Cyanobiphenyl-substituted Polymethylsiloxane Encapsulated Particles for Packed Capillary Column Supercritical Fluid Chromatography

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Abstract. Nine isomeric cyanobiphenyl-substituted (25%) polymethylsiloxane (CBP) stationary phases were coated and immobilized by free-radical crosslinking on silica packing materials for use in supercritical fluid chromatography (SFC). The selectivities of these packing materials to benzene derivatives, polycyclic aromatic hydrocarbons (PAHs), and double bonds in fatty acid methylesters (FAMEs) were determined. Among the nine isomeric CBP-coated packing materials, the *o*, *p*-CBP phase was found to be the most selective to both double bonds and aromatic isomers under SFC conditions. The durability of the packing materials was evaluated by measuring the changes in selectivity with SFC use. The loss in selectivity was less than 0.20% after being continuously washed by supercritical carbon dioxide at 100°C and 300 atm for more than 100 h.

Key words: *supercritical fluid chromatography, stationary phases, packing materials, packed capillary columns, cyanobiphenyl-substituted polymethylhydrosiloxane*

INTRODUCTION

One advantage of packed column supercritical fluid chromatography (SFC) is the ready availability of packing materials which have been developed for high performance liquid chromatography (LC). However, the packing materials used for packed column SFC should be less polar than those used in LC because of the limitation in polarity of supercritical CO_2 which is the most widely used mobile phase. Eliminating strongly polar silanol groups on the silica surface and producing weakly polar and selective groups on the packing materials is an effective means to obtain selective separations while still permitting the use of neat supercritical CO_2 as mobile phase.

Deactivation of the silica surface can be carried out with either small molecule reagents or with polymeric silylation reagents. Using small monomeric silylation reagents with one to several reactive groups, such as chloro-, dichloro-, and trichlorosilanes, monolayer coverage with organic functionalities can be formed on the silica surface [1, 2]. The use of the dehydrocondensation reaction to bond short polymeric chains to silica particles is an effective means of achieving a well-deactivated surface. Previous work has shown that less polar packing materials could be obtained using polymeric deactivations than with small monomeric silylation reagents [3]. Selective packing materials suitable for special applications can be obtained by incorporating some selective groups in the deactivating polymer or by coating and immobilizing a second polymer layer over the deactivation layer which has the selectivity desired. The latter method serves to encapsulate the silanol groups more completely and permits the use of some commercial stationary phases which have been developed for gas chromatography [4, 5].

Typical polar and selective stationary phases used for gas chromatography include polyethylene glycol [6] and the cyanopropyl-substituted

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polysiloxanes [7]. The stability and lifetimes of open tubular columns coated with these two types of stationary phases have not been reported under the conditions of SFC; however, it is known that the polyethylene glycol phases have been satisfactorily used in certain instances. Cyanobiphenyl-substituted polymethylsiloxane stationary phases (CBPs) were recently developed by Rossiter et al. [8]. The GC evaluation of these stationary phases showed that they could be coated and crosslinked more easily and completely than Carbowax 20M and the 50% cyanopropyl SP series of stationary phases [9]. The thermal stability of the cyanobiphenyl stationary phases is sufficient for an allowable operating temperature of as high as 300°C. GC evaluation also showed that the selectivity of the CBP phases to aromatic isomers was greater than those of Carbowax 20M and 50% cyanopropyl-substituted polymethylsiloxane. The selectivity to double bonds was less than Carbowax 20M or SP 2380. The elution order of the fatty acid methylesters (FAMEs) with carbon number 18 was 18:1, 18:0, 18:2, and 18:3 on a CBP column. In contrast, the elution order on Carbowax 20M and SP 2380 was 18:0, 18:1, 18:2, and 18:3 [5,6]. It is clear from these previous studies that the CBPs are medium polar stationary phases.

Shape/size selectivity is often required for the separation of complex mixtures of aromatic isomers. Long organic chains in the stationary phase, such as is characteristic of the polymeric C_{18} bonded phases, provide shape/size selectivity in LC [10,11], while liquid crystalline stationary phases give a similar effect in GC [12,13]. SFC separations should provide greater speed and better resolution than LC, because of the faster mass transfer of solutes in the supercritical mobile phase [14]. Cyanobiphenyl-substituted stationary phases exhibit liquid crystalline properties and have been shown to possess shape/size selectivity [9].

In this article, porous silica particles (10 μm, 80 Å pore size) were deactivated by dehydrocondensation of polymethylhydrosiloxane and silanol groups on the silica surface, and then coated with the 25% cyanobiphenyl-substituted polymethylsiloxane stationary phases. Using a free radical reaction, the cyanobiphenyl-substituted polymethylsiloxane stationary phase was crosslinked within the stationary phase layer and with the polymer layer which was bonded to the silica surface in the deactivation step. The shape/size selectivity of the resultant columns to positional isomers of benzene and polycyclic aromatic hydrocarbons (PAHs) was investigated. The selectivity to double bonds of FAMEs was also measured under SFC conditions.

