ELSEVIER

Journal of Chromatography A, 813 (1998) 239–246

JOURNAL OF CHROMATOGRAPHY A

# Characteristics of octadecylsilylated silica gels end-capped by high-temperature silylation

Yoshihisa Sudo[1,*], Takeharu Wada[2]

*Chemicals Inspection and Testing Institute, Division of Research and Development, 4-1-1 Higashimukojima Sumida-ku, Tokyo 131-0032, Japan*

Received 10 February 1998; received in revised form 28 April 1998; accepted 28 April 1998

**Abstract**

Octadecylsilylated silica gels (ODSs) that were end-capped by high-temperature silylation (HTS) were shown to be effective in the elimination of the undesirable secondary interaction with chelating compounds. ODSs prepared from high-purity or low-purity silica gel treated with HCl, or untreated, were end-capped by HTS or by liquid-phase silylation. ODSs that were end-capped by HTS and prepared from high-purity silica gel treated with HCl showed little secondary interaction with chelating compounds. The secondary interaction with pyridine was eliminated by HTS, regardless of the purity of the base silica. HTS also improved the stability of packing material at high pH, by reducing the dissolution rate of the base silica, and prevented hydrolysis of the bonded phase at low pH values. 

*Keywords:* Stationary phases, LC; High-temperature silylation; Silylation; End-capping; Silica gel, octadecylsilylated

## 1. Introduction

In high-performance liquid chromatography (HPLC) using octadecylsilylated silica gel (ODS), the main factor in retention is the hydrophobic interaction between analytes and stationary phases [1]. However, a secondary interaction due to residual silanol groups on the ODS affects the retention and the peak shape of some analytes [2,3]. Moreover, since ODS prepared from silica gel containing large amounts of metal impurities shows strong peak tailing of basic analytes and chelating compounds, metal impurities in silica gel are also regarded as a cause of the secondary interaction [4,5]. It has been reported that metal impurities on the surface of silica gel form active centers of adsorption [6], while metal impurities have been considered to change the acidity of silanol groups on the silica gel [7–10]. It has been reported that binary metal oxides, such as silica–alumina, have surface acidity [11], which suggests that metal impurities in the lattice of silica gel enhance the acidity of silanol groups. Acidic silanol groups show undesirable interaction with basic analytes [12–14]. Therefore, it is assumed that metal impurities interact with basic analytes indirectly, through the silanol groups [7,8].

*Corresponding author.

[1]Present address: Chemicals Inspection and Testing Institute, Chemical Biotesting Center, 19-14 Chuo-machi, Kurume, Fukuoka 830-0023, Japan.

[2]Present address: Chemicals Inspecting and Testing Institute, Chemical Biotesting Center, 3-822 ishii-machi, Hita, Oita 877-0061, Japan.

0021-9673/98/$19.00 
PII: S0021-9673(98)00395-1

HPLC of chelating compounds shows that they sometimes interact directly with the metal impurities on ODS. In such cases, the interaction is eliminated the addition of either metal ions [15] or chelating reagents [16,17] to the mobile phase. However, the additives in the mobile phase interfere with UV detection at low wavelengths and with liquid chromatography–mass spectrometry (LC–MS) analysis.

It is therefore necessary to remove the metal impurities from silica gel. Silica gel is treated with acids prior to octadecylsilylation [18]. Verzele et al. [19] recommended boiling the silica gel with 1 *M* HCl, to eliminate the metal impurities in it. Treatment of silica gel with EDTA [12] and treatment of ODS with a mixture of methanol and HCl (60:40, v/v) [4] were also reported. However, treatment with HCl cannot remove the metal impurities adequately [5,19]. It was recently revealed that the use of high-purity silica gel is effective in diminishing the undesirable secondary interaction [5,20,21], whereas it has been reported that high-purity silica gel also contains active acid silanol groups [12]. Preparations of packings by polymer coating [5] and with a trifunctional silylating reagent [22] were also reported for the elimination of undesirable effects of the metal impurities. However, these methods cannot completely eliminate the secondary interaction [5,22].

