Short communication

In situ silylation of silica-based packings using supercritical fluid as reaction medium

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Abstract

A method for in situ silylation was developed using supercritical carbon dioxide as a reaction medium and was applied to an endcapping of ODS-silica gels as well as a trimethylsilylation of silica gels. The results of pyridine/phenol test and surface coverage of silica-based packings were improved by the proposed method. It was indicated that supercritical fluid is more effective than organic solvents as the silylation medium.

Keywords: Supercritical fluid chromatography; Stationary phases, LC; Stationary phases, SFC; Phenol; Pyridine

1. Introduction

In LC, silica-based packings are widely used as a stationary phase. Some of the weak points of silica-based packings are a limitation of pH of mobile phase and an adsorption of polar compounds (especially in bases), based on the silanol groups on the silica gel surface. In the case of reversed-phase silica gel packings (e.g. ODS-silica gel), the packings are not completely inert, even after the endcapping of residual silanol groups. Various deactivation procedures are reported in order to improve the inertness of reversed-phase silica gel packings [1–7].

The reversed-phase silica gel packings are used in supercritical fluid chromatography (SFC) as well as LC. The inertness of the column packing has a large influence on the retention of solutes in SFC [8–10]. In SFC, polar solvents, such as methanol, are frequently added to the carbon dioxide mobile phase as

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a modifier. However flame ionization detection, which is one of the advantages of SFC, cannot be used in this case. More deactivation of reversed-phase silica gel packings is required for their use in SFC.

The in situ (on-column) silylation procedure is a simple technique for derivatizing and/or regenerating column packings [11–13]. In previous studies, this technique was applied to the endcapping of ODS-silica gels using organic solvents as the reaction media [10,14]. However, the inertness of the packings endcapped by this procedure was poor in comparison with inert packings on the market. This in situ silylation procedure would be useful as a simple improvement technique for column packings if it had a higher effectiveness.

Supercritical fluids have liquid-like solubilities and gas-like viscosities. The mass transfers in supercritical fluids are faster than those in liquids. In the field of analytical chemistry, supercritical fluids are widely used, such as a mobile phase of SFC and an

extraction medium of supercritical fluid extraction (SFE). A number of investigations were reported concerning the use of an in situ derivatization technique in SFE in order to improve the extraction efficiency [15].

In this study, we demonstrated in situ silylation of silica-based packings using supercritical fluid as the reaction medium. The performance of this procedure was evaluated by the chromatographic behavior.

2. Experimental

2.1. Apparatus

Silylation of silica-based packings using supercritical fluid as the reaction medium was carried out using a Suprex (Pittsburgh, PA, USA) SFC-200A modified as shown in Fig. 1. The silylating agent was introduced into a syringe pump via a Valco (Houston, TX, USA) C6UW injector together with carbon dioxide. Carbon dioxide mixed with silylating agent was introduced into a column placed inside an oven. The flow rate of supercritical fluid was controlled by a restrictor made of a capillary tube of 400 mm × 25 μm I.D.

2.2. Materials

Silica gels (particle size, 10 μm; pore diameter, 130 Å) and monomeric type ODS-silica gels (particle size, 10 mm; pore diameter, 130 Å; without endcapping) were supplied by Fuji Silysia Chemical (Kasugai, Aichi, Japan). Hexamethyldisilazane
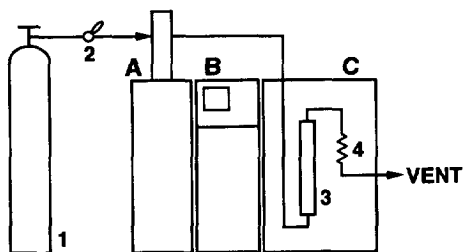

Fig. 1. Schematic diagram of in situ silylation system. The system consisted of a pump unit (A), a control unit (B) and an oven unit (C). 1=carbon dioxide cylinder; 2=injector; 3=column; 4= restrictor.

(HMDS) used as the silylating agent was of silylation grade for GC. Carbon dioxide was of standard grade. Acetonitrile was of HPLC grade. Toluene was dehydrated by molecular sieve (5A). Other reagents were of reagent grade and used without purification.

2.3. Silylation of silica-based packings

Silylation in toluene

Silica gels and ODS-silica gels (ODS-N) were trimethylsilylated with HMDS in toluene for 12 h under reflux with stirring, respectively (SIL-T and ODS-T). According to the elemental analysis, 12 h of reaction time was sufficient for the trimethyl-silylation. After washing with toluene and dichloromethane, both packings were dried under vacuum at 110°C for 6 h. Both packings were packed into empty columns (150 mm × 4.6 mm I.D.) by a slurry method.

In situ silylation using supercritical fluid

SIL-T and ODS-T were trimethylsilylated by an in situ procedure using carbon dioxide containing 1.5% (v/v) of HMDS (SIL-SF and ODS-SF). The reaction conditions were as follows: temperature, 110°C; pressure, 25 MPa; time, 4 h.