EXPERIMENTAL

Materials and instrumentation. Nine cyanobiphenyl-substituted (25%) polymethylsiloxane stationary phases were previously synthesized according to ref. [8]. Porous silica particles (10 μm, 80 Å pores) were purchased from Alltech Associates (Deerfield, IL, USA), azo-*tert*-butane was purchased from Lancaster (Windham, NH, USA), and polymethylhydrosiloxane was purchased from Hüle (Bristol, PA, USA). The fused silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA). Column connections were made using zero dead volume unions (Valco Instruments, Houston, TX, USA). The column packing and SFC analyses were performed using a Lee Scientific Model 600 SFC instrument (Dionex, Salt Lake City, UT, USA). SFC grade carbon dioxide was purchased from Scott Specialty Gases (Plumsteadville, PA, USA), lime oil was purchased from BERJE (Bloomfield, NJ, USA) and all other chemicals used were purchased from Aldrich (Milwaukee, WI, USA).

Deactivation reaction. Polymethylhydrosiloxane was used for the deactivation according to the procedure described in ref. [4].

Coating and crosslinking reaction. Cyanobiphenyl-substituted polymethylsiloxane stationary phases (0.03 g) and 0.001 g azo-*tert*-butane were dissolved in 20 mL of methylene chloride, and then transferred into a reaction vessel (described in ref. [3]). Polymethylhydrosiloxane deactivated silica particles (0.2 g) were also transferred into the reaction vessel and argon gas was bubbled through the mixture at room temperature. After the solvent had vaporized, the crosslinking reaction was carried out by heating from room temperature to 210°C at 2°C min^{-1} and then holding for 5 h at 210°C under a continual argon gas purge. After the reaction was complete, the product was washed with 50 mL of methylene chloride.

Packing of the capillary columns. Fused silica capillary columns (40 cm × 320 μm i.d.) were packed using carbon dioxide at room temperature and 80 atm pressure according to the procedure described in ref. [15].

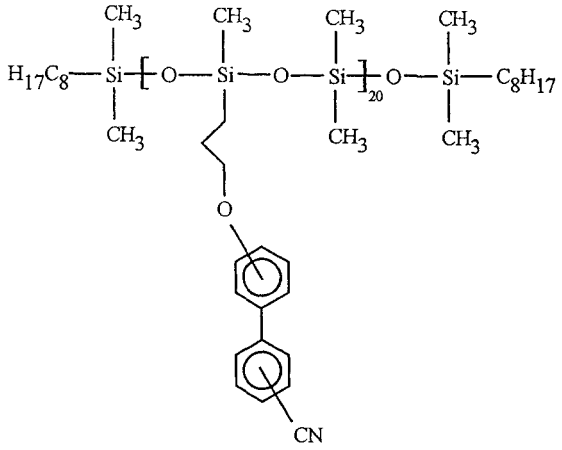

Figure 1. *Structure of the 25% cyanobiphenyl-substituted polymethylsiloxane. The percentage of cyano (w/w) in the polymer is 6.72%.*

RESULTS AND DISCUSSION

Figure 1 shows the structures of the 25% cyanobiphenyl-substituted polymethylsiloxane stationary phases. There are nine different isomers (*p,p*-CBP, *m,p*-CBP, *o,p*-CBP, *p,m*-CBP, *m,m*-CBP, *o,m*-CBP, *p,o*-CBP, *m,o*-CBP, and *o,o*-CBP). The content of the cyano groups in the polymers was 6.72% (w/w).

Selectivity to aromatic isomers. Table I lists the selectivities of the capillary columns packed with untreated porous silica particles, CBP coated particles, and SE-54 (5% phenyl, 1% vinyl) coated particles [4] to the dodecylxylene isomers under the conditions of SFC. All nine CBP coated particles showed greater selectivity to the positional isomers of the benzene derivatives than the SE-54 coated particles or the untreated porous silica. The *o,p*-CBP phase gave the greatest selectivity among all nine CBP phases. Figure 2 shows SFC separations of six dodecylxylene isomers with three packed capillary columns. The elution order of the isomers was the same on the column containing SE-54 as on the CBP coated particles. However, a better separation of the dodecylxylene isomers could be obtained with the CBP coated particles; 2,5-dimethyldodecylbenzene (isomer 2) and 2,4-dimethyldodecylbenzene (isomer 3) could be separated with the column packed with *o,p*-CBP coated particles, but could not be separated with the column packed with SE-54 coated material. The selectivity of CBP to benzene derivatives probably arises from the higher concentration of benzene rings in the CBP stationary phase than in the SE-54 stationary phase (1% vinyl, 5% phenyl). Polar groups have minor contribution to the separation selectivity of aromatic isomers [16]. The elution order of the isomers on the column packed with untreated silica particles was different from those observed on the columns packed with SE-54 or CBP coated particles. This is because of the different separation mechanism on these two types of columns. For the column packed with untreated silica particles, the separation is influenced mainly by an adsorption mechanism, while for the columns packed with SE-54 and CBP coated particles, the separation is based on the selective partitioning of analytes between the supercritical mobile phase and polymeric stationary phase.

