



ELSEVIER

Journal of Chromatography A, 828 (1998) 75–81

JOURNAL OF
CHROMATOGRAPHY A

Synthesis and characterization of a polymer-coated C_{18} stationary phase with high carbon content for liquid chromatography

Shinichi Kobayashi*, Isao Tanaka, Osamu Shirota, Taketoshi Kanda, Yutaka Ohtsu

Shiseido Research Center, 1050 Nippa-cho, Kohoku, Yokohama 223-8553, Japan

Abstract

High-purity porous silica with a large surface area (particle diameter 5 μm , mean pore diameter 8.4 nm, surface area 350 m^2/g) was coated with a homogeneous silicone polymer film, which was thereafter modified with octadecyl groups to be used as a stationary phase for reversed-phase chromatography. The stationary phase had a high carbon content (18%), and showed a relatively large overall retention for various analytes among other stationary phases available in the market. It also showed preferable characteristics in separation of basic compounds, such as pyridine, and hinokitiol, a substance that easily coordinates with metals, and also had a high durability under an alkaline mobile phase. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Stationary phases, LC; Polymer coatings; Pyridine; Phenol; Hinokitiol

1. Introduction

Stationary phases for reversed-phase liquid chromatography (RP-LC) can roughly be divided into several types [1]. One of the most common phases are silicas chemically modified with relatively long alkyl groups. Silica, as a chromatographic support, provides a high mechanical strength and a flexibility on designing particle diameter and pore size, and makes it possible to prepare high-efficiency packed columns, which are now available from hundreds of different commercial suppliers [2]. Many important works were reported in the field of the stationary phase characterization [3–7]. Another common type is those based on organic polymers. Organic polymers generally provide a possibility for a variety of chemical modifications, and also a chemical stability under a wide pH range [8].

Both types of phases, however, have certain

limitations in their performance in RP-LC applications. Silica, by nature, dissolves in an alkaline solution, and a mobile phase pH is usually adjusted between acidic and neutral regions [9]. Undesirable secondary effects originated from silica surface, such as deterioration of peak shape for basic or chelating compounds, are often observed. Although organic-polymer phases show simple separation characteristics as a stationary phase for RP-LC, being free from any secondary effect, their physical natures, such as the existence of meso-pores and a swelling tendency, result in limited separation efficiency in comparison with silica-based phases [10].

Several attempts to improve the performance of silica-based phases have been reported. Trimethylsilane has been used to “end-cap” the silica surface after the introduction of octadecyl (or octyl) groups in order to minimize the effects caused by residual silanol groups [11]. An attempt to use purified silica with minimized metal contents as a starting material has shown some improvements on

*Corresponding author.

the separation of chelating compounds and proteins [12,13]. Efforts to improve organic polymer phases also have been tried, such as an increase of a cross-linking ratio to decrease the extent of swelling in an organic-containing mobile phase [14].

As another attempt, a “hybrid” type of stationary phases have been synthesized, where a thin layer of organic silicone polymer was formed on silica surface through gradual supply of vaporized silicone monomers, and thereafter long alkyl chains were introduced to the polymer-coated silica by hydrosilylation. Polymer-coated silica phases, the final products, were intended to possess advantages of both silica- and polymer-based phases, and actually showed several improvements over conventional silica phases [15].

While the polymer-coating process solved problems generating from original silica surface, a certain decrease of overall retention for analytes was observed. It seemed that the polymer coating process decreased a density of alkyl chains of the final product in comparison with conventional C₁₈ bonded silicas. As a continuing study, this paper describes the synthesis and characterization of a polymer-coated C₁₈ phase with a silica, a starting material, of a larger surface area (pore size, 8.4 nm, relative surface area, 350 m²/g) to see if an expected increase of retention of analytes and other additional advantages can be obtained.

2. Experimental

2.1. Materials

High-purity silica (Shiseido, Tokyo, Japan) (particle size, 5 μm; pore diameter, 8.4 nm; surface area, 350 m²/g; metal impurities <5 ppm) was used as a starting material for the polymer-coated C₁₈ stationary phase (PC-C₁₈). 1,3,5,7-Tetramethylcyclotetrasiloxane (H4), the silicone monomer used for the polymer coating, was purchased from Toshiba Silicone (Tokyo, Japan). All the reagents and solvents used to synthesize the packing material were of special grade from Nakarai Tesque (Kyoto, Japan), and were used as received. Acetonitrile and methanol used for the mobile phases were of HPLC grade

from Nakarai Tesque. Water was purified with a Milli-Q system (Nihon Millipore, Tokyo, Japan).

