mm i.d. × 10 cm) (Merck, Darmstadt, Germany) was also used for comparison.

Van Deemter curves were plotted from 2-nitroacetanilide peak width using non-linear least squares regressions.

3. Results and discussion

The principle of the cladding process developed in this study is based on the application of a radial pressure generated by PTFE shrinkage on heated PEEK tubing. It relies then on the fine control of temperature. PEEK has typically a glass transition temperature of 143 °C and a melting temperature of 343 °C. Independent tests have shown that PEEK exhibits a heat distortion temperature up to 315 °C [17], while the heat shrinkable PTFE tubing contracts at 327 °C. Combining all these characteristics, the temperature program should allow PTFE to shrink and deform PEEK which will take the shape of the monolith and adhere to its surface.

3.1. Cladding temperature effect

Experiments were performed in order to determine the optimal temperature for cladding. Two identical silica monoliths (macropores: 1.7 µm, skeleton: 1.3 µm, mesopores: 16 nm) were cladded independently. For the first one, the final temperature was chosen to be 330 °C, i.e. between the shrinking temperature of PTFE (327 °C) and the melting temperature of PEEK (343 °C), and for the second one, the temperature (345 °C) was slightly exceeding the melting temperature of PEEK.

The column cladded at a final temperature of 330 °C, shows no separation (column: 10 cm × 4.9 mm i.d.). Both the total absence of resolution and the low pressure drop (83 psi) indicate the existence of preferential paths probably occasioned by a poor cladding quality.

When the cladding procedure was performed at a temperature higher than the melting temperature of PEEK, the separation profile changed (column 8.8 cm × 4.9 mm i.d.). The separation of the test mixture was realised in <5 min. The associated pressure drop, normalised for 10 cm length, was in this case 160 psi, which suggests a better cladding.

Since the radial pressure applied by the PTFE tubing onto the whole length is weak, this may require the PEEK being less viscous and more malleable in order to be able to deform and adhere to the monolith surface.

3.2. Verification of the cladding quality by physical methods

Apart from the achievement or not of a chromatogram, it was necessary to verify the cladding quality. The difficulty arises from the contribution of the quality of both the cladding and the monolith itself to the chromatographic performances, which cannot be easily assessed separately. For that purpose, columns were checked by several methods.

3.2.1. SEM analysis

Several columns were cut at different places in order to verify by SEM the absence of dead volume. This column had already been used in chromatography and submitted to a pressure drop of at least 1500 psi. As shown in Fig. 1, no dead volume was observed by

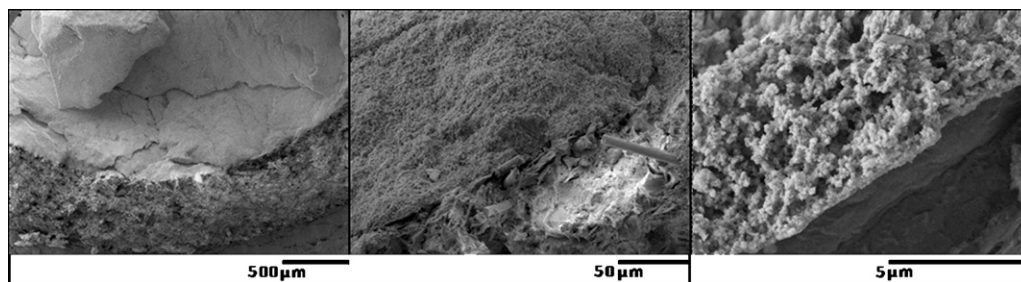


Fig. 1. SEM images at different scales showing the interface between the monolith and the PEEK cladding. The sample is a section of a previously served column (max backpressure reached 1500 psi).

SEM analysis after the application of high pressure. Furthermore no PEEK penetration inside the monolithic structure was observed. The PEEK resin seems to marry the surface without altering the structure of the monolithic material.

3.2.2. Mercury intrusion analysis

To corroborate SEM analysis results, mercury intrusion analyses were also realised on longer pieces of columns, and the pore size distribution of the column was compared to the pore size distribution of both bare monolithic material from the same batch and PEEK tubing, all heated the same way.