Table I. *Selectivities of capillary column packing materials to benzene derivatives (dodecylxylene isomers) under SFC conditions.*[a]

Stationary phase	$\alpha_{5/1}$[b]	$\alpha_{4/3}$[b]	$\alpha_{4/1}$[b]
p,p-CBP	1.183	1.026	1.139
o,p-CBP	1.208	1.037	1.147
m,p-CBP	1.152	1.017	1.103
p,m-CBP	1.199	1.025	1.140
o,m-CBP	1.184	1.019	1.131
m,m-CBP	1.189	1.027	1.128
p,o-CBP	1.185	1.025	1.127
o,o-CBP	1.170	1.024	1.108
m,o-CBP	1.161	1.018	1.106
SE-54	1.117	1.000	1.080
silica	1.418	1.000	1.130

[a]Neat CO_2; 85°C; 170 atm; 40 cm × 320 μm i.d. fused silica capillary columns. The dead time was measured using methane.
[b]Compounds used for the determination of selectivity were (1) 3,5-dimethyldodecylbenzene, (3) 2,4-dimethyldodecylbenzene, (4) 2,3-dimethyldodecylbenzene, (5) 1,5-dimethyldodecylbenzene.

Figure 3 shows an SFC separation of a mixture of PAHs. The phenyl and quaterphenyl isomers can be separated from each other using columns packed with silica particles (300 Å pores) as well as with the *o,p*-CBP coated material. However, the *m*-terphenyl and *p*-terphenyl isomers could not be separated from each other on the column packed with SE-54 coated particles. The aromatic isomers, benz[a]anthracene, benzo[c]phenanthrene, and chrysene could not be separated on columns packed with untreated silica or SE-54 coated particles. However, good separation could be obtained on the column packed with *o,p*-CBP

