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Development of a new cyano-bonded column for high-performance liquid chromatography

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Abstract

A unique cyano-bonded column for high-performance liquid chromatography was developed by chemically bonding cyanopropyl groups to the silica gel support, and its chromatographic performance was described. Eight cyanopropyl-bonded silica gels were prepared under different conditions. These packing materials were packed into a stainless steel column, the chromatographic properties and durability of which were investigated in both normal- and reversed-phase partition modes. The separating selectivity and the durability of cyanopropyl-bonded silica (CN) columns were dependent on the preparation conditions. Particularly, the relationship between the density of cyanopropyl groups on the surface of the silica gel and the irreversible adsorption of basic compounds was examined. Also, the experimental results indicated that endcapping resulted in the poor separating selectivity and durability of the CN columns.

Keywords: Stationary phases, LC; Selectivity

1. Introduction

A cyanopropyl-bonded (CN) column has been widely applied in the separation of polar compounds from biological samples, because it showed specific selectivity and is suitable for use in both normal- or reversed-phase high-performance liquid chromatography (HPLC) [1–9]. The difference in the retention mechanism and the separating selectivity between the CN column and other types of columns (e.g., amino, diol and unmodified silica columns) has been investigated for a normal-phase partition mode [10–16]. The properties of the CN column are governed by the type of cyano phase (trifunctional or monofunctional) on the silica gel support and endcapping [17]. The specific properties of the CN column have also been shown in reversed-phase partition mode when the chromatographic results of commercially available CN columns and ODS columns were compared [18,19]. Although the CN column is commercially available now, and has a number of advantages, its use is not so widespread due to the problems with durability and irreversible adsorption. In order to improve chromatographic performance of the column, we attempted to develop a new cyano-bonded column. The packing material was synthesized by bonding cyanopropyl groups to the silica support with a high density, and non-endcapping.

2. Experimental

2.1. Materials

Cyanopropyl-bonded silica packings were synthesized using silica gel (particle size 5 μm, surface area 450 m^2/g, pore diameter 100 Å) manufactured

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by GL Sciences (Tokyo, Japan) and silylation reagents. 3-Cyanopropyldimethylchlorosilane, 3-cyanopropyltrichlorosilane and trimethylchlorosilane were purchased from Tisso (Tokyo, Japan). Acetonitrile, methanol, ethanol and hexane used for the mobile phases were of HPLC-grade and purchased from Kishida (Osaka, Japan). Purified water was obtained from a Milli-Q water purification system (Millipore, Milford, MA, USA). All other chemicals were of analytical-reagent grade, and obtained from Kishida.

2.2. Preparation of the cyanopropyl-bonded silica column

Silica gel was dried at 130°C for 3 h and then the silylation reagent (3-cyanopropyldimethylchlorosilane or 3-cyanopropyltrichlorosilane) and *n*-decane were added. After 6 h refluxing *n*-decane, the mixture was cooled to room temperature. The solvent was filtered off and the cyanopropyl-bonded silica gel was sequentially rinsed with methylene chloride and acetone. Furthermore, silylation as described above was done again using the cyanopropyl-bonded silica gel in place of silica gel. In a silylation procedure, so-called endcapping, the cyanopropyl-bonded silica gel and trimethylchlorosilane and toluene were mixed and then refluxed for 3 h. Finally, eight packings which were prepared under different conditions were obtained (Table 1). These packing materials were packed into a stainless steel column (150×4.6 mm I.D.).

2.3. Chromatographic test

2.3.1. Reversed-phase partition mode

Aromatic compounds (ethylbenzene, 5 mg/ml; naphthalene, 0.5 mg/ml; *n*-propylbenzene, 5 mg/ml; *n*-butylbenzne, 6 mg/ml; anthracene, 0.05 mg/ml) and pyridine–phenol (pyridine, 0.09 mg/ml; phenol, 0.41 mg/ml) were used as the samples to evaluate the selectivity and surface activity of cyanopropyl-bonded silica gel, respectively.