2.2. Synthesis of PC-C₁₈

The polymer-coated silica was prepared according to the method of Fukui et al. [16]. The high-purity silica (100 g) was placed in contact with the vapor of H4 (40 g) at 100°C for 24 h under a nitrogen atmosphere. The deposited H4 molecules were polymerized on the silica surface. The reactive SiH groups left on the silicone polymer were utilized for the subsequent modification. 1-Octadecene was attached to the polymer formed on silica through hydrosilylation. Details of the synthetic process were described in the previous report [15]. Pore distribution, pore diameter, and surface area of products were measured with Autosorb-1 (Yuasa Ionics, Tokyo, Japan). Alkyl chain density was calculated by dividing a mass increase after the introduction of octadecyl groups by their molecular mass and the surface area of the starting silica.

2.3. Elemental analysis

Elemental analysis was carried out with an Organic Elemental Analyzer 2400CHN (Perkin-Elmer Japan, Yokohama, Japan).

2.4. Chromatography

The PC-C₁₈ was packed into a stainless steel column (150 mm×4.6 mm I.D.). Measurements were performed using a Nanospace high-performance liquid chromatography (HPLC) system (Shiseido). Data acquisition and integration were performed with an S-MC data system (Shiseido). Commercial columns used in this study were listed in Table 3. Unless otherwise stated, all characterization tests were performed on the following conditions; mobile phase, methanol–water (50:50), flow-rate of 1.0 ml/min, detection at 254 nm and column oven temperature at 40°C. Test solution [containing uracil (U), caffeine (C), phenol (P), 2-ethylpyridine (EP), methyl benzoate (MB), benzene (B), dimethylaniline (DA), toluene (T), phenylacetylacetone (PAA), and naphthalene (N)] was injected. The definitions of

Table 1
Definition of chromatographic parameters

Hydrophobicity	$\alpha(T/B)$	$=k'(\text{toluene})/k'(\text{benzene})$
Surface polarity	$\alpha(MB/T)$	$=k'(\text{methyl benzoate})/k'(\text{toluene})$
Ion-exchangeability	$\alpha(EP/B)$	$=k'(2\text{-ethylpyridine})/k'(\text{benzene})$
Hydrogen bonding capacity	$\alpha(C/P)$	$=k'(\text{caffeine})/k'(\text{phenol})$
Effect of residual surface metals	PA(PAA/N)	$=\text{Peak area}(\text{phenylacetylacetone})/\text{peak area}(\text{naphthalene})$
Steric selectivity ^a	$\alpha(T/O)$	$=k'(\text{triphenylene})/k'(\text{o-terphenyl})^a$

^a Steric selectivity tests were performed under the following conditions: mobile phase, methanol–water (80:20), flow-rate, 1.0 ml/min, detection at 254 nm, and column oven temperature; 35°C. The test solution, which contained triphenylene and *o*-terphenyl in methanol, was injected.

chromatographic parameters are summarized in Table 1.

Column durability tests were performed under the following conditions: mobile phase containing 3.5 mmol/l $\text{Na}_2\text{B}_4\text{O}_7$ (pH 10) in methanol–water (10:90), flow-rate of 1.0 ml/min, detection at 254 nm and column oven temperature at 50°C. The test solution of benzyl alcohol (100 ppm) was injected. Percentage of k' (retention factor) at 20 h over the initial one was calculated.

3. Results and discussion

Structural images of the polymer-coated silica phase (PC-C₁₈) and a conventional C₁₈ bonded phase are illustrated in Fig. 1. PC-C₁₈ differs from conventional bonded phases in that the original silica surface is treated with the polymer coating process prior to the introduction of long alkyl groups.