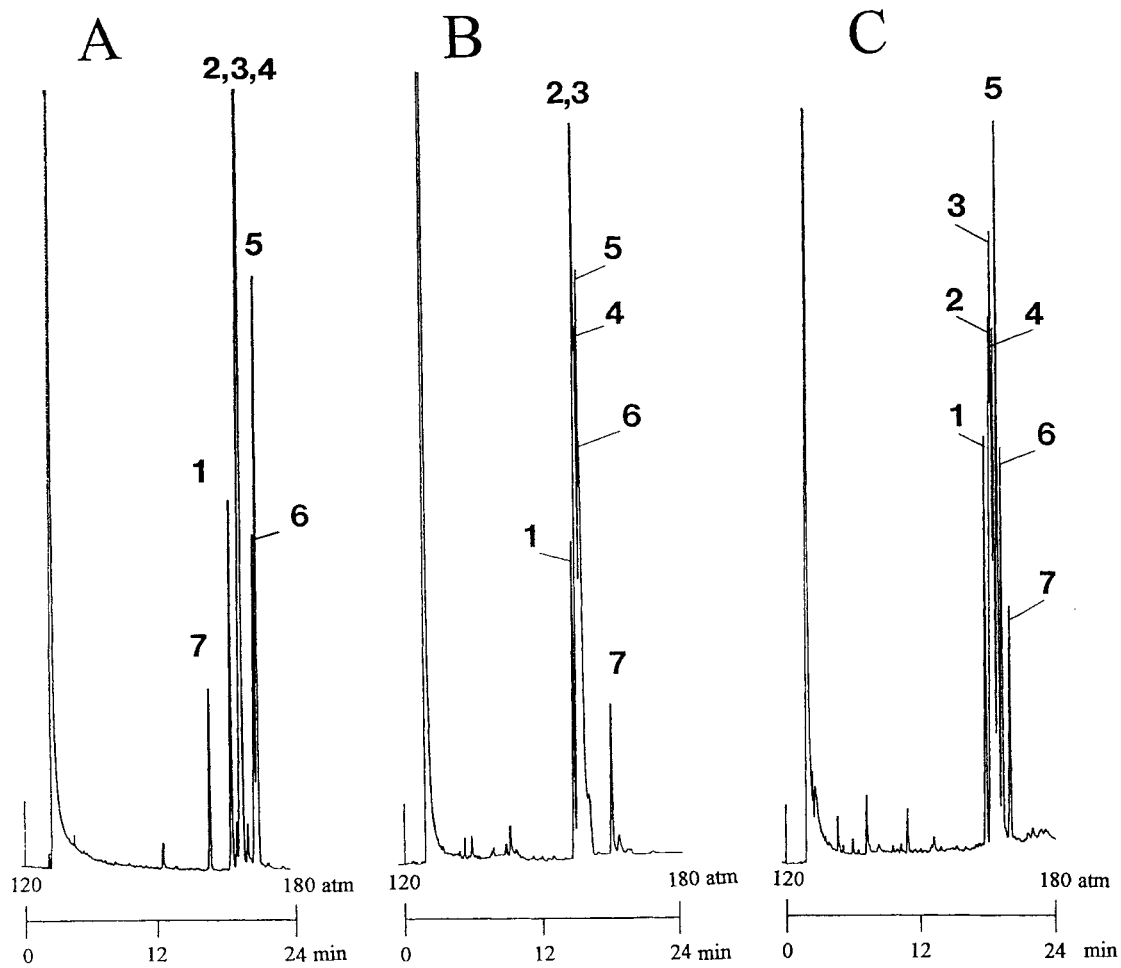

Figure 2. *SFC chromatograms of positional isomers of dodecylxylene. Conditions: 40 cm × 320 μm i.d. fused silica capillary columns packed with (A) untreated silica particles, (B) SE-54 coated silica particles, and (C) o, p-CBP coated silica particles; 85°C; pressure programmed from 120 atm to 180 atm at 2.5 atm min^{-1}; neat CO_2 mobile phase. Peak identifications: (1) 3,5-dimethyldodecylbenzene, (2) 2,5-dimethyldodecylbenzene, (3) 2,4-dimethyldodecylbenzene, (4) 2,3-dimethyldodecylbenzene, (5) 1,5-dimethyldodecylbenzene, (6) 1,2-dimethyldodecylbenzene, (7) unknown.*

coated particles. The elution order was benzo[c]phenanthrene, benz[a]anthracene, and chrysene, which is the same as in LC with polymeric C_{18} bonded packing materials [10, 11] and in GC with liquid crystalline and CBP stationary phases [9, 12, 13]. However, the elution of triphenylene was different. With the polymeric C_{18} bonded phase in LC and the liquid crystalline stationary phase in GC, it elutes between benzo[c]phenanthrene and benz[a]anthracene according to the L/B value [10–13]. With the CBP stationary phase in GC, it elutes between benz[a]anthracene and chrysene [9]. Under the conditions of SFC with the CBP-coated particles, it elutes later than chrysene. This result suggests that the shape recognition mechanism is probably not the sole interaction between the CBP stationary phase and the PAHs.

Selectivity to double bonds. The polar cyano group has been found to provide selective interaction with double bonds [17, 18]. Table II lists the selectivities of the CBP phases to the double bonds of FAMEs. Under SFC conditions, the *o, p*-CBP coated particles showed the greatest selectivity to double bonds compared to the other CBP coated particles. Figure 4 shows the separation of a mixture of saturated and unsaturated FAMEs with columns packed with untreated, *o, p*-CBP coated, and SE-54 coated particles. The saturated stearic acid methylester could not be separated from the oleic acid methylester using capillary columns packed with CBP coated particles under SFC