Another disadvantage of silica-based stationary phases is that modifier groups, such as alkylsilyl groups, and base silica are easily hydrolyzed in aqueous solution [23–26]. Several reports are concerned with hydrolysis of bonded phases in low-pH mobile phases with trifluoroacetic acid [27–31]. The hydrolysis reaction can be prevented through the steric hindrance effect of the modifier groups; this effect is provided by more bulky or longer-chain modifier groups [28,29,32], by increasing the amount of modifier groups [31], by sufficient end-capping [33] or by polymer coating of silica [5,34]. In addition, high-purity silica [20] and pretreatment of the silica support [35,36] also improve the hydrolytic stability.

Previously, we showed that the secondary interaction caused by the residual silanol groups is eliminated by end-capping the ODS by high-temperature silylation (HTS) [37–41]. The ODS end-capped by HTS is commercially produced and its packed column, L-column™ ODS, is available on the market. The IR spectrum of the ODS revealed that HTS achieves very high surface coverage. Since this high surface coverage inhibits solutes from approaching the surface of the base silica, HTS is expected to reduce the effect of the metal impurities and to improve the stabilities of the stationary phase and the end-capping groups against hydrolysis. In the present study, ODS was prepared from high-purity silica gel and was end-capped by HTS, and its characteristics were examined.

## 2. Experimental

### *2.1. Reagents and materials*

All silicon chemicals were purchased from Shin-etsu Chemicals (Tokyo, Japan). The solvents used as mobile phases for HPLC were of HPLC grade from Wako (Tokyo, Japan). Hydrofluoric acid and nitric acid were of trace analysis grade from Kanto Chemicals (Tokyo, Japan). The low-purity silica gel used was Develosil (particle size, 5 μm; pore size, 115 Å and surface area, 350 $m^2/g$) from Nomura Chemicals (Seto, Japan). The high-purity silica gel used was MSgel (particle size, 5 μm; pore size, 120 Å and surface area, 350 $m^2/g$) from Dokai Chemicals (Kitakyushu, Japan). The silica gels were heated in 20% HCl at 100°C for 16 h, washed with water until free of acid, and dried under vacuum at 140°C.

### *2.2. Determination of metal impurities in silica gels*

Water (5 g) was added to 1 g of silica gel in a PTFE beaker. A 5-ml volume of a 50% aqueous solution of hydrofluoric acid was added to the silica gel to dissolve it. The solution was heated on a hot plate and evaporated to dryness. The residue was dissolved using 0.25 ml of conc. $HNO_3$ and the solution was made up to 25 ml with water. The final solution was analyzed using an inductively coupled plasma mass spectrometer (PMS200, Yokogawa Analytical Systems, Tokyo, Japan).

### 2.3. Preparation of ODS

Silica gels that had been dried under vacuum at 140°C for 8 h were silylated with octadecyltrichlorosilane by refluxing in toluene in the presence of pyridine, and the residual chloro groups were hydrolyzed to give ODSs. Then, end-capping of the ODSs by HTS was performed using 1,3-dimethoxytetramethyldisiloxane (DMTMDS) or hexamethylcyclotrisiloxane ($D_3$) in a glass ampoule at 360°C for 24 h. End-capping of the ODSs by liquid-phase silylation (LPS) was performed with hexamethyldisilazane (HMDS) under refluxing conditions in toluene. Details of the octadecylsilylation of silica gels and of the end-capping of the ODSs have been given previously [40].

### 2.4. Chromatographic measurements

The prepared ODSs were packed into stainless steel tubes (150×4.6 mm I.D.) by a high-pressure slurry-packing procedure. The HPLC system consisted of a pump (LC-10AD, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), a data processor (C-R7A, Shimadzu) and an injector (model 7125, Rheodyne, Cotati, CA, USA). Uracil was used as a void volume marker.

## 3. Results and discussion

### 3.1. Silica gel and ODS

Table 1 shows the metal content of the silica gels. Treatment with HCl eliminated a large amount of metal from the Develosil and a small amount from the MSgel. Table 2 shows the carbon content of each ODS. The carbon content of the ODS prepared from silica gel treated with HCl was less than that of the ODS prepared from untreated silica gel.

