Silver-complexed Dicyanobiphenyl-substituted Polymethylsiloxane Encapsulated Particles for Packed Capillary Column Supercritical Fluid Chromatography

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Abstract. High nitrile content cyano-substituted polymethylsiloxane encapsulated particles were prepared for double bond selectivity in packed capillary column supercritical fluid chromatography (SFC). Silica particles were deactivated with 50% cyanopropyl-substituted polymethylhydrosiloxane, and coated with 25% *o,p*-dicyano-*p*-biphenyl-substituted polymethylsiloxane (*o,p,p*-DCBP), and 25% *m,m*-dicyano-*p*-phenyl-substituted polymethylsiloxane (*m,m,p*-DCBP) stationary phases. Argentation of the particles was carried out by complex formation between silver ions and the cyano groups on the particles. Unsaturated fatty acid methylesters (FAMEs) were used to investigate the selectivity of these packing materials to double bonds using packed capillary column SFC. Separations were performed according to the degree, position, and geometrical configuration of the double bonds. The effect of temperature on selectivity was also investigated. The stability of the packing materials was evaluated by monitoring the loss in selectivity of the oleate/elaidate isomers after washing the column with supercritical CO_2 at 300 atm and 85°C. The separation of FAMEs in a commercial fish oil was compared using capillary columns packed with DCBP coated and silver-complexed particles.

Key words: *supercritical fluid chromatography, packed capillary columns, dicyanobiphenyl-substituted polymethylsiloxanes, silver chelated packing materials, polymer encapsulated packing materials, fatty acid methylesters, double bond selectivity*

INTRODUCTION

Highly efficient separations of special analytes necessitate selective stationary phases. SFC allows the possibility for highly efficient separations to be carried out at relatively low temperatures. One of the advantages of packed column SFC is the ready availability of column packing materials. However, the practical use of small particles in packed column SFC has been mainly limited to packing materials used in high performance liquid chromatography (LC). Since supercritical CO_2 usually has characteristics of faster mass transfer and lower polarity than liquids, packing particles providing fast mass transfer and low polarity are needed to match these characteristics. Polymer coated particles are desirable for this propose because of the thin layer of polymeric stationary phase that either eliminates or masks polar groups on the silica surface.

Unsaturated fatty acids, underivatized and as methylesters (FAMEs), have been separated to various degrees of satisfaction using gas chromatography (GC) [1] and LC [2]. Polyester, polyethylene glycol, free fatty acid phase (FFAP), and cyano-substituted polysiloxane stationary phases have been found to demonstrate selective interaction with unsaturated double bonds [3–6] in GC. This selectivity can be increased by increasing the polarity of the stationary phases [3, 7]. Cyano-substituted

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polysiloxanes have become the most useful stationary phases for the separation of unsaturated FAMEs [4, 5]. While FAMEs can be separated according to both degree and position of double bonds using the previously mentioned polar stationary phases, they can be further separated according to geometrical configuration (*cis/trans*) around the double bonds using cyano-substituted polysiloxane stationary phases.

The use of elevated temperature in GC is a disadvantage for the separation of thermally labile esters such as polyenic FAMEs and reinyl and cholesterol esters. LC, on the other hand, is suitable for the separation of relatively simple mixtures of FAMEs in which thermally labile esters are present [8]. The separation can be carried out according to the degree, position, or geometrical configuration of the double bonds using columns packed with certain polar or nonpolar packing materials, or a specialized argentation packing [9–12]. However, separation efficiency is relatively low and detection can be a problem [13, 14].

SFC can be used to perform separations at lower temperature than GC and with higher efficiency and more sensitivity than LC. The use of polyester stationary phases in SFC has not been reported to date. Commercial cyanopropyl-substituted polysiloxane stationary phases, such as SP-2340, are difficult to immobilize, which limits their application in SFC [15]. Direct bonding of a cyanopropyl hydrosiloxane polymer on the inner surface of fused silica tubing via a dehydrocondensation reaction was used for the selective separation of compounds with double bonds under SFC conditions [16]. Cyanobiphenyl (CBP) polymethylsiloxane stationary phases can be immobilized easily and have been used for both open tubular and packed capillary column SFC [17–19]. Columns were stable, and little loss of selectivity was found under typical SFC conditions [19]. However, the selectivity of the previously prepared cyano-containing stationary phases to double bonds appears to be insufficient to separate challenging monoenic isomers, because they generally have had a relatively low nitrile content in the stationary phases. Recently, packed capillary argentation columns using silica-based cation exchangers were successfully used in SFC [20, 21]. Using an argentation column, geometrical *cis/trans* isomers of FAMEs could be separated under SFC conditions, but modifiers in the CO_2 mobile phase were needed [21].