Pore distribution curves of a silica (starting material), a polymer-coated silica (intermediate product), and PC-C₁₈-8 [final product, the last “8” stands for a pore diameter (/nm) of starting silica] are shown together in Fig. 2. The distribution curve shifted to a smaller direction without a significant change in peak

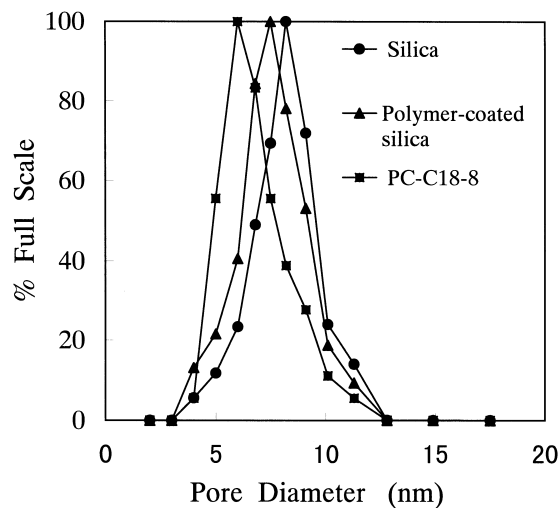


Fig. 2. Micropore distribution at each step.

width, as each synthetic step proceeded. Although direct observation of the polymer layer or measurements of its homogeneity with an appropriate methodology might be necessary to obtain clear conclusions, the results suggest that homogenous polymer coating and alkyl chain introduction seem to have been done as expected.

Similar results to above ones have already been

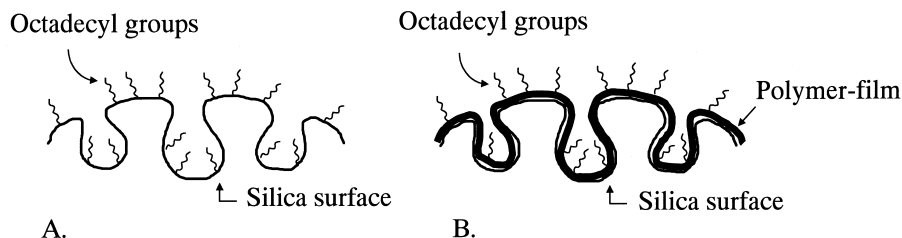


Fig. 1. Structural images of reversed-phases. (A) Conventional C₁₈-bonded silica phase, (B) polymer-coated C₁₈ silica phase.

obtained with a polymer-coated phase from a starting silica with 12-nm pore diameter (PC-C₁₈-12) [15]. The primary objective of this study is to prepare a polymer-coated phase with a larger overall retention for various analytes by using silica of a larger surface area to accommodate a wide range of applications.

Elemental analysis was carried out for PC-C₁₈-8. A carbon content was measured as 18%, which was 3% larger than that of PC-C₁₈-12 (Table 2). Since carbon contents of other commercial bonded phases collected in this study ranged around 15–19% (from their specifications), PC-C₁₈-8 was expected to show a relatively large overall retention among other phases.

The separation of the test mixture (ten standard compounds) was examined. Chromatograms obtained with the two types of polymer-coated phases, PC-C₁₈-8 and -12, are compared in Fig. 3. Significant increases in retention time were observed for all the substances except for uracil, a substance included for a zero-retention marker. A retention time of naphthalene, the last eluting substance, was increased by 10 min, approximately. The *k'* (retention factor) values of naphthalene are listed with those of all the phases tested in the study (Table 3). PC-C₁₈-8 seems to successfully belong to the group of large overall retention within the tested phases.

For detailed characterization, peaks of various substances were closely examined by the method Kimata et al. suggested [17]. Definitions of parameters (Table 1) to be used in the following discussions were described earlier. All the parameters were based on the comparison (in *k'* or peak area) of a pair of substances sharing a close structure, but differing in one structural aspect.

$\alpha(T/B)$, hydrophobicity, represents an extent of retention increase caused by one additional methyl group on an analyte molecule. Values obtained in the