Table 2
Carbon content of ODSs and end-capped ODSs

| Silica[a] | Treatment of silica | Carbon content (%) | | |
|---|---|---|---|---|
| | | ODS | End-capped ODS | |
| | | | LPS[b] | HTS[c] |
| 1 | – | 18.25 | 19.68 | 18.34 |
| 2 | HCl | 17.19 | 18.26 | 17.61 |
| 3 | – | 17.07 | 18.26 | 16.84 |
| 4 | HCl | 16.75 | 17.74 | 16.65 |

[a] Compare with Table 1.
[b] Liquid-phase silylation with HMDS.
[c] High-temperature silylation with DMTMDS.

### 3.2. Chromatography of chelating compounds

Oxine and hinokitiol were used to evaluate the effect of metal impurities on the chromatography of chelating compounds. The structures of these compounds are shown in Fig. 1. Theophylline and benzene were used as references for these compounds, respectively, and the separation factors ($\alpha$) of the chelating compounds and the references were

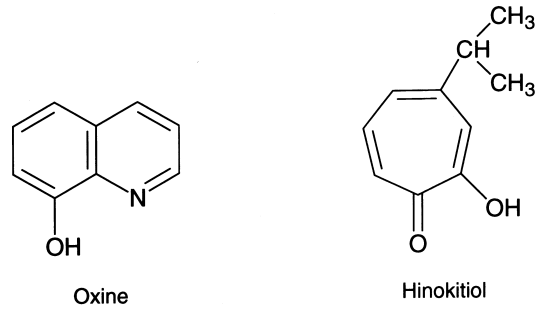


Fig. 1. Structures of the chelating compounds.

Table 1
Metal content of the silica gels used

| Number | Silica | Treatment | Metal content (mg/l) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Na | Mg | Ca | Al | Ti | Fe |
| 1 | Develosil | No | 54 | 188 | 1050 | 442 | 132 | 34 |
| 2 | Develosil | HCl | <3 | 90 | 440 | 185 | 69 | 5.4 |
| 3 | MSgel | No | <3 | 0.33 | 2.6 | <3 | 0.13 | 6.4 |
| 4 | MSgel | HCl | <3 | 0.08 | 0.4 | <3 | 0.07 | 0.8 |

used as indices in the evaluation of the ODSs. The secondary interaction was evaluated to be less when the value obtained was larger.

Table 3 shows the $\alpha$ values for the ODSs. Two chelating compounds were not eluted within the analytical time in HPLC using any of the ODSs prepared from the low-purity silica gel. When the low-purity silica gel was used, neither pretreatment with HCl nor end-capping by HTS could reduce the effect of the metal impurities. On the other hand, when the high-purity silica gel was used, the chelating compounds were eluted faster than the reference compounds because of the treatments used (Fig. 2). Fig. 3 show the chromatograms of the chelating compounds on the ODSs prepared from the high-purity silica gel treated with HCl (silica 4). The peak shapes of the chelating compounds on these chromatograms clearly reveal the difference in the effectiveness of the two end-capping methods. The degree of secondary interaction with oxine was slightly less on the ODS end-capped by HTS than by LPS, whereas that with hinokitiol was much less on the ODS that was end-capped by HTS rather than by LPS. Therefore, HCl treatment of the high-purity silica gel could not adequately reduce the undesirable effect of the metal impurities in HPLC for chelating compounds when it was used alone, but it could adequately reduce the undesirable effect when combined with end-capping by HTS. These results indicate that the analytes were effectively prevented from approaching the silica surface, due to the higher surface coverage achieved by HTS end-capping, which agrees well with previous results [41] using IR spectra, which indicated that only a small number of the silanol groups remained on ODSs that were end-capped by HTS. In addition, these results indicate that the heating treatment with HCl could not adequately eliminate even a small amount of metal impurities from the surface of the high-purity silica gel.