The obtained results show no difference in pore size distribution between the bare monolith and the column piece, i.e. no appearance of pores presenting of larger diameter which would reveal a poor cladding.

The relative density and the porosity of the PEEK material as well as of the bare monolith were determined by analysing the samples separately. The results are recapitulated in Table 1. It should however be noted that values of pore volume and porosity obtained for PEEK are to be taken carefully since they contain an important measuring error due to the PEEK low porosity. From the low pressure analysis on the column segment and the obtained data from the analysis of the PEEK and the monolith separately, the weight of the monolith in the column segment can be deduced as well as the weight of the PEEK cladding. From these determined masses the corresponding pore volume for each constituent of the column segment could be calculated and compared to the total injected mercury volume (Table 1).

The theoretical total pore volume of the column segment calculated from the measurements on the two constituents is $0.58 \text{ cm}^3 \text{ g}^{-1}$. It can be compared to the measured pore volume, obtained directly from the mercury analysis, which is $0.59 \text{ cm}^3 \text{ g}^{-1}$. However, this difference (1.5%) cannot be assimilated to an extra void volume since the comparison of pore size distribution profiles of the bare monolith and the column segment does not show any significant difference. Consequently, this difference is attributed to the experimental error, classically estimated around 5% [18] and these pore volumes values can be considered as identical.

Table 1

Results obtained by mercury intrusion porosimetry for PEEK material, bare monolith and a column segment.

	Weight (g) ^a	Density (g cm^{-3}) ^b	Porosity (%) ^b	Sample volume (cm^3) ^b	Experimental pore volume ($\text{cm}^3 \text{ g}^{-1}$) ^c	Theoretical pore volume ($\text{cm}^3 \text{ g}^{-1}$) ^a
PEEK	0.7223	1.32	4.2			
Monolith	0.1962	0.24	89.3			
Encased monolith	1.1901	0.79	46.8	1.50	0.59	0.58

^a Calculated from the densities and porosities determined for each material separately, from the total weight and from the total volume of the sample determined from the low pressure analysis.

^b Determined from low pressure analysis.

^c Result of the product of the both sample porosity and volume reported to the sample mass.

3.3. Verification of the cladding quality by chromatography

3.3.1. Permeability of the columns

The monoliths were applied in normal phase chromatography.

Fig. 2 represents the plots of pressure drop, normalised to a 10 cm column length, as a function of the mobile phase velocity.

Columns were run at different flow rates, till mobile phase velocities 4–8 times greater than the optimal one. The corresponding backpressures were not found to damage the monolith, for example a maximum backpressure of 1250 psi was applied to the column SM-2.2 without altering the stationary phase.

The permeability of the prepared columns was calculated according to Eq. (1) and compared to the Chromolith's permeability in order to verify the quality of the cladding.

$$B_0 = \frac{u_0 \eta L}{\Delta P} \quad (1)$$

where B_0 is the permeability (m^2), u_0 the linear velocity (m s^{-1}), η the mobile phase viscosity (Pa s), L the column length (m) and ΔP the pressure drop (Pa).

The dependence of the permeability on the domain size allowed us to use it as a control parameter of the cladding quality. The calculated permeability of the prepared columns was then plotted against the domain size (ranging $1.8 \mu\text{m}$ from $5.1 \mu\text{m}$). The latter was assimilated to an effective particle size by Minakuchi et al. [8,9], the columns used for the plot all presented good separation profiles. The Chromolith permeability, calculated the same way, was included for comparison [19]. It can be noted that the permeability increases linearly with the domain size (correlation coefficient = 0.9929). Furthermore the commercial column belongs to the linear regression. Columns not fitting this line (more than 15% of deviation) were rejected since they did not show any separation capacity in chromatography.

3.3.2. Performances of the columns

Different silica monoliths were prepared from various amounts of F68 porogen. The use of F68 as a porogen instead of the classical polyethylene glycol (PEG) led to the preparation of monoliths having smaller domain sizes over a wider composition range [15].