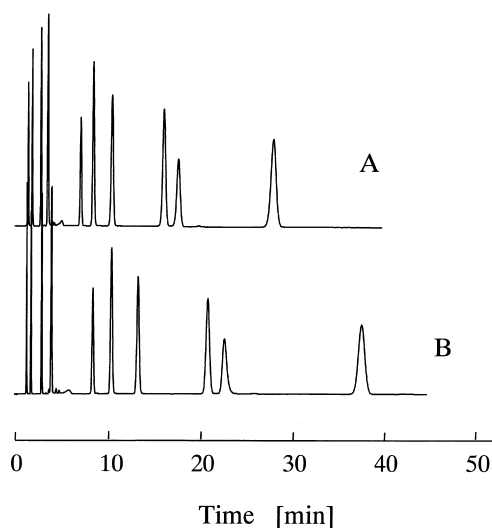


Fig. 3. Chromatograms of test mixture obtained with two polymer-coated phases. (A) PC-C₁₈-12; (B) PC-C₁₈-8. Column size: 150 × 4.6 mm I.D., mobile phase: methanol–water (50/50), flow-rate: 1.0 ml/min, temperature: 40°C, detection: 254 nm. Sample: uracil, caffeine, phenol, 2-ethylpyridine, methyl benzoate, benzene, dimethylaniline, toluene, phenylacetylacetone, naphthalene (in this order in chromatograms).

tested phases did not differ significantly, ranging 2.06 to 2.15. This can be explained by the fact they are all C₁₈-based. PC-C₁₈-8 showed a relatively large value (2.14) among them.

Caffeine is known to form a hydrogen bond with a silanol group. $\alpha(C/P)$, hydrogen bond capacity, represents an amount of neutral silanol groups left on the surface. $\alpha(MB/T)$, surface polarity, is a value to evaluate an extent of dipole moment interaction between the carboxyl ester moiety of methyl benzoate and silica surface. $\alpha(EP/B)$, ion exchangeability, is supposed to be a large value, when 2-ethylpyridine, a cationic substance, interacts with acidic silanol groups. All of these three parameters,

Table 2
Characteristic values of two polymer-coated packing materials

	Micropore diameter (nm)	Micropore volume (ml/g) ^a	Surface area (m ² /g) ^a	Total carbon (%)	Alkyl density ^b (μmol/m ²)
PC-C ₁₈ -8	8.4	0.83	350	18	2.4
PC-C ₁₈ -12	12.6	0.99	330	15	2.1

^a Vales of starting silica.

^b Alkyl density is a quotient of the mass increase to molecular mass of octadecyl group and surface area.

Table 3
Characterization of various silica-based C₁₈ packing materials

No.	Stationary phase	$\alpha(T/B)$	$\alpha(MB/T)$	$\alpha(C/P)$	$\alpha(EP/B)$	$\alpha(T/O)$	PA(PAA/N) ^a	<i>k'</i> (N)	Column durability (%) ^c
1	PC-C ₁₈ -8	2.14	0.36	0.29	0.28	1.35	0.65	26.8	90.4
2	PC-C ₁₈ -12	2.09	0.39	0.31	0.31	1.27	0.58	17.7	80.0
3	Inertsil ODS3V	2.12	0.42	0.33	0.28	1.33	0.01	32.0	78.9
4	LiChrospher RP18	2.08	0.46	0.53	* ^b	1.84	* ^b	25.2	51.5
5	LiChrospher RP18(e)	2.10	0.41	0.36	* ^b	1.66	* ^b	26.2	70.3
6	Nucleosil ODS	2.06	0.48	0.49	0.60	1.75	* ^b	19.9	46.8
7	Symmetry C ₁₈	2.11	0.39	0.33	0.31	1.61	0.41	28.3	87.2
8	TSKgel ODS 80TsQA	2.09	0.47	0.39	0.43	1.21	0.53	18.2	79.3
9	YMC Pro C ₁₈	2.15	0.41	0.33	0.36	1.34	0.47	21.3	75.4
10	Zorbax ODS	2.14	0.38	0.35	* ^b	1.60	* ^b	30.8	88.7

^a PA (PAA/N) was measured exceptionally by the conditions below. Column size: 150×4.6 mm I.D., mobile phase: methanol–water (80:20), flow-rate: 1.0 ml/min, temperature: 35°C, detection: 254 nm.

^b Peaks not observed.

^c Mobile phase: 3.5 mmol/l Na₂B₄O₇ (pH 10) in methanol–water (10:90), temperature: 50°C, detection: 254 nm. Percent of *k'* at 20 h over the initial one.

$\alpha(C/P)$, $\alpha(MB/T)$ and $\alpha(EP/B)$, directly or indirectly, represent the amount of silica surface left even after a process of chemistry to form a hydrophobic surface.