2.3.2. Normal-phase mode

Benzene–naphthalene–anthracene (benzene, 15 mg/ml; naphthalene, 1 mg/ml; anthracene, 0.1 mg/ml) and benzylamine–pyridine–aniline (benzylamine, 4 mg/ml; pyridine, 0.5 mg/ml; aniline, 1 mg/ml) were used as the samples to evaluate the retention ability of low polar compounds and surface activity of cyanopropyl-bonded silica gel, respectively.

2.4. Instrumentation

The HPLC system consisted of an auto-sample injector AS640, a pump PU610, a themostated column oven CO630, a UV–Vis detector UV620 and a data analyzing processor V Station. An ODS column Inertsil ODS-3 (150×4.6 mm I.D., particle size 5 μm) was used for comparison with the CN columns. All instruments and columns were from GL Sciences.

Table 1
List of prepared CN columns

CN column	Reagent of cyanopropylsilylation	Number of times of silylation	Endcapping	Carbon loading (C, %, w/w)
A	3-Cyanopropyldimethylchlorosilane	2	No	9.0
B	3-Cyanopropyldimethylchlorosilane	2	Yes	8.2
C	3-Cyanopropyldimethylchlorosilane	1	No	7.3
D	3-Cyanopropyldimethylchlorosilane	1	Yes	7.0
E	3-Cyanopropyltrichlorosilane	2	No	14.1
F	3-Cyanopropyltrichlorosilane	2	Yes	14.0
G	3-Cyanopropyltrichlorosilane	1	No	12.7
H	3-Cyanopropyltrichlorosilane	1	Yes	13.0

3. Results and discussion

3.1. Properties of cyanopropyl-bonded silica gel

3.1.1. Selectivity

In general, the selectivity of a CN column is different from that of an ODS column for reversed-phase HPLC; therefore, a CN column is useful to separate some compounds which cannot be separated by an ODS column, especially when only one organic solvent can be used (e.g., low-wavelength UV detection may be required, in which case only acetonitrile and water are usable). In order to evaluate the selectivity of eight CN columns prepared under different conditions, aromatic compounds were used. The separation factors between the aromatic compounds are shown in Fig. 1. The ODS column was superior for the separation of ethylbenzene and *n*-butylbenzene than the CN columns because the separation was based on hydrophobic interaction. On the other hand, the CN columns E–H, synthesized with 3-cyanopropyltrichlorsilane, exhibited stronger retention towards polyaromatic hydrocarbons than that to alkylbenzenes due to dipolar interaction; therefore, they exhibited higher selectivity in comparison with Inertsil ODS-3. The selectivity of CN columns A–D, prepared with cyanopropyldimethylchlorsilane, were similar to Inertsil ODS-3. Moreover, columns E and G showed the highest selectivity due to the absence of endcapping.

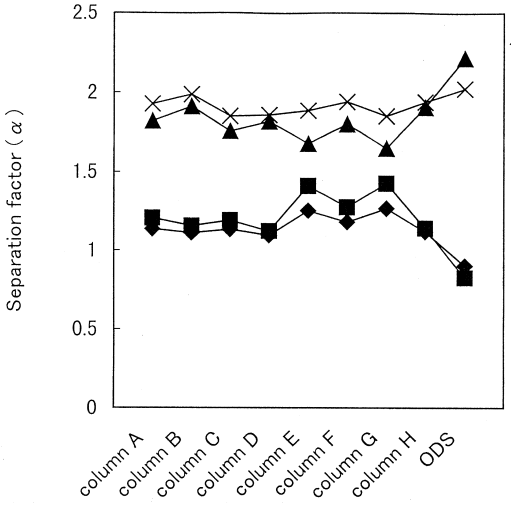

Fig. 1. The separation factor between aromatic compounds using CN columns and ODS column. Mobile phase: acetonitrile–water (40:60); flow-rate: 1.0 ml/min; column temperature: 40°C; sample volume: 1 μl; detection: UV at 254 nm. ♦=α(naphthalene–ethylbenzene); ■ α(anthracene–*n*-butylbenzene); ▲=α(*n*-butylbenzene–ethylbenzene); ×=α(anthracene–naphthalene).