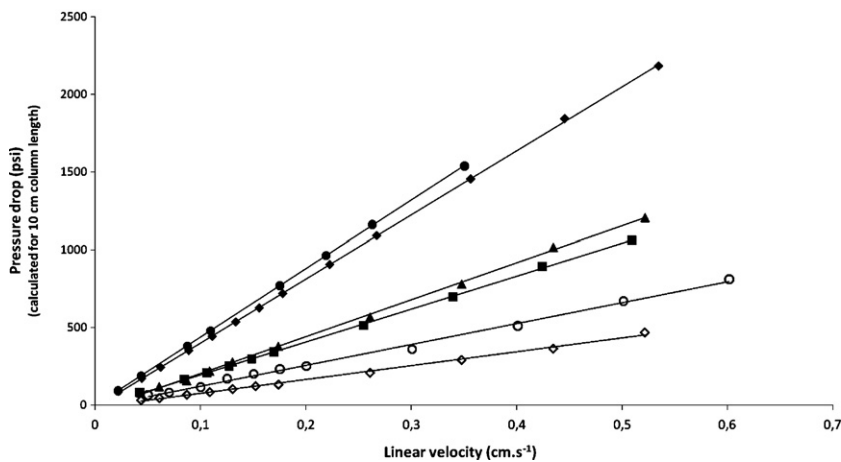


Fig. 2. Plots of the pressure drop against linear velocity for a normalised 10 cm column length. The prepared columns (domain size varying from 1.8 μm to 5.1 μm) are compared to the Chromolith (domain size = 3.5 μm). (●) SM-1.8 ($L = 6.6$ cm; $d_i = 4.9$ mm); (◆) SM-2.2 ($L = 5.7$ cm; $d_i = 4.9$ mm); (■) SM-2.8 ($L = 8.8$ cm; $d_i = 4.9$ mm); (▲) SM-3.0 ($L = 10$ cm; $d_i = 4.9$ mm); (○) Chromolith ($L = 10$ cm; $d_i = 4.6$ mm); (◇) SM-5.0 ($L = 9.6$ cm; $d_i = 4.9$ mm).