PC-C₁₈-8 showed the small values for all of the

above three parameters, which can be explained by the existence of the polymer layer covering the silica surface. Effects of residual silanols sometimes play an important role in separating analytes in certain applications, and should not be considered categori-

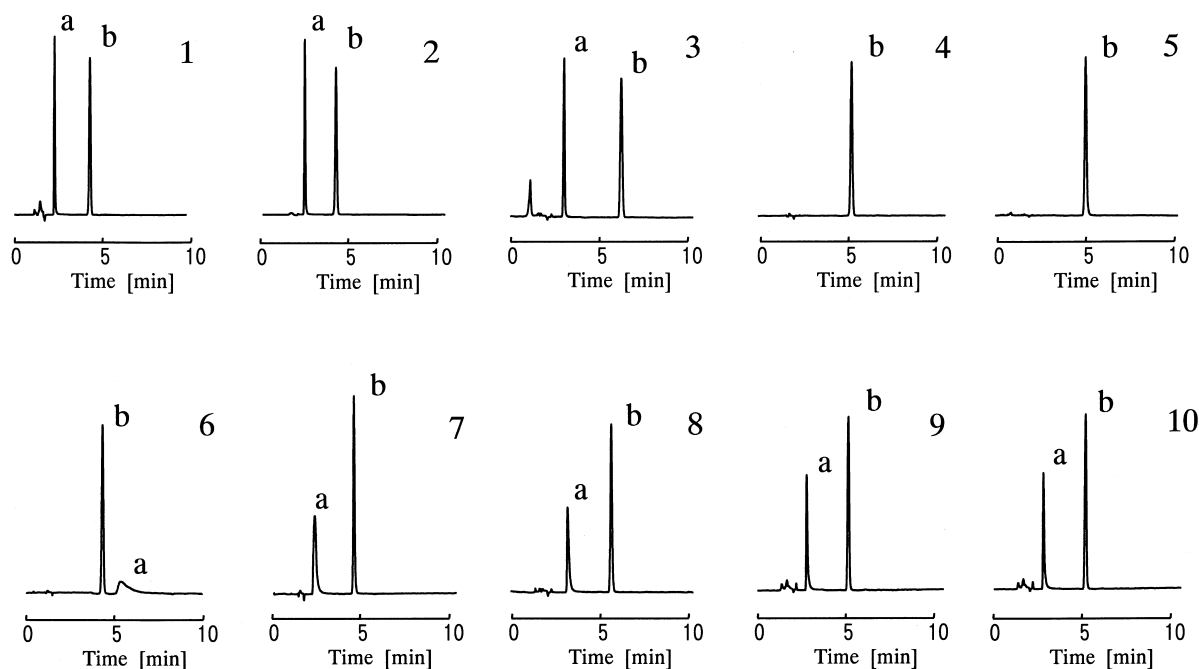


Fig. 4. Peak profiles of pyridine (peak a) and phenol (peak b). Column size: 150×4.6 mm I.D., mobile phase: acetonitrile–water (30:70), flow-rate: 1.0 ml/min, temperature: 40°C, detection: 254 nm. Columns: 1=PC-C₁₈-8, 2=PC-C₁₈-12, 3=Inertsil ODS-3V, 4=LiChrospher RP-18, 5=LiChrospher RP-18(e), 6=Nucleosil ODS, 7=Symmetry C₁₈, 8=TSKgel ODS 80TsQA, 9=YMC Pro C₁₈, 10. Zorbax ODS.

cally as negative aspects. However, it is also true that phases of strong silanol effects have limitations on their applications: it was impossible to observe a peak of 2-ethylpyridine in several commercial phases, probably due to its irreversible adsorption (shown by asterisks in Table 3). In fact, most of commercial suppliers tend to “improve” their products by tighter end-capping procedures [18].

$\alpha(T/O)$, steric selectivity, will be a large value, when triphenylene, a planer molecule, is retained longer than *o*-terphenyl, a flexible counterpart. It is empirically known that polymeric phases (bonded phases prepared with a multifunctional silane) generally show a value more than 1.6 [17]. The observed values here (including that of PC-C₁₈-8), range from 1.21 to 1.84. As Sander and Wise used another pair of substances to study a steric selectivity [19], it might be interesting to see if the similar results can be obtained.