3.1.2. Irreversible adsorption in reversed-phase HPLC

The residual silanol groups on the surface of packing materials are acidic and strongly retain basic samples. The retained basic samples elute late or elute as tailing peaks, causing difficulties with analyzing the basic samples [20]. In this study, pyridine and phenol were used to evaluate CN columns and the chromatograms are shown in Fig. 2. From columns prepared using 3-cyanopropyldimethylchlorsilane (columns A–D), pyridine is eluted as a sharp peak. On the other hand, from columns prepared using 3-cyanopropyltrichlorsilane (columns E–H), the retention time and peak shape of pyridine changed according to the density of cyanopropyl groups on the silica support. In the case of column G, pyridine is eluted late after phenol as a tailing peak due to low density (Table 1) of cyanopropyl groups on silica support.

3.1.3. Capacity factors of less polar compounds

The capacity factors of benzene, naphthalene and anthracene using hexane as mobile phase are shown in Fig. 3. Generally, a CN column was less useful in comparison with an unmodified silica column because of the weak retention of less polar solutes when using hexane as a mobile phase [10,21]. However, the CN columns used non-endcapped packing like column E have capacity factors that can separate these compounds sufficiently.

3.1.4. Irreversible adsorption in normal-phase HPLC

Basic compounds were also applied to evaluate CN columns for normal-phase partition mode. The chromatograms of benzylamine, pyridine and aniline are shown in Fig. 4. Excepting pyridine, the peak shapes of benzylamine and aniline were similar, even if the different types of the CN columns were used.

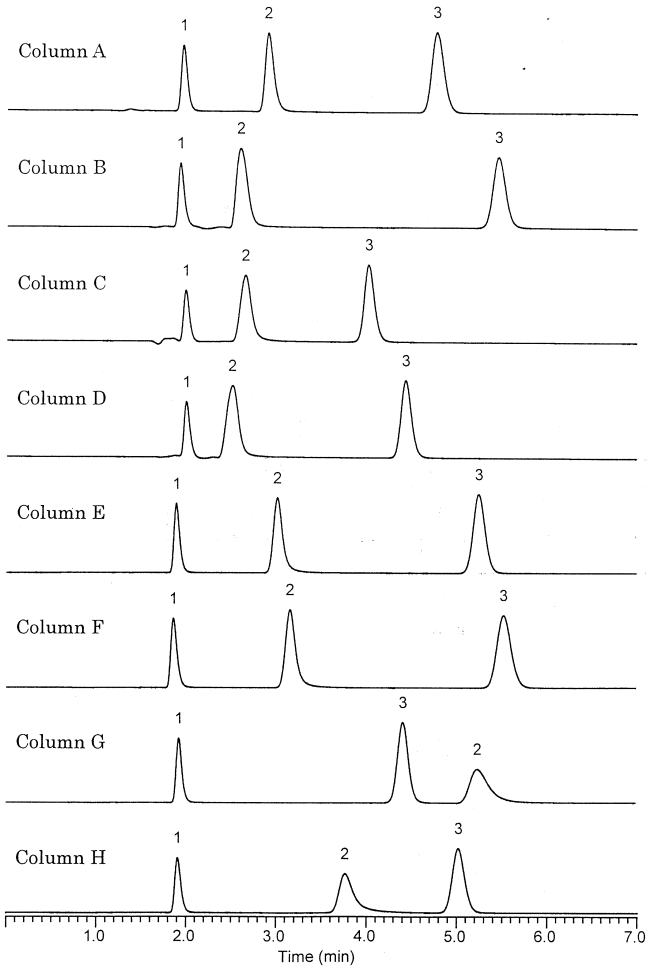

Fig. 2. Chromatograms of uracil, pyridine and phenol. Mobile phase: methanol–water (30:70); flow-rate: 1.0 ml/min; column temperature: 40°C; sample volume: 1 μl; detection: UV at 254 nm. Peaks: 1=uracil, 2=pyridine, 3=phenol.