Although FAMEs are weakly polar, there is strong interaction between FAMEs and bare silica particles. Long times and high pressures were needed to elute FAMEs, and serious peak tailing was produced when neat CO_2 was used as the mobile phase [22]. Deactivation of the silica particles to eliminate silanol groups on their surface was needed to carry out acceptable SFC separation of FAMEs with symmetrical peaks using neat CO_2 as mobile phase. Surface deactivation using a cyano-substituted deactivating reagent has been found to be suitable for coating selective polar stationary phases [23].

In this paper, we report new polymer coated packing materials containing high nitrile content for complexation with silver for the selective separation of compounds with double bonds according to the degree, position, and geometrical configuration of the double bonds under SFC conditions with neat CO_2. The selectivity and stability of these new packing materials were evaluated in packed capillary SFC using FAME standard compounds.

EXPERIMENTAL

Materials and Instrumentation. Porous silica particles (10 μm, 80 Å pores) were purchased from Alltech Associates (Deerfield, IL, USA). The previously reported 50% cyanopropyl polymethylhydrosiloxane (Figure 1A) was synthesized in our laboratory [23]. The 25% *o,p*-dicyano-*p*-biphenyl and *m,m*-dicyano-*p*-biphenyl polymethylsiloxane (DCBP) stationary phases (Figure 1B) were also prepared in our laboratory according to the same procedure as reported in ref. [17]. Azo-*tert*-butane (ATB) was purchased from Lancaster (Windham, NH, USA). The fused silica capillary tubing used for the packed columns was purchased from Polymicro Technologies (Phoenix, AZ, USA). Silver nitrate was purchased from Baker Chemical (Phillipsburg, NJ, USA). Column connections were made using zero dead volume unions (Valco Instruments, Houston, TX, USA). The column packing and SFC analyses were carried out 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). FAME standards were purchased from Sigma (St. Louis, MO, USA). A commercial fish oil (CPL-30) was purchased from Larodan Fine Chemicals (Malmö, Sweden). Methane gas was used for the determination of the unretained

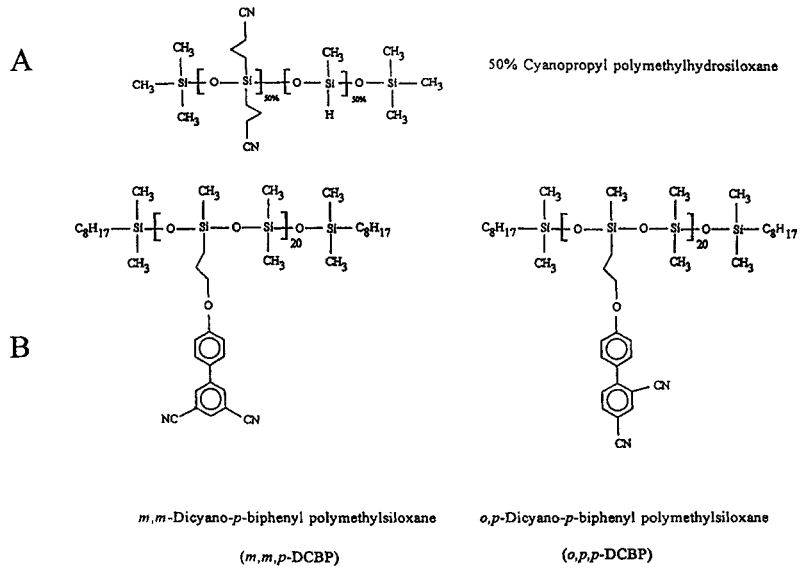

Figure 1. *Structures of the deactivating reagent and the new dicyanobiphenyl polymethylsiloxane stationary phases.*