Effects of metal impurities were examined next. A

peak area, PA(PAA/N) value is related to a recovery of phenylacetylacetone, a substance with a strong interaction with metals, and is supposed to indirectly indicate a purity concerning metals [20]. PC-C₁₈-8 showed the largest value (0.65), which seems to be resulted from the use of high-purity silica as a starting material in addition to the existence of the polymer coating. Four of the tested columns could not elute the substance as a chromatographic peak (shown by asterisks in Table 3).

Peak profiles of other representing standard compounds were obtained for each stationary phase. Fig. 4 shows the separation of pyridine, a cationic compound under the condition, and phenol as a reference compound. Pyridine seemed totally adsorbed in several columns, and a partial deterioration of peak shape was observed in some of other columns. Both PC-C₁₈-8 and -12 showed a symmetric peak profile, which seems to agree with the above discussion concerning the $\alpha(EP/B)$ value.

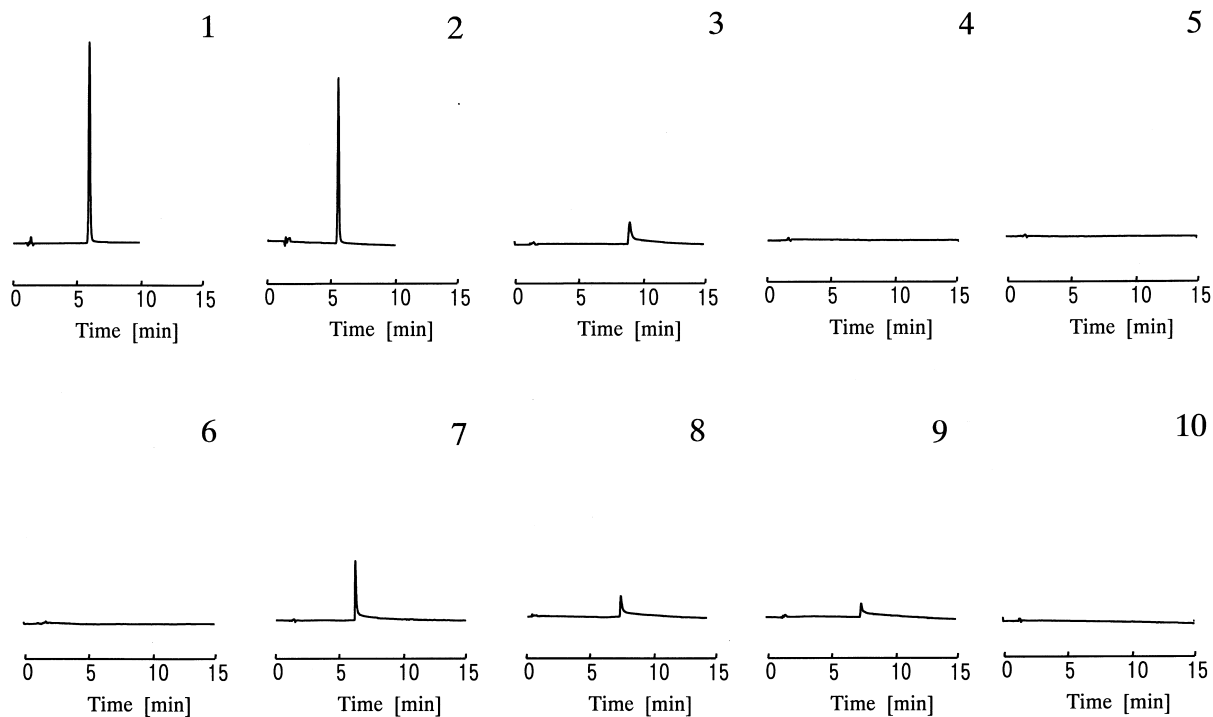


Fig. 5. Peak profiles of hinokitiol. Column size: 150×4.6 mm I.D., mobile phase: 20 mmol/l KH₂PO₄, 1 mmol/l EDTA-2Na in acetonitrile–water (40:60), flow-rate: 1.0 ml/min, temperature: 40°C, detection: 320 nm. Columns: 1=PC-C₁₈-8, 2=PC-C₁₈-12, 3=Inertsil ODS-3V, 4=LiChrospher RP-18, 5=LiChrospher RP-18(e), 6=Nucleosil ODS, 7=Symmetry C₁₈, 8=TSKgel ODS 80TsQA, 9=YMC Pro C₁₈, 10=Zorbax ODS.