The retention and peak shape of pyridine were influenced by the residual silanol groups and the density of cyanopropyl groups on the silica support.

3.2. Durability

As shown in Fig. 5, the durability of CN columns was compared at a condition of acidic eluent (0.1% trifluoroacetic acid). The columns packed with non-endcapped packing material and synthesized using 3-cyanopropyltrichlorsilane (columns E and G) showed relatively high durability. The chromatograms of aromatic compounds and relationship between the selectivity and the capacity factor were compared among columns E, F and F′ (column F′ was column F exposed to 0.1% trifluoroacetic acid for 500 h), the results of which are shown in Figs. 6 and 7. In column F, trimethylsilylmethyl groups were hydrolyzed by exposure to acidic eluent; therefore, the column showed properties similar to the non-endcapped column, and a decrease in retention and an increase in selectivity.

4. Conclusion

The analytical results of aromatic compounds and basic compounds using CN columns were strongly

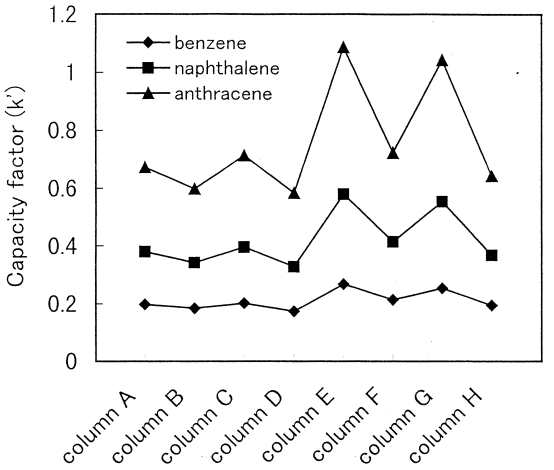

Fig. 3. The capacity factor of benzene, naphthalene, anthracene using CN columns. Mobile phase: *n*-hexane; flow-rate: 1.0 ml/min; column temperature: 40°C; sample volume: 1 μl; detection: UV at 254 nm.

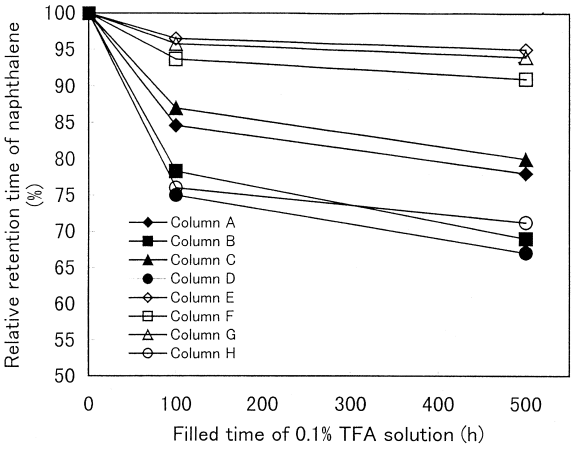

Fig. 5. The durability of CN columns. Naphthalene was analyzed under the following conditions: mobile phase: acetonitrile–water (30:70); flow-rate: 1.0 ml/min; column temperature: 40°C; detection: UV at 254 nm.