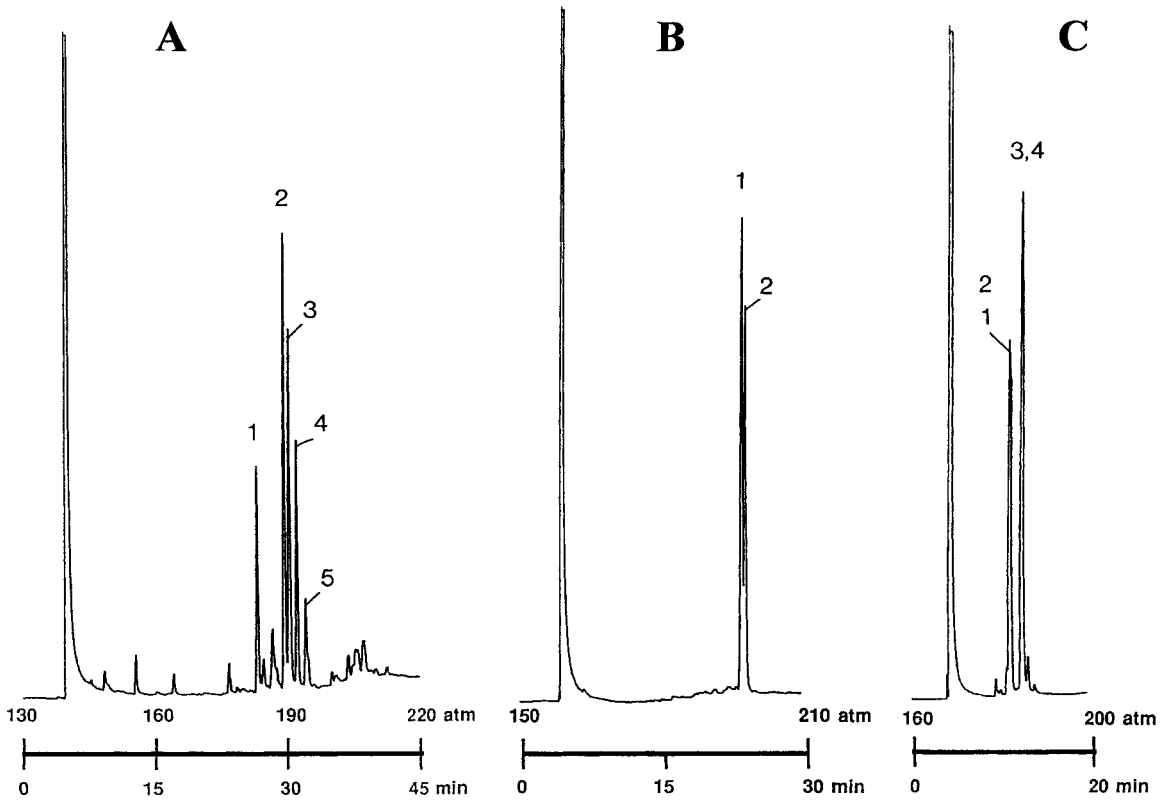

Figure 2. *SFC chromatograms of FAMEs on cyanopropyl deactivated and m,m,p-DCBP coated particles. Conditions: 50 cm × 250 μm i.d. fused silica capillary column packed with cyanopropyl polymethylhydrosiloxane deactivated and m,m,p-DCBP coated particles; neat CO_2; (A) 85°C, pressure programmed from 130 atm to 220 atm at 2 atm min^{-1}. Peak identifications: (1) methylpalmitate, (2) methylstearate, (3) methyloleate, (4) methyllinoleate, (5) methyllinolenate. (B) 85°C, pressure programmed from 150 atm to 200 atm at 2 atm min^{-1}. Peak identifications: (1) γ-methyllinolenate, (2) methyllinolenate. (C) 60°C, pressure programmed from 160 atm to 210 atm at 2.0 atm min^{-1}. Peak identifications: (1) methylpalmitelaidate, (2) methylpalmitoleate, (3) methylelaidate, (4) methyloleate.*

time. All other chemicals used were purchased from Aldrich (Milwaukee, WI, USA).

Preparation of the silica particles. Silica particles (0.2 g) and 50% cyanopropyl polymethylhydrosiloxane (0.04 g) were used for particle deactivation. The highest temperature used for the dehydrocondensation reaction was 250°C. All other deactivation conditions were the same as those reported in ref. [22]. The ratio of DCBP stationary phase to deactivated particles was 15% (w/w). Other coating conditions were the same as those in ref. [19]. Silver nitrate (0.3 g) was dissolved in HPLC grade water (2 mL). The particles (0.1 g) in the reaction vessel were then washed with the silver nitrate solution in a sonic bath to facilitate the argentation. After 15 min, the solution was suctioned out of the inlet of the reaction vessel with a water vacuum pump. The particles were finally dried at 150°C for 2 h.

Fused silica capillary columns (50 cm × 250 μm i.d.) were packed using carbon dioxide slurries at room temperature and a pressure of 80 atm according to the procedure described in ref. [24].

Saponification of the fish oil. The procedure described in ref. [25] was followed for the methylation of the CPL-30 fish oil.