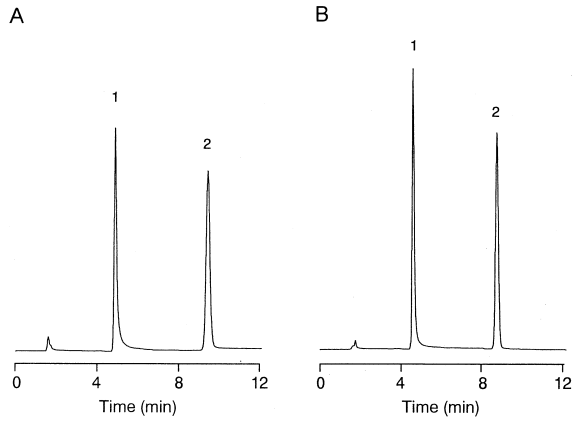


Fig. 2. Chromatograms of oxine on (A) the ODS that was end-capped by LPS and (B) the ODS that was end-capped by HTS with HMDMDS. HPLC conditions: mobile phase, acetonitrile–20 mmol/l $H_3PO_4$ (5:95, v/v); flow-rate, 1 ml/min; temperature, 40°C; detection, UV 250 nm. Peak: 1=oxine (5 mg/l); 2=theophylline (50 mg/l).

Table 3
Activity of ODSs for chelating compounds and pyridine

| Silica[a] | Treatment of silica | $\alpha$ | | | | | |
|---|---|---|---|---|---|---|---|
| | | Theophylline/oxine[b] | | Benzene/hinokitiol[c] | | Phenol/pyridine[d] | |
| | | LPS[e] | HTS[f] | LPS[e] | HTS[f] | LPS[e] | HTS[f] |
| 1 | no | –[g] | –[g] | –[g] | –[g] | –[g] | 1.59 |
| 2 | HCl | –[g] | –[g] | –[g] | –[g] | 1.81 | 3.02 |
| 3 | no | –[g] | 2.41 | –[g] | 1.75 | 1.18 | 3.02 |
| 4 | HCl | 2.38 | 2.39 | 1.44 | 1.76 | 2.44 | 3.10 |

[a] Compare with Table 1.
[b] Mobile phase, acetonitrile–20 mmol/l phosphoric acid (5:95, v/v); temperature, 40°C; detection, UV 250 nm.
[c] Mobile phase, acetonitrile–20 mmol/l phosphoric acid (4:6, v/v); temperature, 40°C; detection, UV 254 nm.
[d] Mobile phase, acetonitrile–water (3:7, v/v); temperature, 25°C; detection, UV 254 nm.
[e] End-capped by liquid-phase silylation with HMDS.
[f] End-capped by high-temperature silylation with DMTMDS.
[g] The testing compound was not eluted within the analytical time.

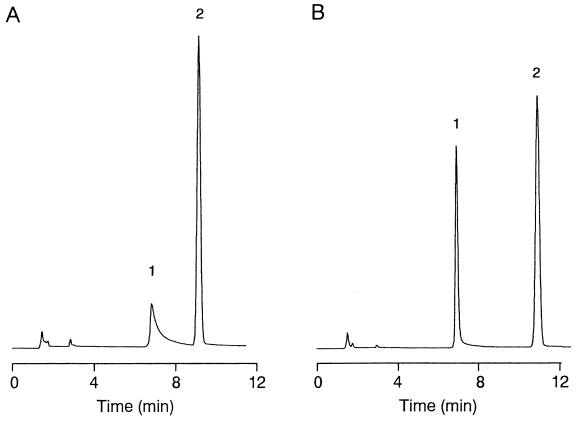


Fig. 3. Chromatograms of hinokitiol (A) on the ODS that was end-capped by LPS and (B) on the ODS that was end-capped by HTS with DMTMDS. HPLC conditions: mobile phase, acetonitrile–20 mmol/l $H_3PO_4$ (4:6, v/v); flow-rate, 1 ml/min; temperature, 40°C; detection, UV 254 nm. Peak: 1=hinokitiol (200 mg/l); 2=benzene (2%).