The peak shape of hinokitiol, another compound known to form a complex with metals, was examined for each stationary phase. Four out of the ten stationary phases could not elute hinokitiol as a recognizable peak, and a peak tailing was observed more or less in other phases (Fig. 5). On the contrary, PC-C₁₈-8 and -12 showed a highly symmetrical peak profile. The peak profiles observed here could be correlated to PA(PAA/N) values discussed earlier.

Finally, column durability under an alkaline mobile phase was studied. The test examines a retention time decrease of benzyl alcohol after an accelerated damaging conditions (50°C, pH 10). As a result, all of the stationary phases were damaged to some extent, and the retention time decreased to a half the original one in one case (Table 3). PC-C₁₈-8 was found the most durable among these phases, and showed an improvement over PC-C₁₈-12, a counterpart of less carbon content. It seems that the increased hydrophobicity lowers the accessibility of the aqueous buffer that attacks the surface of the stationary phase. The amount of residual silanol groups also seems to be an influencing factor. The durability of LiChrospher RP-18(e) was much higher than that of RP-18, its non-end-capped version.

4. Conclusions

PC-C₁₈-8, the polymer-coated silica, had a high carbon content (18%), and showed a large overall retention for analytes among other stationary phases tested in this paper. The characterization study with various standard compounds found that PC-C₁₈-8 had a highly inert surface free from residual silanol groups and metal impurities. In addition, it was found that PC-C₁₈-8 showed an improvement in

durability under an alkaline condition over PC-C₁₈-12, a previous version of the polymer-coated phase.

References

- [1] K.K. Unger (Ed.), Packings and Stationary Phases in Chromatographic Techniques (Chromatographic Science Series, Vol. 47), Marcel Dekker, New York, 1990.
- [2] S.A.B. Anal. Chem. 58 (1986) 876A.
- [3] H. Hanai, H.F. Walter, J.D. Navatil, D. Warren, J. Chromatogr. 155 (1978) 261.
- [4] H. Engelhardt, M. Jungheum, Chromatographia 29 (1990) 59.
- [5] H. Engelhardt, H. Low, W. Gotzinger, J. Chromatogr. 544 (1990) 371.
- [6] S.J. Schmitz, H. Zwanziger, H. Engelhardt, J. Chromatogr. 544 (1990) 381.
- [7] T. Hamoir, F. Cuesta Sanchez, B. Bourguignon, D.L. Massart, J. Chromatogr. Sci. 32 (1994) 488.
- [8] E. Cruz, M.R. Euerby, C.M. Johnson, C.A. Hackett, Chromatographia 44 (1997) 151.
- [9] J. Kohler, P.D. Chase, R.D. Farlee, J. Vega, J.J. Kirkland, J. Chromatogr. 352 (1986) 275.
- [10] N. Tanaka, K. Hashizume, M. Araki, J. Chromatogr. 400 (1987) 33.
- [11] A. Pryde, J. Chromatogr. Sci. 12 (1974) 486.
- [12] B. Pfeleiderer, E. Bayer, J. Chromatogr. 468 (1989) 67.
- [13] J. Koyama, J. Nomura, Y. Ohtsu, O. Nakata, M. Takahashi, Chem. Lett. 687 (1990)
- [14] H. Wada, Chromatographia 22 (1986) 194.
- [15] Y. Ohtsu, H. Fukui, T. Kanda, K. Nakamura, M. Nakano, O. Nakata, Y. Fujiyama, Chromatographia 24 (1987) 380.
- [16] H. Fukui, T. Ogawa, M. Nakano, M. Yamaguchi, Y. Kanda, Controlled Interphases in Composite Materials, Elsevier, New York, 1990, p. 469.
- [17] K. Kimata, K. Iwaguchi, S. Onishi, K. Jinno, R. Eksteen, K. Hosoya, M. Araki, N. Tanaka, J. Chromatogr. Sci. 27 (1989) 721.
- [18] Y. Sudo, J. Chromatogr. A 737 (1996) 139.
- [19] L.C. Sander, S.A. Wise, LC-GC 5 (1990) 377.
- [20] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, N. Tanaka, J. Chromatogr. 481 (1989) 147.