RESULTS AND DISCUSSION

The selectivity of the new stationary phases according to the extent of unsaturation (number of double bonds) was evaluated with a standard mixture of methyl stearate (C18:0), methyloleate (*cis*-9-C18:1), methyllinoleate (*cis*9,12-C18:2), and methyllinolenate (*cis*-9,12,15-C18:3). The selectivity to the position of double bonds was evaluated with a pair of positional isomers, methyllinolenate (*cis*-9,12,15-C18:3) and γ-methyllinolenate (*cis*-6,9,12-C18:3). The selectivity to geometrical configuration was evaluated with two pairs of challenging monoenic FAMEs, oleate (*cis*-9-C18:1)/elaidate (*trans*-9-C18:1) and palmi-

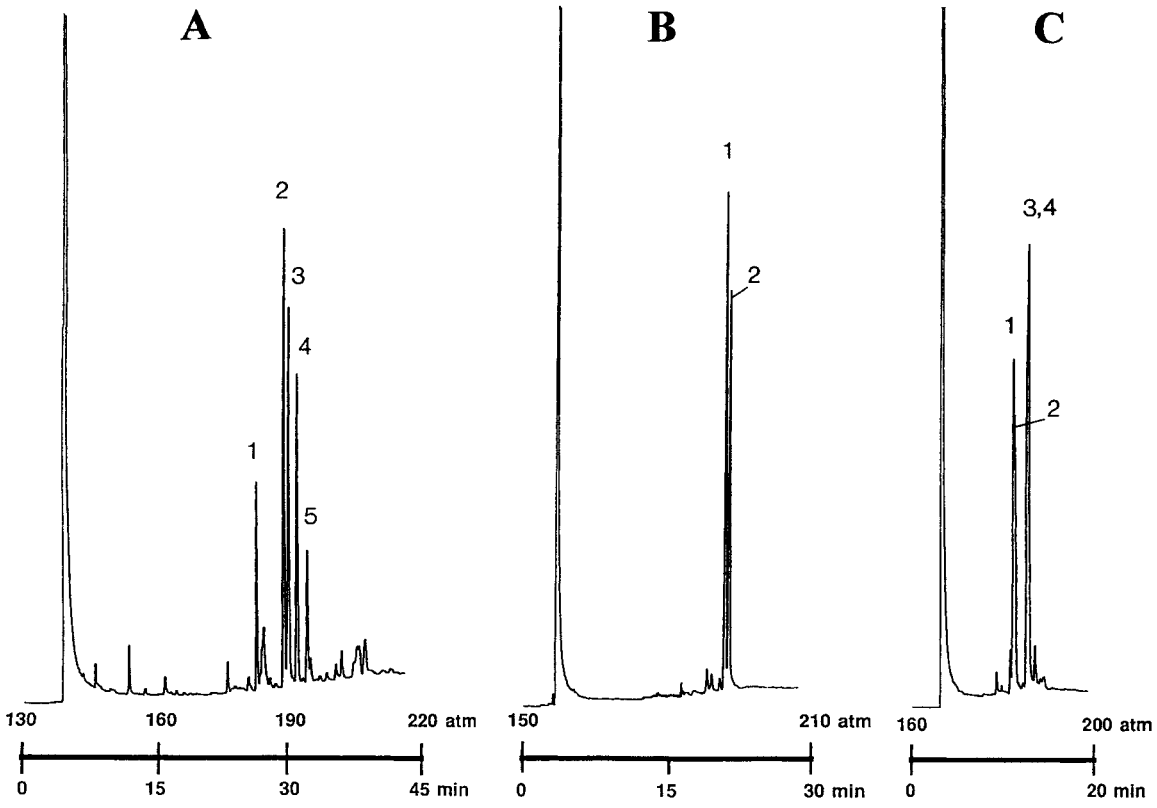

Figure 3. *SFC chromatograms of FAMEs on cyanopropyl deactivated and o,p,p-DCBP coated particles. Conditions: 50 cm × 250 μm i.d. fused silica capillary column packed with cyanopropyl polymethylhydrosiloxane deactivated and o,p,p-DCBP coated particles. Other conditions and peak identifications are the same as those listed in Figure 2.*