### *3.3. Chromatography of basic compounds*

Pyridine interacts more strongly with the residual silanol groups than aniline and its derivatives [21,40,43], which have often been used as test compounds in the evaluation of column properties [12,42]. The retention of pyridine is affected by even a small number of residual silanol groups [41]. Pyridine was therefore employed in this study to evaluate the effect of the residual silanol groups on the ODSs prepared. Phenol was used as the reference, and the separation factor ($\alpha_{ph/py}$) was measured, to evaluate the inertness of the ODSs for basic compounds, because there is a good inverse correlation between the $\alpha_{ph/py}$ value and the concentration of silanol groups on the ODS [41]. Table 3 shows the $\alpha_{ph/py}$ values for the ODSs. HPLC of pyridine was less affected by the purity of the silica gels than that of chelating compounds. The purity of the silica gel had no effect on the adsorptive activity of ODS for pyridine when the silica gel was treated with HCl and the ODS was end-capped by HTS, whereas high-purity silica gel was required when the ODS was end-capped by LPS.

The strong interaction between the chelating compounds and the silica-2-based ODS that was end-capped by HTS means that pyridine also came into contact with the residual metal impurities on the surface of this ODS. However, this ODS was as inert for pyridine as the silica-4-based ODS that was end-capped by HTS. These results suggest that the residual metals, after HCl treatment, did not directly interact with basic compounds. On the contrary, the silica-2-based ODS that was end-capped by LPS was more active for pyridine than the silica-4-based ODS that were end-capped by LPS, suggesting that the residual silanol groups on the former are more active than those on the latter. Therefore, it is concluded that, after HCl treatment, the residual metals activated silanol groups rather than interacted directly with basic compounds. On the other hand, the ODS prepared from low-purity untreated silica gel (silica 1) showed adsorptive activity for pyridine despite end-capping by HTS. The metal impurities on the surface of the untreated silica gel may be active points of adsorption or may inhibit end-capping.

Snyder et al. [44] noted that end-capping cannot completely overcome the disadvantage of an acidic silica gel. However, we have shown that HTS can almost completely deactivate ODS, even if the silica support has a large number of metal impurities and acidic silanol groups.

The properties of the ODSs differ between batches as well as between manufacturers, especially in the HPLC of basic solutes [45,46]. This is attributed to differences in the properties of the base silica [12,14,45]. However, the $\alpha_{ph/py}$ value for the silica-2-based ODS end-capped by HTS and that for the silica-4-based ODS end-capped by HTS were almost the same, which indicates that HTS end-capping contributes to reproducibility in the preparation of ODSs.

### *3.4. Stability of ODSs*

ODSs prepared from high-purity silica gel were end-capped by HTS with $D_3$ or by LPS with HMDS. The effects of each end-capping method on the stability of the stationary phase and end-capping groups against hydrolysis were compared using these ODSs. An acidic or alkaline mobile phase was passed through columns that were packed with each ODS, and the performance of the columns was examined periodically. The acidic mobile phase used was methanol–20 mmol/l $H_3PO_4$ (pH 2.1) (1:1,

v/v), the alkaline mobile phase was methanol–20 mmol/l $K_2HPO_4$ (pH 9.3) (1:1, v/v), and the flow-rate was 1 ml/min. The columns were purged sequentially with 10 ml of methanol–water (1:1, v/v) and 30 ml of methanol at 1 ml/min before the performance of the columns was periodically examined. The stability of the octadecylsilyl groups, the base silica and the end-capping groups against hydrolysis were evaluated using the retention factor ($k'$) of naphthalene, the number of theoretical plates ($N$) and the $\alpha_{ph/py}$ values, respectively.

The results are shown in Fig. 4 Fig. 5 Fig. 6. The $k'$ value, $N$ and the $\alpha_{ph/py}$ value for the ODS end-capped by HTS were constant for more than 600 h in the acidic mobile phase and for 400 h in the alkaline one. In contrast, for the ODS that was end-capped by LPS, the $k'$ and $\alpha_{ph/py}$ values decreased rapidly in both mobile phases. $N$ decreased rapidly and a gap was formed at the top of the ODS bed after 300 h from the start of the test in the alkaline mobile phase. However, $N$ did not decrease in the acidic mobile phase.