2.4. Evaluation of column packings

The column packings were evaluated by the pyridine/phenol (Py/Ph) test in SFC as well as HPLC.

The HPLC system consisted of a GL Sciences (Tokyo, Japan) Model 576 pump, a Reodyne (Cotati, CA, USA) Model 7125 injector, a Shimadzu (Kyoto, Japan) LTO-6A column oven and a Shimadzu SPD-6A UV detector.

The conditions for the Py/Ph test in HPLC were as follows: mobile phase, water–acetonitrile (70:30, v/v); flow rate, 1 ml/min; column temperature, 30°C; detection, UV 254 nm.

The SFC system consisted of a Shimadzu LC-6A pump, the head of which was cooled, a Shimadzu SLC-6A pump controller, a Reodyne Model 7125 injector, a column oven from a Shimadzu LC-1 system, a flame ionization detector from a Shimadzu GC-7A system, and a restrictor made of a capillary

tube of 400 mm × 50 μm I.D. The details of this system were described in previous work [16].

The conditions of the Py/Ph test in SFC were as follows: mobile phase, carbon dioxide; pressure, 25 MPa; column temperature, 40°C.

The carbon content of column packings was obtained using a EA1108 elemental analyzer Carlo Erba (Milan, Italy).

3. Results and discussion

It is considered that the efficiency of in situ silylation depends on the various factors, such as reagent, column temperature and reaction medium. The column temperature was set at 110°C, which corresponds to the boiling point of toluene, in order to evaluate the effectiveness of the supercritical fluid as a reaction medium. HMDS was used as the silylating agent throughout the experiment. In this study, two kinds of silica-based packings were trimethylsilylated by the in situ procedure using supercritical fluid as the reaction medium.

3.1. Trimethylsilylation of silica gels

Silica gels were trimethylsilylated by two kinds of procedures. Table 1 shows the results of the Py/Ph test and carbon content analysis for SIL-T and SIL-SF. According to the literature [17], the surface coverages were calculated from the carbon content as follows: SIL-T, 3.91 μmol/m²; SIL-SF, 4.13 μmol/ m². Silica gels were efficiently trimethylsilylated by additional silylation using supercritical fluid as the reaction medium.

The definition of the separation factor (α), shown in Table 1, is the ratio of k' of phenol to that of pyridine. In general, pyridine, used as a typical base, is strongly adsorbed to residual silanol groups on the
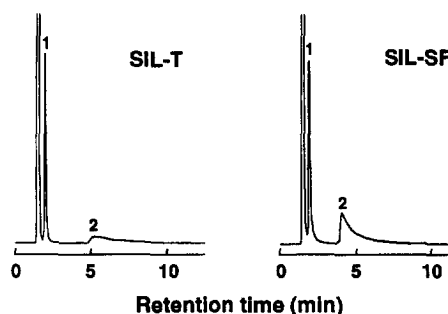

Fig. 2. Chromatograms from the pyridine/phenol test of trimethylsilylated silica gels in SFC. Peaks: 1=phenol; 2=pyridine.

silica gel surface. Therefore, larger values of α indicate satisfactory deactivations. The results for α in Table 1 indicate that silica gels were deactivated by additional trimethylsilylation.

In the Py/Ph test in HPLC, pyridine was eluted faster than phenol. In SFC, on the other hand, the elution order of pyridine and phenol was reversed. In SFC using pure carbon dioxide as the mobile phase, the retention behavior of polar solutes (especially in bases) was more critical than that in HPLC. In the HPLC chromatograms of the Py/Ph test, there were no large differences between SIL-T and SIL-SF. Fig. 2 shows SFC chromatograms for SIL-T and SIL-SF in the Py/Ph test. The advantages of in situ silylation were more clearly observed in SFC than in HPLC.

3.2. Endcapping of ODS-silica gels

In situ trimethylsilylation using supercritical fluid as the reaction medium was also applied to the endcapping of ODS-silica gels. Table 2 shows the results of the Py/Ph test and carbon content analysis for ODS-N, ODS-T and ODS-SF. The value of α was increased by the additional endcapping. The

Table 1
Effect of in situ trimethylsilylation of silica gel using supercritical carbon dioxide as the reaction medium

Columns	Carbon content (%)	α (phenol/pyridine)	
		HPLC	SFC
SIL-T	4.42	2.49	0.12
SIL-SF	4.64	2.54	0.17

Table 2
Effect of in situ endcapping of ODS-silica gel using supercritical carbon dioxide as the reaction medium

Columns	Carbon content (%)	α (phenol/pyridine)	
		HPLC	SFC
ODS-N	17.04	–	–
ODS-T	17.82	2.10	0.09
ODS-SF	18.13	2.28	0.17

surface coverages of ODS-N, ODS-T and ODS-SF with octadecylsilyl and trimethylsilyl groups were 2.65, 3.29 and 3.55 μmol/m^2, respectively. It is considered that 0.26 μmol/m^2 of trimethylsilyl groups were additionally combined to the residual silanol groups by in situ endcapping. These results indicate that the effectiveness of supercritical fluid as the reaction medium for silylation is higher than that of toluene. The surface coverage of ODS-SF is poorer than that of SIL-SF: probably because tri-methylsilylation was sterically hindered by octa-decylsilyl groups on the silica gel surface.