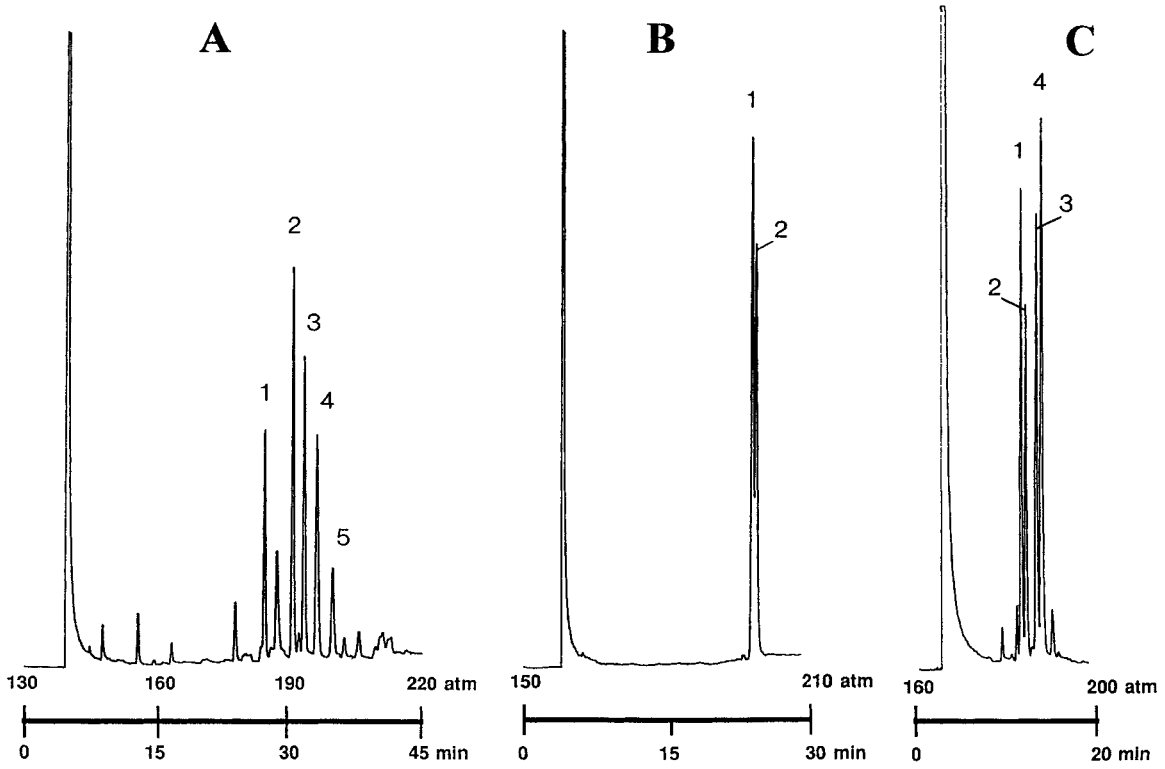

Figure 4. *SFC chromatograms of FAMEs on cyanopropyl deactivated, o,p,p-DCBP coated, and silver complexed particles. Conditions: 50 cm × 250 μm i.d. fused silica capillary column packed with cyanopropyl polymethylhydrosiloxane deactivated, o,p,p-DCBP coated, and silver-complexed particles. Conditions and peak identifications are the same as those listed in Figure 2.*

toleate (*cis*-9-C16:1)/palmitelaidate (*trans*-9-C16:1).

SFC separations of mixtures of FAMEs using a capillary column packed with 50% cyanopropyl-substituted polymethylhydrosiloxane deactivated particles, without a stationary phase coating, showed low selectivity. The saturated methyl stearate and monoenic oleate were only partially separated. The *cis/trans* isomers of the monoenic FAMEs could not be separated. Partial separation of the positional isomers, methyllinolenate and γ-methyl linolenate, was obtained. Although there was a high nitrile content in the cyanopropyl deactivation reagent (21.7% in one repeating unit), the selectivity of the packing materials was relatively low. This result suggests that only a small amount of the cyano functionality remains bonded on the silica surface after heat treatment during the dehydrocondensation reaction.

SFC separations of FAMEs using a deactivated and silver complexed capillary column, again with no stationary phase coating, showed only slightly improved selectivity. The separation according to the number of double bonds was somewhat improved. The selectivity according to position was little improved after argentation of the column. The *cis/trans* isomers of C16:1 were partially separated; however, the *cis/trans* isomers of C18:1 were not separated at all. The improvement in selectivity after argentation was not large enough to separate various types of isomers of FAMEs. This leads to the conclusion that insufficient silver was fixed on the particles.

While the selectivity was not acceptable for FAMEs using the packing materials that were deactivated but not coated with a stationary phase film, the peak shapes were sharp and symmetrical. This indicates that the particles were well deactivated. Therefore, argentation did not occur through an ion exchange mechanism with surface silanol groups. After the particles were encapsulated with a dicyanobiphenylpolysiloxane coating, a high concentration of nitrile groups were available to