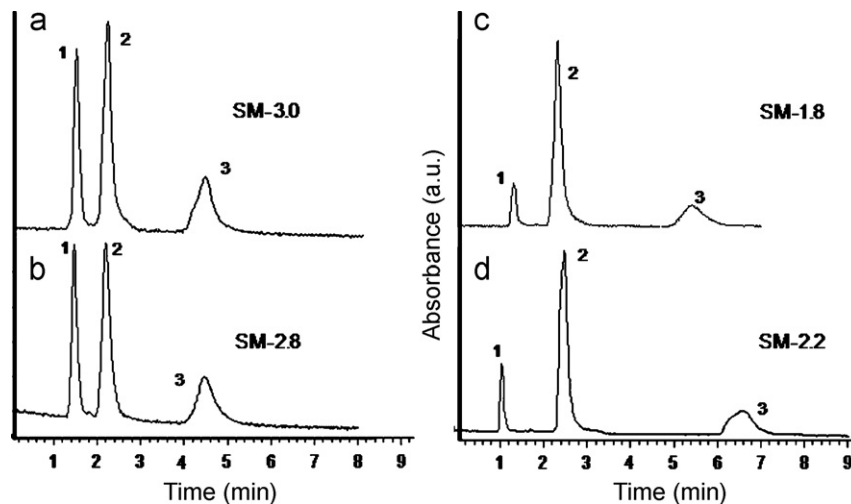


Fig. 3. Chromatograms of the test mixture (toluene (1), 2,3-dimethylanthraquinone (2) and 2-nitroacetanilide (3)) on the four selected columns at a flow rate of 1 mL min^{-1} . Mobile phase: 95% n-heptane, 5% 1,4-dioxane. (a) SM-3.0: $d_{\text{macropore}} = 1.7 \mu\text{m}$, $d_{\text{skeleton}} = 1.3 \mu\text{m}$, $d_{\text{mesopore}} = 15 \text{ nm}$, $L = 10$ cm, $d_i = 4.9$ mm; (b) SM-2.8: $d_{\text{macropore}} = 1.5 \mu\text{m}$, $d_{\text{skeleton}} = 1.3 \mu\text{m}$, $d_{\text{mesopore}} = 15 \text{ nm}$, $L = 8.8$ cm, $d_i = 4.9$ mm; (c) SM-1.8: $d_{\text{macropore}} = 0.9 \mu\text{m}$, $d_{\text{skeleton}} = 0.9 \mu\text{m}$, $d_{\text{mesopore}} = 13 \text{ nm}$, $L = 6.6$ cm, $d_i = 4.9$ mm; (d) SM-2.2: $d_{\text{macropore}} = 1.3 \mu\text{m}$, $d_{\text{skeleton}} = 0.9 \mu\text{m}$, $d_{\text{mesopore}} = 7 \text{ nm}$, $L = 5.7$ cm, $d_i = 4.9$ mm. UV detection at 254 nm, injected volume = $10 \mu\text{L}$.

The prepared columns were then tested in adsorption chromatography mode. Fig. 3 shows the obtained chromatograms. We can notice an improvement of the chromatographic efficiency with both smaller domains and smaller mesopores columns.

Fig. 4 shows the van Deemter plot of the column presenting the best performances compared to the Chromolith's. The curve exhibits a minimum plate height of about $10 \mu\text{m}$ for the commercial column while it is around $50 \mu\text{m}$ for the best performing prepared column (SM-2.2).

Nevertheless, these results present a significant improvement compared to published results obtained with a PTFE cladding [11,12]. Indeed, calculations performed from the published chromatograms lead to about 2500 plates/m in adsorption chromatography while our columns present 18,000 plates/m in the same conditions.

A possible explanation of the low efficiency compared to the Chromolith can be drawn from the calculation of the van Deemter parameters. It demonstrates a 75% contribution of the A term to the minimum plate height. Since the A term is directly related to the structure of the stationary phase and the contribution of the wall effect, the high value obtained might be attributed to a radial heterogeneity in the monolithic structure.

This radial heterogeneity might come from a non-equivalent heat distribution during the gel stage, as reported by Nakanishi and Soga [20] who showed that a weak temperature gradient between the centre and the border of the monolith might cause a structural

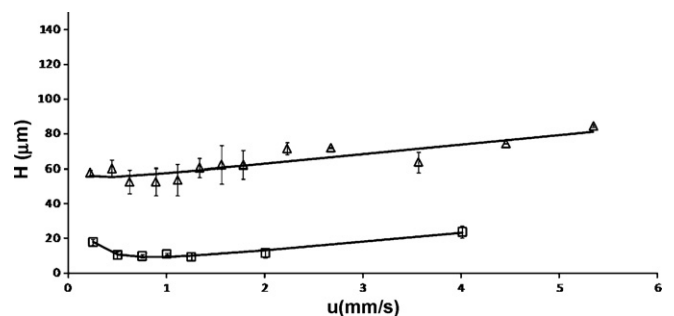


Fig. 4. van Deemter plots obtained with 2-nitroacetanilide, Δ SM-2.2, \square Chromolith. Mobile phase: 95% n-heptane, 5% 1,4-dioxane. Stationary phase: (a) SM-2.2: $d_{\text{macropore}} = 1.3 \mu\text{m}$, $d_{\text{skeleton}} = 0.9 \mu\text{m}$, $d_{\text{mesopore}} = 7 \text{ nm}$, $S_{\text{BET}} = 700 \text{ m}^2 \text{ g}^{-1}$, $L = 9.6$ cm, $d_i = 4.9$ mm; (b) Chromolith: $d_{\text{macropore}} = 2 \mu\text{m}$, $d_{\text{skeleton}} = 1.5 \mu\text{m}$, $d_{\text{mesopore}} = 13 \text{ nm}$, $S_{\text{BET}} = 300 \text{ m}^2 \text{ g}^{-1}$, $L = 10$ cm, $d_i = 4.6$ mm. UV detection at 254 nm, injected volume = $10 \mu\text{L}$.

difference between those two regions. This radial heterogeneity was also demonstrated by Mriziq et al. [21].