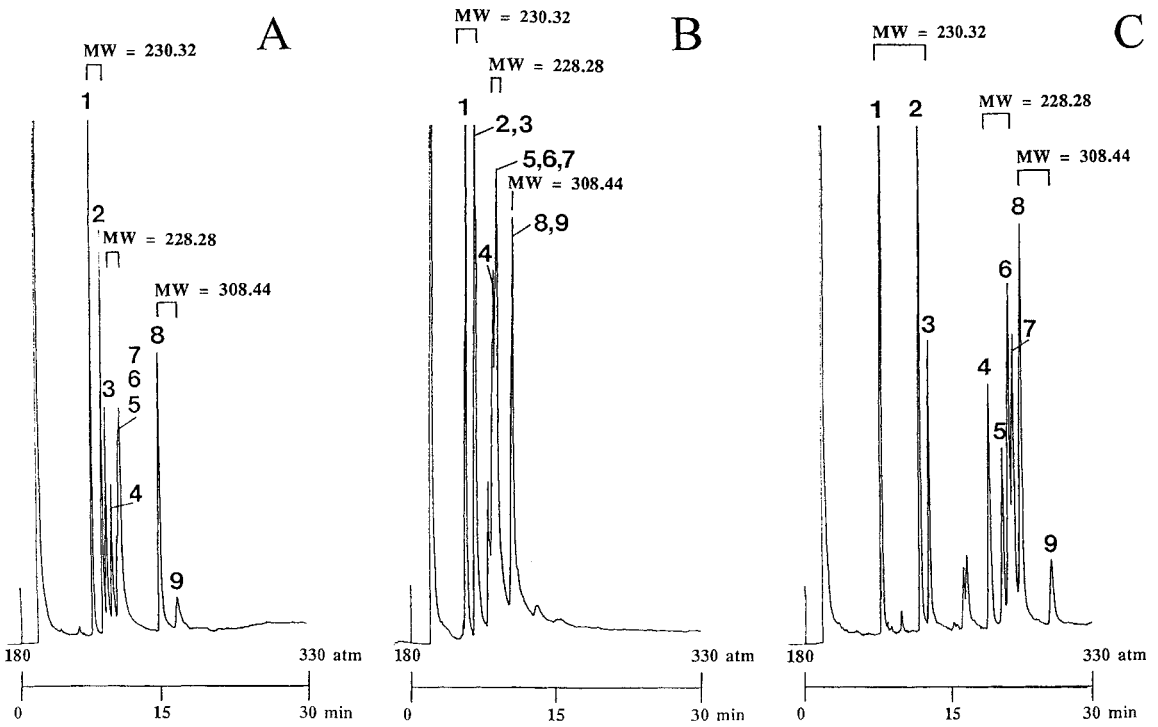

Figure 3. *SFC chromatograms of a mixture of PAHs. Conditions: 40 cm × 320 μm i.d. fused silica capillary columns packed with (A) untreated silica particles, (B) SE-54 coated silica particles, and (C) o, p-CBP coated silica particles; 85°C; pressure programmed from 180 atm to 330 atm at 5 atm min^{-1}; neat CO_2 mobile phase. Peak identifications: (1) o-terphenyl, (2) m-terphenyl, (3) p-terphenyl, (4) benzo[c]phenanthrene, (5) benz[a]anthracene, (6) chrysene, (7) triphenylene, (8) o-quaterphenyl, (9) p-quaterphenyl.*

Table II. *Selectivities of capillary column packing materials to fatty acid methylesters (FAMEs) under SFC conditions.*[a]

Stationary phase	$\alpha_{2/1}$[b]	$\alpha_{3/2}$[b]
p, *p*-CBP	1.015	1.050
o, *p*-CBP	1.052	1.085
m, *p*-CBP	1.013	1.047
p, *m*-CBP	1.050	1.075
o, *m*-CBP	1.046	1.072
m, *m*-CBP	1.033	1.062
p, *o*-CBP	1.042	1.072
o, *o*-CBP	1.038	1.060
m, *o*-CBP	1.038	1.057
SE-54	1.000	1.000

[a]Conditions were the same as those in Table I.
[b](1) methyloleate, (2) methyllinoleate, (3) methyllinolenate.

conditions. The elution order of the FAMEs on columns packed with the CBP phase was according to the carbon number of the FAMEs. Within the set of compounds with the same carbon number, the elution order was according to the number of double bonds. There was no overlap among the FAMEs with different carbon number and double bonds, and symmetrical peak shapes could be obtained with neat CO_2 as mobile phase. This result indicates that the CBP coated particles represent medium polar stationary phases. Although FAMEs are weakly polar compounds, untreated silica particles exhibit strong adsorption toward them and produce seriously tailing peaks [Figure 4(A)]. With particles coated with metals, the separation of FAMEs was carried out in accordance to the number of double bonds; however, polar modifiers in the mobile phase were needed [19, 20]. Nonpolar SE-54 coated silica particles gave no separation of compounds with the same carbon number but with different number of double bonds.

Effect of SFC conditions on selectivity. Since SFC typically operates at a lower temperature than GC, the selectivity of SFC should be larger than that of GC. From the results in Table I and the data from open tubular column GC in ref. [7], it can be seen that the selectivities of