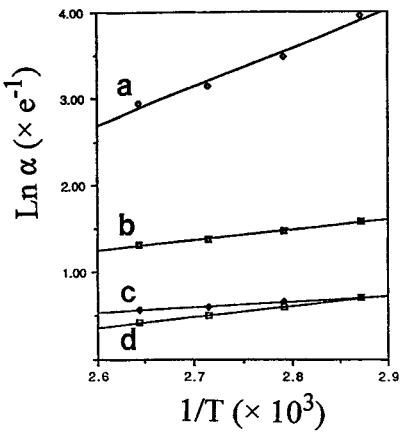

Figure 5. *Effect of temperature on selectivity (ρ = 0.500 g mL^{-1}). Conditions: neat CO_2; 50 cm × 250 μm i.d. fused capillary column packed with cyanopropyl polymethylhydrosiloxane deactivated, o,p,p-DCBP coated, and silver-complexed particles;*

(a) $Ln\ \alpha_{methyl\ stearate/methyl\ palmitate} = -0.8803 + 0.4421 \times 10^3\ T^{-1}$, $R = 0.99$;

(b) $Ln\ \alpha_{methyl\ oleicate/methyl\ stearate} = -0.1879 + 0.1201 \times 10^3\ T^{-1}$, $R = 1.00$;

(c) $Ln\ \alpha_{methyl\ oleate/methyl\ elaidate} = -0.1136 + 0.0641 \times 10^3\ T^{-1}$, $R = 1.00$;

(d) $Ln\ \alpha_{methyl\ linolenate/\gamma\text{-}methyl\ linolenate} = -0.2819 + 0.1224 \times 10^3\ T^{-1}$, $R = 1.00$.

complex with the silver ions. It is well known that cyano groups in organic compounds can interact strongly with transition metals such as silver [26, 27].

Figures 2 and 3 show SFC chromatograms of FAMEs using the capillary columns packed with deactivated and *o,p,p*-DCBP or *m,m,p*-DCBP coated particles. The asymmetric isomer, *o,p,p*-DCBP, shows higher selectivity for double bonds than the symmetric isomer, *m,m,p*-DCBP. Compared with the results obtained using the deactivated and silver complexed columns, it can be seen that the selectivity to number of double bonds was improved after coating the *o,p,p*-DCBP stationary phase on the deactivated particles. The separation of saturated methyl stearate and monoenic methyl oleate is approximately the same as that obtained from the argentation column with only a deactivation layer on the particles. The most obvious improvement after coating with the *o,p,p*-DCBP stationary phase is seen in the separation of positional isomers. Methyllinolenate and γ-methyllinolenate were baseline separated. However, the selectivity for *cis/trans* shows only slight improvement after coating with the *o,p,p*-DCBP stationary phase. Therefore, the *o,p,p*-DCBP stationary phase has greater selectivity for positional isomers than for geometrical isomers of FAMEs.

Figure 4 shows SFC chromatograms of FAMEs using a capillary column which had been deactivated, coated with *o,p,p*-DCBP and complexed with silver. The selectivities for the number and geometrical configurations of double bonds were improved greatly, and monoenic *cis/trans* isomers were separated very well. The selectivity for positional isomers became poor after argentation. The silver ions in the argentation column exhibit special interactions with certain geometrical configurations. In fact, they demonstrate greater selectivity for geometrical isomers than for positional isomers. On the other hand, although silver ions were fixed on the stationary phase and the FAME peaks became broader, symmetrical peak shapes were obtained under SFC conditions using neat CO_2.

Figure 5 shows the effect of temperature on stationary phase selectivity according to carbon number, and degree, position, and geometrical configuration of the double bonds of the FAMEs on the *o,p,p*-DCBP coated and silver complexed packing materials. At constant density (0.500 g mL^{-1}), all selectivities increased with decreasing temperature. However, the effect of temperature on various selectivities was different. The effect on carbon number selectivity was the largest, on geometrical configuration was the smallest, and on the number and position of double bonds was approximately the same. Therefore, SFC has one distinguishing advantage in that different types of selectivity can be adjusted by changing the temperature. This is significant for the separation of a complex mixture of FAMEs because overlap of certain critical peaks can be avoided by adjusting the operating temperature. Figure 6 shows SFC chromatograms obtained at different temperatures with the same pressure program. By increasing the temperature, separation of groups containing different carbon numbers becomes better, but the separation of components of the same carbon number becomes worse. The overlap of peaks 9, 10, and 11 can be avoided at either higher or lower temperatures