In HPLC, pyridine was eluted faster than phenol in both cases using ODS-T and ODS-SF. The elution time of pyridine became shorter with additional in situ endcapping. Fig. 3 shows SFC chromatograms in the Py/Ph test for ODS-T and ODS-SF. The advantages of the in situ silylation procedure were more clearly observed in SFC than in HPLC. As regards pyridine, the peak shape is improved and elution time becomes shorter with additional endcapping. In addition, phenol, used as a typical acid, is also eluted faster on ODS-SF than on ODS-T. In SFC, however, pyridine is eluted far ahead of phenol and their peak shape is poor, even after in situ endcapping. Therefore, ODS-SF is not good enough and more deactivation is required for its successful use in SFC.

As described before, silica-based packings were more deactivated by the reaction with HMDS in supercritical fluid rather than in liquid. The advantages of supercritical fluid as the reaction medium are probably due to its physical properties, such as lower viscosities of the fluid and/or faster mass transfer of solutes. It is well known that these

properties can be widely varied by changing the temperature and the pressure. It is considered that an investigation of silylation under various conditions of supercritical fluid would be required to obtain optimum conditions.

In this study, we demonstrated in situ silylation of silica-based packings using supercritical fluid. The results from the Py/Ph test and elemental analysis of packings lead to the conclusion that supercritical fluid is more effective than organic solvent as the silylation medium .

References

[1] Y. Sudo, Chromatography, 12 (1991) 38.
[2] N. Nagae, Y. Hatano and D. Ishii, Chromatography, 14 (1993) 45R.
[3] Y. Ohtsu, Y. Shiojima, T. Okumura, J. Koyama, K. Naka-mura, O. Nakata, K. Kimata and N. Tanaka, J. Chromatogr., 481 (1989) 147.
[4] G. Schomburg, J. Köhler, H. Figge, A. Deege and U. Bien-Vogelsang, Chromatographia, 18 (1984) 265.
[5] A. Kurganov, O. Kuzmenko, V.A. Davankov, B. Eray, K.K. Unger and U. Trüdinger, J. Chromatogr., 506 (1990) 391.
[6] M. Hanson, K.K. Unger and G. Schomburg, J. Chromatogr., 517 (1990) 269.
[7] M.J.J. Hetem, J.W. de Haan, H.A. Claessens, C.A. Cramers, A. Deege and G. Schomburg, J. Chromatogr., 540 (1991) 53.
[8] A. Nomura, J. Yamada, K. Tsunoda, K. Fukushima and K. Nobuhara, Anal. Sci., 5 (1989) 335.
[9] A. Nomura, J. Yamada, K. Tsunoda, K. Sakaki and T. Yokochi, Anal. Chem., 61 (1989) 2076.
[10] M. de Weerdt, C. Dewaele, M.Verzele and P. Sandra, J. High Resolut. Chromatogr., 13 (1990) 40.
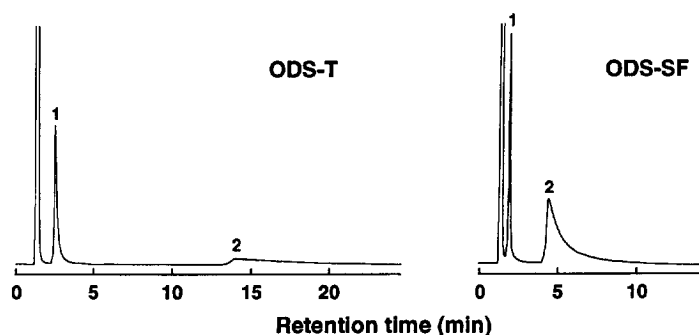[11] R.K. Gilpin, D.J. Camillo and C.A. Janicki, J. Chromatogr., 121 (1976) 13.

Fig. 3. Chromatograms from the pyridine/phenol test of ODS-silica gels in SFC. Peaks: 1=phenol; 2=pyridine.

[12] C. Olieman, E. Sedlick and D. Voskamp, J. Chromatogr., 207 (1981) 421.

[13] T.D. Wilson, J. Chromatogr., 253 (1982) 260.

[14] M. Verzele, M. de Coninck, C. Dewaele and E. Geeraert, Chromatographia, 19 (1984) 443.

[15] T.L. Chester, J.D. Pinkston and D.E. Raynie, Anal. Chem., 66 (1994) 106R.

[16] T. Yarita, Y. Horimoto, J. Yamada and A. Nomura, Anal. Sci., 8 (1992) 811.

[17] G.E. Berendsen, K.A. Pikaart and L. de Galan, J. Liq. Chromatogr., 3 (1980) 1437.