4. Conclusion

A simple method of cladding monolithic silica using PEEK was presented and verified through various determinations of potential in-between spaces. As a consequence, this method allows the study of new monolithic silica rods performances in chromatography. This was exemplified by the use of silica monoliths prepared using F68 polymer in chromatographic analysis. These results show good performances of the columns, which could however be improved by a better control of the drying stage causing an inhomogeneity of the macroporous network.

Acknowledgements

This work was supported by the French Ministry of Research and Higher Education, Paris, France (grant to JC). The authors wish to thank the mechanics services of ECPM and IPHC for their help as well as the CRITT Matériaux Alsace for the access to mercury porosimetry.

References

- [1] M. Motokawa, H. Kobayashi, N. Ishizuka, H. Minakuchi, K. Nakanishi, H. Jinnai, K. Hosoya, T. Ikegami, N. Tanaka, *J. Chromatogr. A* 961 (2002) 53.
- [2] D. Allen, Z. El Rassi, *Electrophoresis* 24 (2003) 408.
- [3] N. Tanaka, H. Nagayama, H. Kobayashi, T. Ikegami, K. Hosoya, N. Ishizuka, H. Minakuchi, K. Nakanishi, K. Cabrera, D. Lubda, *J. High Resolut. Chromatogr.* 23 (2000) 111.
- [4] D. Lubda, K. Cabrera, in *Merck Patent* (Ed.), WO, 2001, p. 19.
- [5] K. Cabrera, A. Kraus, W. Neuroth, D. Lubda, in *G. Merck Patent GmbH* (Ed.), DE, 2000, p. 8.
- [6] G. Hauke, G. Saettler, D. Lubda, E. Dicks, W. Neuroth, K. Cabrera, A. Kraus, in *Merck Patent* (Ed.), DE, 1999, p. 8.
- [7] D. Lubda, G. Wieland, K. Cabrera, H.D. Harders, E. Dicks, G. Sattler, W. Neuroth, D. Heuser, in *Merck Patent* (Ed.), WO, 1998, p. 23.
- [8] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *J. Chromatogr. A* 797 (1998) 121.
- [9] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *J. Chromatogr. A* 762 (1997) 135.
- [10] H. Minakuchi, N. Ishizuka, K. Nakanishi, N. Soga, N. Tanaka, *J. Chromatogr. A* 828 (1998) 83.
- [11] H. Zhong, G. Zhu, P. Wang, J. Liu, J. Yang, Q. Yang, *J. Chromatogr. A* 1190 (2008) 232.
- [12] C. Liang, S. Dai, G. Guiochon, *Chem. Commun.* (2002) 2680.
- [13] H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, N. Tanaka, *Anal. Chem.* 68 (1996) 3498.
- [14] K. Morisato, S. Miyazaki, M. Ohira, M. Furuno, M. Nyudo, H. Terashima, K. Nakanishi, *J. Chromatogr. A* 1216 (2009) 7384.
- [15] J. Chamieh, Y. Zimmermann, A. Boos, A. Hagège, *J. Colloid Interface Sci.* 340 (2009) 225.
- [16] E.P. Barrett, L.G. Joyner, P.P. Halenda, *J. Am. Chem. Soc.* 73 (1951) 373.
- [17] Victrex, Victrex documents: PEEK properties guide, 2004, p. 1.
- [18] J. Kloubek, *Powder Technol.* 29 (1981) 63.
- [19] M. Kele, G. Guiochon, *J. Chromatogr. A* 960 (2002) 19.
- [20] K. Nakanishi, N. Soga, *J. Non-Cryst. Solids* 139 (1992) 14.
- [21] K.S. Mriziq, J.A. Abia, Y. Lee, G. Guiochon, *J. Chromatogr. A* 1193 (2008) 97.