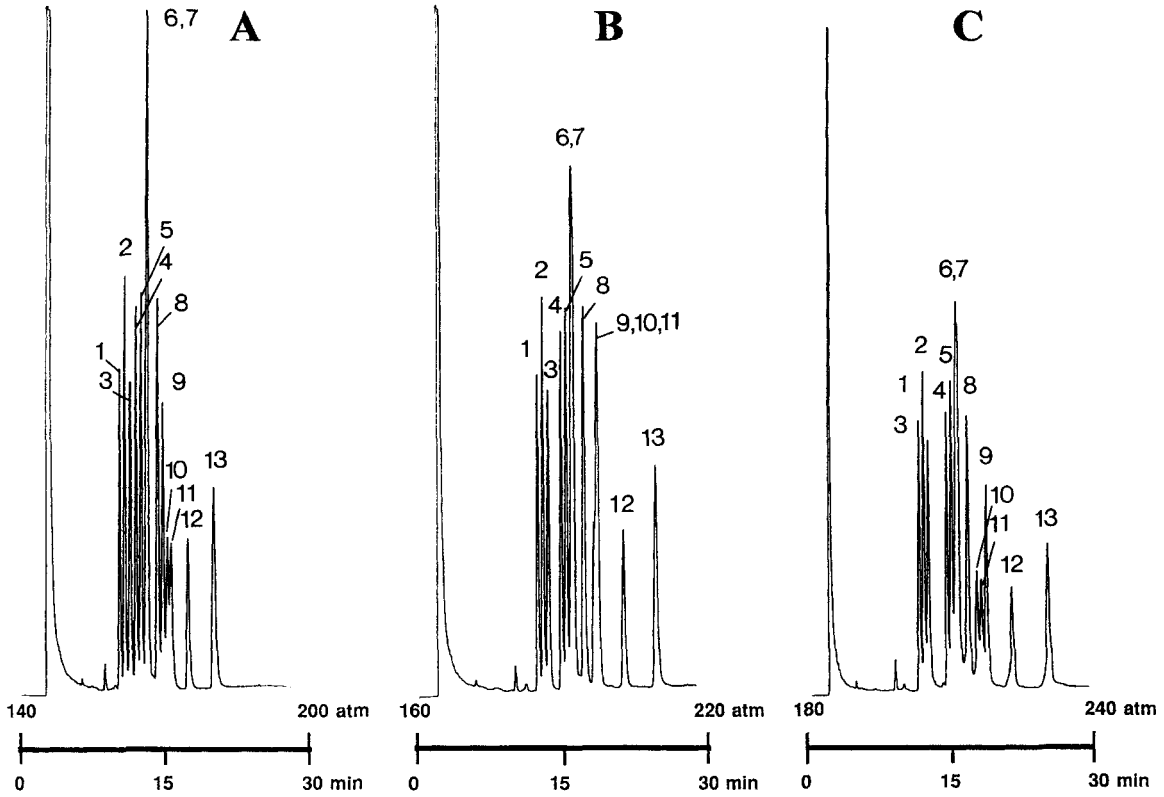

Figure 6. *Effect of temperature on the separation of FAMEs. Conditions: (A) neat CO_2; 60°C; pressure programmed from 140 atm to 200 atm at 2 atm min^{-1}. (B) 85°C; pressure programmed from 160 atm to 220 atm at 2 atm min^{-1}. (C) 110°C; pressure programmed from 180 atm to 240 atm at 2 atm min^{-1}. Peak identifications: (1) methylpalmitate, (2) methylpalmitelaidate, (3) methylpalmitoleate, (4) methylstearate, (5) methylelaidate, (6) methyloleate, (7) methyllinolelaidate, (8) methyllinoleate, (9) methylgondoate, (10) γ = methyllinolenate, (11) methyllinolenate, (12) cis-11,14,17-methyleicosatrienoate, (13) cis-7,10,13,16-methyldocosatetraenoate. Other conditions are the same as those listed in Figure 5.*

than 85°C because of the different types of selectivity (carbon number and degree of double bonds). The overlap of peaks 6 and 7 cannot be avoided by changing temperature because of their close similarity (degree of double bonds).

The stability of the packing materials was evaluated by monitoring the loss in selectivity with use. The loss of silver ions reduces the properties of the column to those of the column packed with *o,p,p*-DCBP coated particles in which there was no separation of methyloleicate/methylelaidicate isomers. The loss of *o,p,p*-DCBP stationary phase reduces the column to the deactivated and silver complexed column for which there was no separation of the geometrical isomers. Our results indicate that increasing the supercritical CO_2 washing time from 24 to 48 h led to a decrease in selectivity from 1.090 to 1.085. Additional washing up to 144 h led to only minor selectivity loss (1.081). The treatment conditions were: 300 atm, 85°C, and 60 cm min^{-1} linear velocity.