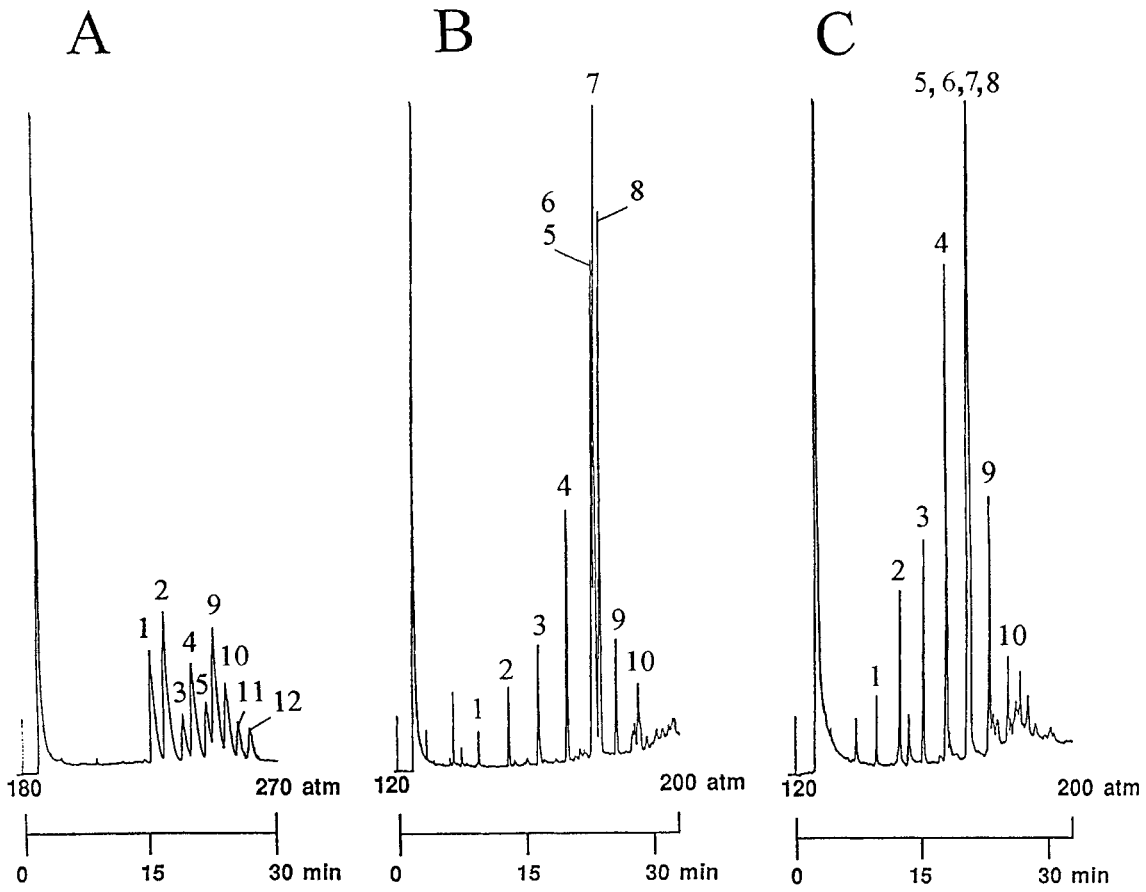

Figure 4. *SFC chromatograms of FAMEs. Conditions: 40 cm × 320 μm i.d. fused silica capillary columns packed with (A) untreated silica particles, (B) o,p-CBP coated silica particles, and (C) SE-54 coated silica particles; 85°C; pressure programmed from (A) 180 atm to 270 atm at 3 atm min^{-1}, (B), and (C) 120 atm to 300 atm at 2.5 atm min^{-1}. Peak identifications: (1) methylcaprate, (2) methyllaurate, (3) methylmyristate, (4) methylpalmitate, (5) methylstearate, (6) methyloleate, (7) methyllinoleate, (8) methyllinolenate, (9) methylarachidate, (10) methylbehenate, (11) methyllignocerate, (12) unknown.*

Table III. *Effect of SFC conditions on packed capillary column selectivity.*[a]

Temperature (°C)	$^{\alpha}$2, 3-dimethyldodecylbenzene/2,5-dimethyldodecylbenzene			
	140 atm	160 atm	180 atm	200 atm
70	1.058	1.051	1.042	1.036
85	1.066	1.058	1.050	1.045
100	1.072	1.062	1.054	1.046

[a]40 cm × 320 μm i.d. capillary column packed with *o*, *p*-CBP coated particles.

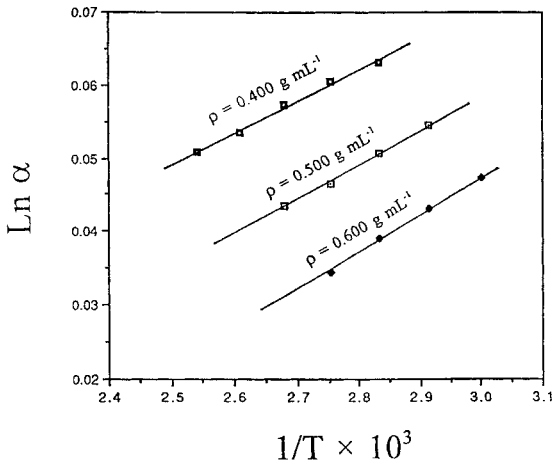

Figure 5. *Effect of temperature on the selectivity of the o,p-CBP coated particles. Conditions: 40 cm × 320 μm i.d. fused silica capillary column packed with o,p-CBP coated silica particles; neat CO_2 mobile phase. The isomers used for the determination of α are 2,3-dimethyldodecylbenzene and 2,5-dimethyldodecylbenzene.*