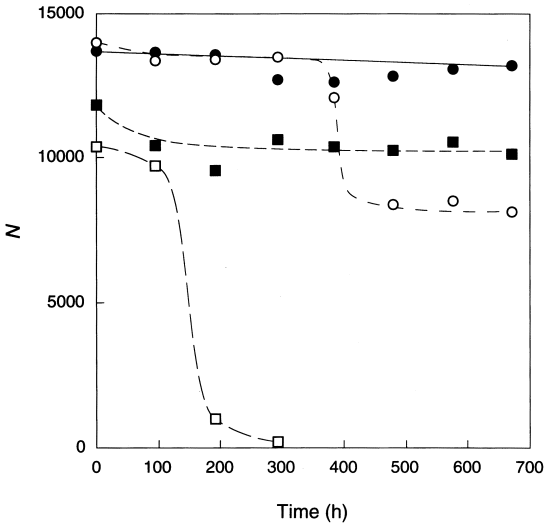


Fig. 5. Change in the number of theoretical plates for the ODS that was end-capped by HTS (circles) and for the ODS that was end-capped by LPS (squares) in the stability test. Test conditions and HPLC conditions are as for Fig. 4.

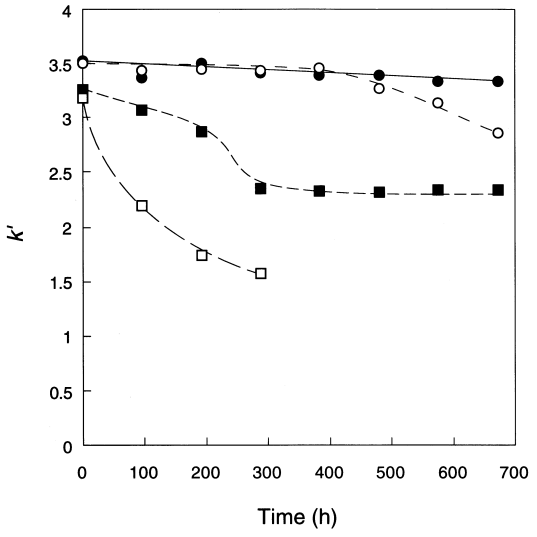


Fig. 4. Change in $k'$ value of naphthalene for the ODS that was end-capped by HTS (circles) and for the ODS that was end-capped by LPS (squares) in the stability test. Test conditions: acidic mobile phase, methanol–20 mmol/l $H_3PO_4$ (pH 2.1) (1:1, v/v; black); alkaline mobile phase, methanol–20 mmol/l $K_2HPO_4$ (pH 9.3) (1:1, v/v; white); flow-rate, 1 ml/min; temperature, 40°C. HPLC conditions: test compound, naphthalene; mobile phase, acetonitrile–water (6:4, v/v); flow-rate, 1 ml/min; temperature, 25°C; detection, UV 254 nm.

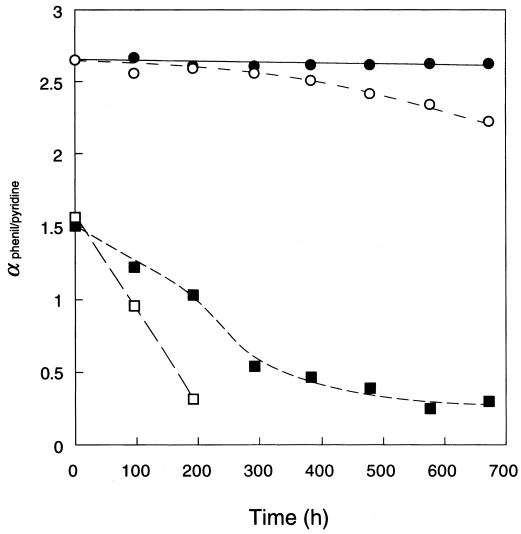


Fig. 6. Change in the $\alpha_{phenol/pyridine}$ value for the ODS that was end-capped by HTS (circles) and for the ODS that was end-capped by LPS (squares) in the stability test. Test conditions: as for Fig. 4. HPLC conditions: test compounds, pyridine and phenol; mobile phase, acetonitrile–water (3:7, v/v); flow-rate, 1 ml/min; temperature, 25°C; detection, UV 254 nm.