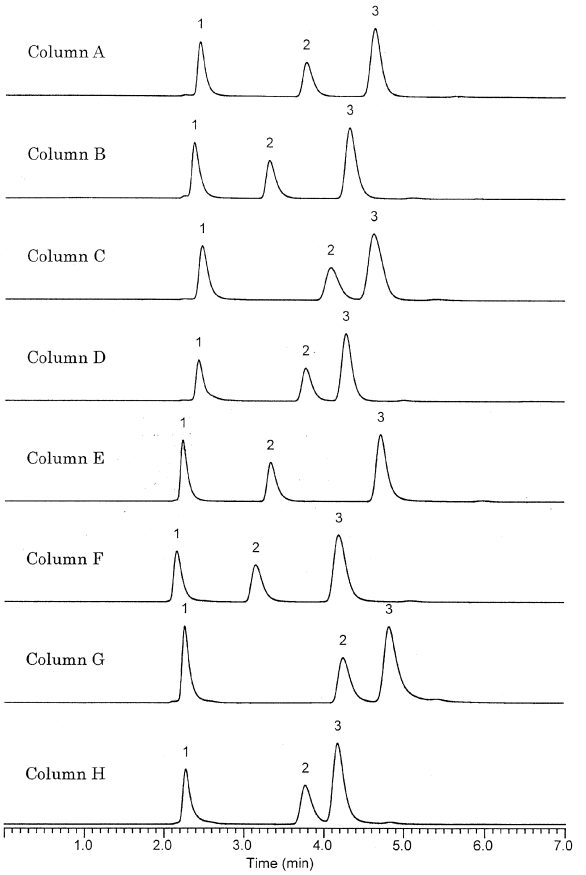

Fig. 4. Chromatograms of benzylamine, pyridine and aniline. Mobile phase: ethanol–*n*-hexane (5:95), flow-rate: 1.0 ml/min; column temperature: 40°C; sample volume: 1 μl; detection: UV at 254 nm. Peaks: 1=benzylamine, 2=pyridine, 3=aniline.

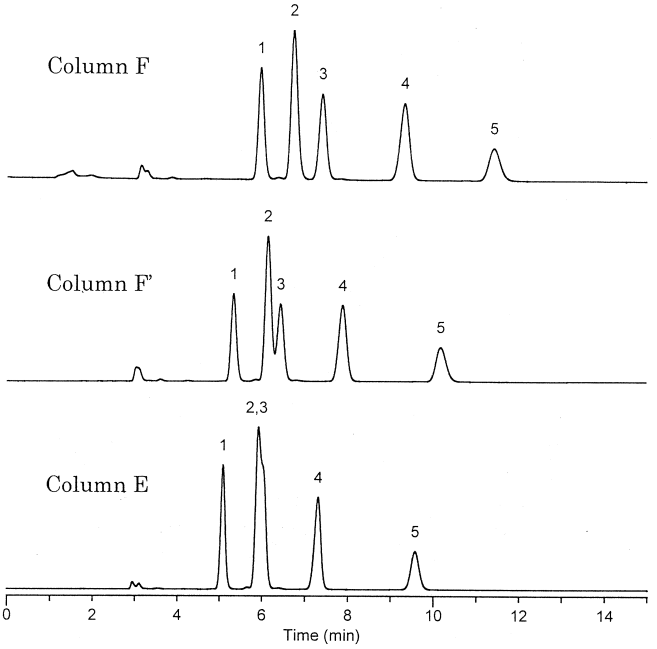

Fig. 6. Chromatograms of aromatic compounds. Mobile phase: acetonitrile–water (40:60); flow-rate: 1.0 ml/min; column temperature: 40°C; sample volume: 1 μl; detection: UV at 254 nm. Peaks: 1=ethylbenzene, 2=naphthalene, 3=*n*-propylbenzene, 4=*n*-butylbenzene, 5=anthracene.

controlled by the type of cyanopropylsilylation reagent used and by the degree of cyanopropylsilylation and endcapping. For the evaluation of column performance, the analytical method using the compounds (i.e., ethylbenzene, naphthalene, pyridine) is very effective. The packing materials synthesized using 3-cyanopropyltrichlorsilane showed a greater durability and separation selectivity than