various isomeric CBP coated particles to 2,3-dimethyldodecylbenzene and 2,5-dimethyldodecylbenzene in SFC are greater than those in GC. The typical operating conditions of SFC are controlled by temperature, pressure, and density of the supercritical mobile phase. Table III lists the effects of temperature and pressure on selectivity. At a constant temperature of 85°C, the selectivity of *o*, *p*-CBP coated particles decreases from 1.066 to 1.045 with increasing pressure from 140 to 200 atm. This decrease in selectivity probably arises primarily from the interaction between the supercritical mobile phase and the stationary phase, in which there is a decrease in the interaction between the solute and the stationary phase [21], and to a much lesser extent from interaction between the solute and the mobile phase. At constant pressure, the selectivity of the stationary phase

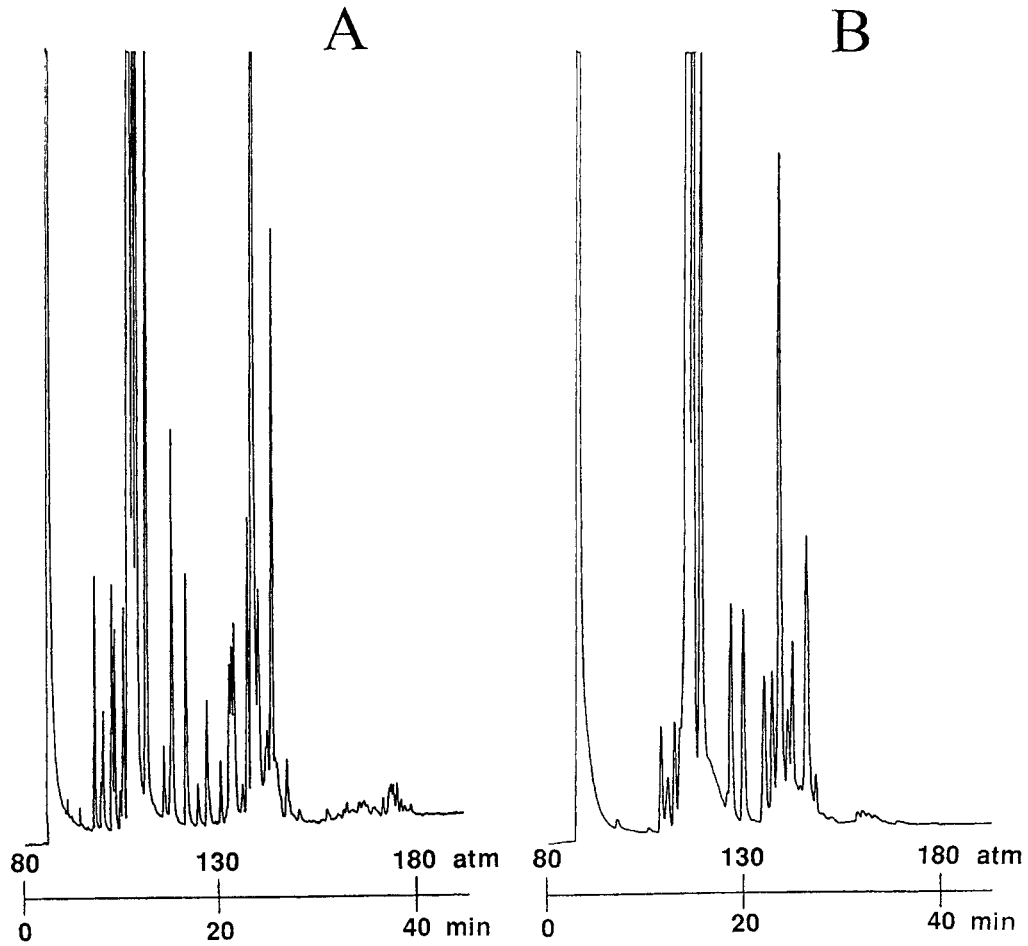

Figure 6. *SFC chromatograms of lime oil. Conditions: 40 cm × 320 fused silica capillary columns packed with (A) o,p-CBP coated silica particles, (B) SE-54 coated silica particles, and (C) untreated silica particles; 85°C; pressure programmed from 80 atm at 2.5 atm* min^{-1}.