The solubility of silica is about 100 ppm in the pH range 1–8, while it increases exponentially above pH 9 [23]. Therefore, the decrease of $N$ in the alkaline mobile phase may be due to the dissolution of the base silica. The results of this test reveal that HTS end-capping inhibited this dissolution. The decrease in the $k'$ value in the alkaline mobile phase may include the loss of the bonded phase caused by the dissolution of silica. On the other hand, the decrease in the $k'$ value for the ODS end-capped by LPS in the acidic mobile phase was due to hydrolysis of the octadecylsilyl group, because base silica was not dissolved in the acidic mobile phase. However, the octadecylsilyl group on the ODS end-capped by HTS was not hydrolyzed.

Snyder et al. [44] noted that the end-capping group, namely, the trimethylsilyl (TMS) group, is readily hydrolyzed from the packing in reversed-phase separation, making the benefits of end-capping marginal for many long-term applications. However, for the ODS that was end-capped by HTS, the constancy of the $\alpha_{ph/py}$ value in the acidic mobile phase reveals that silanol groups were not formed on its surface and the end-capping groups were very stable under this condition. Moreover, the end-capping groups were sufficiently stable to be of practical use even in the alkaline mobile phase. It is concluded that the high surface coverage achieved by HTS provided effective steric hindrance against hydrolysis on the surface of the ODS although the end-capping groups were not bulky.

In addition, the constancy of the $\alpha_{ph/py}$ value for the ODS that was end-capped by HTS proves that siloxane is not formed through dehydroxylation of vicinal silanol groups during HTS because siloxane is readily hydrolyzed to silanol groups [23]. DMTMDS is also expected to provide ODSs with the same stability as $D_3$ because DMTMDS can bond to silanol groups in the same manner as $D_3$, i.e., crosslinkage, and is more effective for HTS end-capping [41].

## 4. Conclusions

End-capping by high-temperature silylation was effective for the elimination of the secondary interaction between ODSs and chelating compounds as well as basic compounds. Moreover, it improved the stability of the ODS against hydrolysis in acidic and alkaline mobile phases. In an acidic mobile phase, in particular, the effect of end-capping by HTS did not diminish for a long time.

## Acknowledgements

We are grateful to Professor Toshiyuki Hobo of Tokyo Metropolitan University for helpful discussions and revision of the manuscript.

## References

[1] C. Horváth, W. Melander, I. Molnár, J. Chromatogr. 125 (1976) 129.
[2] A. Nahum, C. Horáth, J. Chromatogr. 203 (1981) 53.
[3] K.E. Bij, C. Horáth, W.R. Melander, A. Nanum, J. Chromatogr. 203 (1981) 65.
[4] M. Verzele, C. Dewaele, J. Chromatogr. 217 (1981) 399.
[5] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Nakamura, O. Nakata, K. Kimata, N. Tanaka, J. Chromatogr. 481 (1989) 147.
[6] B. Buszewski, Chromatographia 34 (1992) 573.
[7] P.C. Sadek, C.J. Koester, L.D. Bowers, J. Chromatogr. Sci. 25 (1987) 489.
[8] J. Nawrocki, D.L. Moir, W. Szczepaniak, J. Chromatogr. 467 (1989) 31.
[9] J. Nawrocki, D.L. Moir, W. Szczepaniak, Chromatographia 28 (1989) 143.
[10] J.J. Kirkland, C.H. Dilks Jr., J.J. DeStefano, J. Chromatogr. 635 (1993) 19.
[11] K. Tanabe, T. Sumiyoshi, K. Shibata, T. Kiyoura, J. Kitagawa, Bull. Chem. Soc. Jpn. 47 (1974) 1064.
[12] J. Köhler, D.B. Chase, R.D. Farlee, A.J. Vega, J.J. Kirkland, J. Chromatogr. 352 (1986) 275.
[13] M. Muss, H. Engelhardt, J. Chromatogr. 371 (1986) 371.
[14] J. Köhler, J.J. Kirkland, J. Chromatogr. 385 (1987) 125.
[15] P.J.M. Bergers, A.C. De Groot, Water Res. 28 (1994) 639.
[16] S.M. Cramer, B. Nathanael, C. Horváth, J. Chromatogr. 295 (1984) 405.
[17] W.N. Barnes, A. Ray, L.J. Bathes, J. Chromatogr. 347 (1985) 173.
[18] D.J.I. Kingston, B.B. Gerhart, J. Chromatogr. 116 (1976) 182.
[19] M. Verzele, M. De Potter, J. Ghysels, High Resolut. Chromatogr. Chromatogr. Commun. 2 (1979) 151.
[20] M. Ohhira, F. Ohmura, T. Hanai, J. Liq. Chromatogr. 12 (1989) 1065.
[21] D.V. McCalley, J. Chromatogr. 636 (1993) 213.
[22] K. Kimata, N. Tanaka, T. Araki, J. Chromatogr. 594 (1992) 87.