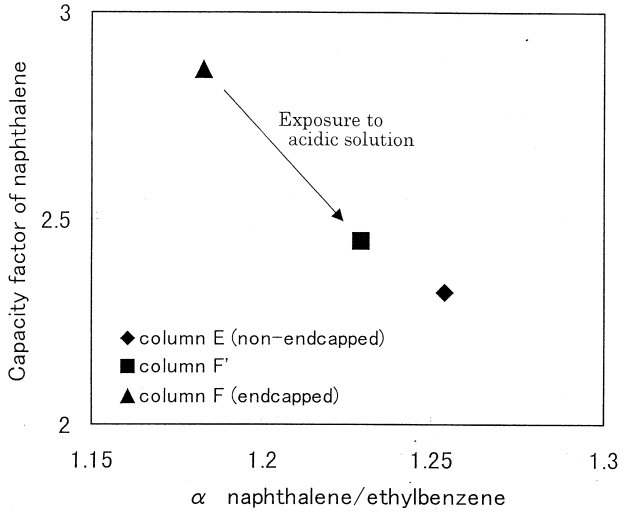

Fig. 7. Change of the retention and the selectivity of column F by exposure to acidic solution.

the packing materials synthesized using 3-cyanopropyldimethylchlorsilane. In reversed-phase mode, the endcapping of cyanopropyl-bonded silica gel caused a decrease in the separation selectivity and durability. In normal-phase mode, endcapping diminished the retention of less polar compounds and was effective for inhibiting adsorption of basic compounds. The adsorption of basic compounds was inhibited by a high loading of cyanopropyl groups onto the silica support in spite of non-endcapping. The experimental results demonstrated that column E is more useful than the other CN columns because it has high selectivity, satisfactory durability and low adsorption activity.

References

[1] S.C. Buist, C.Y.L. Hsu, R.R. Walters, J. Chromatogr. A 828 (1998) 259.
[2] R.R. Walters, S.C. Buist, J. Chromatogr. A 828 (1998) 167.
[3] E. Sottofattori, M. Anzaldi, A. Balbi, G. Tonello, J. Pharm. Biomed. Anal. 18 (1998) 213.
[4] C.L. Boehme, H.W. Strobel, J. Chromatogr. B 718 (1998) 259.
[5] H. Gao, S. Roy, F. Donati, F. Varin, J. Chromatogr. B 718 (1998) 129.
[6] M. Aravagiri, S.R. Marder, D. Wirshing, W.C. Wirshing, Pharmacopsychiatry 31 (1998) 102.
[7] M. Berna, R. Shugert, J. Mullen, J. Mass Spectrom. 33 (1998) 138.
[8] P.K. Kunicki, D. Sitkiewicz, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1169.
[9] S.R. Abbott, J. Chromatogr. Sci. 18 (1980) 540.
[10] E.L. Weiser, A.W. Salotto, J. Chromatogr. 303 (1984) 1.
[11] P.L. Smith, W.T. Cooper, J. Chromatogr. 410 (1987) 249.
[12] M. Lubke, J.L. Quere, D. Barron, J. Chromatogr. A 690 (1995) 41.
[13] E. Grimvall, C. Ostman, J. Chromatogr. A 675 (1994) 55.
[14] J.H. Park, M.H. Yoon, Y.K. Ryu, B.E. Kim, J.W. Ryu, M.D. Jang, J. Chromatogr. A 796 (1998) 249.
[15] J. Li, D.A. Whitman, Anal. Chim. Acta 368 (1998) 141.
[16] P.L. Smith, W.T. Cooper, Chromatographia 25 (1988) 55.
[17] I. Chappel, LC·GC Int. 7 (1994) 282.
[18] R.M. Smith, S.L. Miller, J. Chromatogr. 464 (1989) 297.
[19] P.E. Antle, A.P. Goldberg, L.R. Snyder, J. Chromatogr. 321 (1985) 1.
[20] H. Tanaka, M. Kamada, M. Nyudo, M. Ohira, J. Chromatogr. A 762 (1997) 89.
[21] T. Hamoir, D.L. Massart, J. Chromatogr. A 673 (1994) 1.