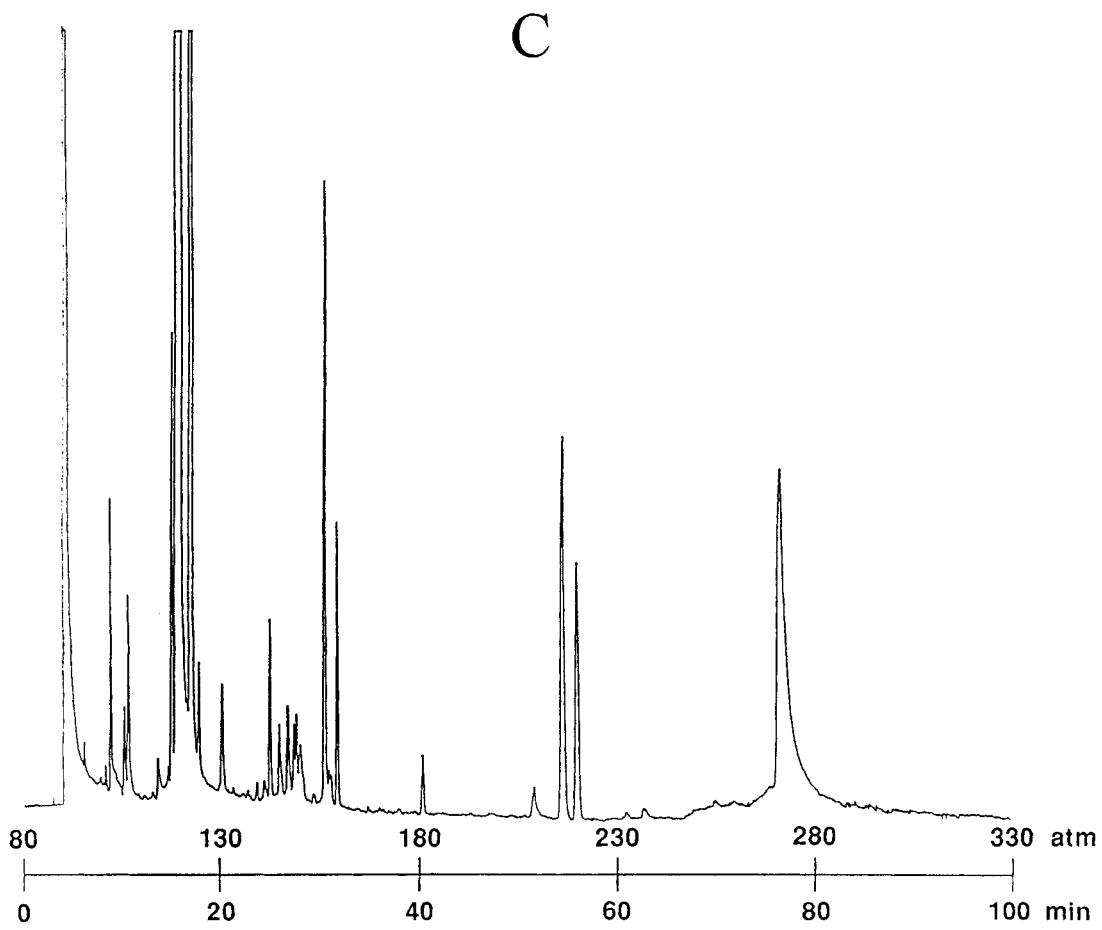

Figure 6. *Continued.*

increases with increasing temperature because the density decreases with increasing temperature. Figure 5 shows the relationship between selectivity and temperature at constant density. A linear relationship exists between the logarithm of selectivity and the reciprocal of temperature.

Lifetime of packing particles. The *o*, *p*-CBP stationary phase was selected to perform lifetime measurements because of its high selectivity. Since untreated silica particles show no separation ($\alpha = 1$) of 3,5-dimethyldodecylbenzene (isomer 2) and 2,3-dimethyldodecylbenzene (isomer 4), the loss of stationary phase would produce a reduction in α. By measuring the decrease in selectivity of the columns, we can determine the loss of polymer from the silica particle surface. When a column packed with *o*, *p*-CBP coated particles was continuously washed with carbon dioxide at 100°C and 300 atm at a linear velocity of 27 cm min^{-1} for more than 100 h, the loss in selectivity between 2,3-dimethyldodecylbenzene and 3,5-dimethyldodecylbenzene was less than 0.2% (1.054 to 1.052). The operating pressure of the CBP columns could be as high as 410 atm.

Separation of lime oil. Figure 6 shows SFC chromatograms of lime oil obtained on capillary columns packed with different types of packing materials. The particles coated with SE-54 produced a poor separation of lime oil. Longer time and higher pressure were needed to elute the sample from the column packed with untreated silica particles. A larger number of peaks could be obtained with the CBP coated particles (medium polarity, high concentration of phenyl groups) in shorter time and lower pressure than with untreated silica particles. This suggests that both moderate polarity and phenyl content in the stationary phase are needed for better separation of the sample. CBP coated particles are ideally suited for this type of application because of their desirable properties.

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