[23] K.K. Unger, Porous Silica: Its Properties and Use as Support in Column Liquid Chromatography, Elsevier, Amsterdam, 1979.
[24] W. Noll, Chemistry and Technology of Silicones, Academic Press, Orlando, FL, 1968.
[25] H.A. Claessens, C.A. Cramers, J.W. de Haan, F.A.H. den Otter, L.J.M. van de Ven, P.J. Andree, G.J. de Jong, N. Lammers, J. Wijma, J. Zeeman, Chromatographia 20 (1985) 582.
[26] H.A. Claessens, J.W. de Haan, L.J.M. van de Ven, P.C. de Bruyn, C.A. Cramers, J. Chromatogr. 436 (1988) 345.
[27] J.L. Glajch, J.J. Kirkland, J. Köhler, J. Chromatogr. 384 (1987) 81.
[28] N. Sagliano Jr., T.R. Floyd, R.A. Hartwick, J.M. Dibussolo, N.T. Miller, J. Chromatogr. 443 (1988) 155.
[29] J.J. Kirkland, J.L. Glajch, R.D. Farlee, Anal. Chem. 61 (1989) 2.
[30] N. Tanaka, K. Kimata, Y. Mikawa, K. Hosoya, T. Araki, Y. Ohtsu, Y. Shiojima, R. Tsuboi, H. Tsuchiya, J. Chromatogr. 535 (1990) 12.
[31] M.J. Wirth, H.O. Fatunmbi, Anal. Chem. 65 (1993) 822.
[32] M.J.J. Hetem, J.W. de Haan, L.J.M. van de Ven, C.A. Cramers, J.N. Kinkel, Anal. Chem. 62 (1990) 2288.
[33] J.J. Kirkland, J.W. Henderson, J.J. DeStefano, M.A. van Straten, H.A. Claessens, J. Chromatogr. A 762 (1997) 97.
[34] M.J.J. Hetem, J.W. de Haan, H.A. Claessens, C.A. Cramers, A. Deege, G. Schomburg, J. Chromatogr. 540 (1991) 53.
[35] J. Köhler, J.J. Kirkland, J. Chromatogr. 385 (1987) 125.
[36] N.T. Miller, J.M. Dibussolo, J. Chromatogr. 499 (1990) 317.
[37] Y. Sudo, T. Takahata, U.S. Patent 5134110, 1992.
[38] Y. Sudo, T. Takahata, European Patent 0443860B1, 1994.
[39] Y. Sudo, T. Takahata, Jpn. Patent 2611545, 1997.
[40] Y. Sudo, J. Chromatogr. A 737 (1996) 139.
[41] Y. Sudo, J. Chromatogr. A 757 (1997) 21.
[42] H. Engelhardt, M. Jungheim, Chromatographia 29 (1990) 59.
[43] P.J. van den Driest, H.J. Ritchie, Chromatographia 24 (1987) 324.
[44] L.R. Snyder, J.L. Glajch, J.J. Kirkland, Practical HPLC Method Development, Wiley-Interscience, New York, 1988, p. 63.
[45] H. Engelhardt, B. Dreyer, H. Schmidt, Chromatographia 16 (1982) 11.
[46] I. Wouters, S. Hendrickx, E. Roets, J. Hoogmartens, H. Vanderhaeghe, J. Chromatogr. 291 (1984) 59.