From the discussion above, it was found that the *o,p,p*-DCBP coated particles exhibited the best selectivity for positional isomers, and their further complexation with silver leads to greater selectivity according to the degree of unsaturation and geometrical configuration of double bonds. The appropriate column can be selected according to the selectivity desired for a certain application. Figure 7 shows chromatograms of FAMEs in a commercial fish oil (CPL-30) using capillary columns packed with *o,p,p*-DCBP coated particles and silver complexed particles using neat CO_2 as mobile phase. The different selectivities according to the number of double bonds results in a different elution order. The overlapping of peaks 8

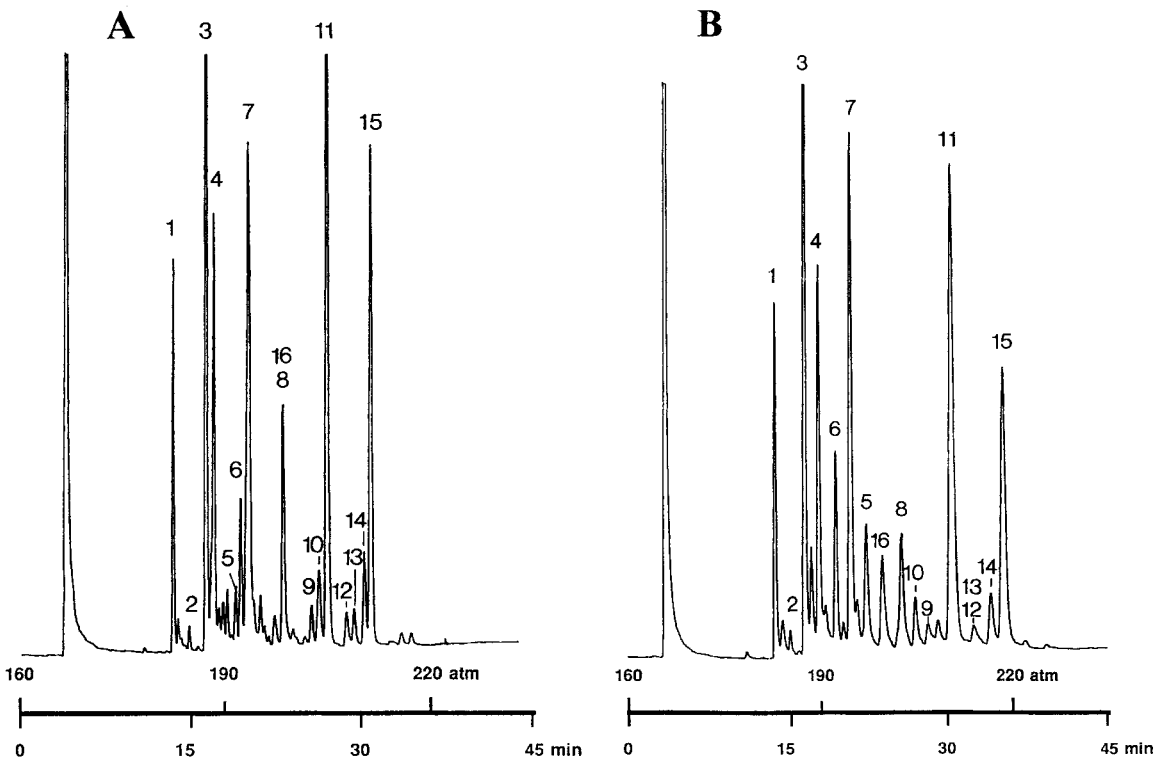

Figure 7. *SFC chromatograms of FAMEs of CPL-30 fish oil. Conditions: neat CO_2; 85°C; pressure programmed from 160 atm at 1.5 atm min^{-1}. The column for (A) is the same as that in Figure 3, and the column for (B) is the same as that in Figure 4. Peak identifications: (1) C14:0, (2) C15:0, (3) C16:0, (4) C16:1 n-7, (5) C16:4 n-4, (6) C18:0, (7) C18:1 n-9, (8) C18:4 n-3, (9) C20:4 n-6, (10) C22:1 n-11, (11) C20:5 n-3, (12) C22:4 n-6, (13) C22:4 n-3, (14) C22:5 n-3, (15) C22:6 n-3, (16) C20:1 n-9.*

and 16 on the *o,p,p*-DCBP coated column was resolved on the silver complexed column.

REFERENCES

1. W. W. Christie, *Gas Chromatography and Lipids: A Practical Guide* (The Oily Press, Ayr, Scotland, 1989).
2. W. W. Christie, *HPLC and Lipids: A Practical Guide* (Pergamon Press, Oxford, England, 1987).
3. J. K. Haken, *J. Chromatogr. Sci.* **13,** 430 (1975).
4. E. S. van Vleet and J. G. Quinn, *J. Chromatogr.* **151,** 396 (1978).
5. K. Grob, Jr., H. P. Neukom, D. Fröhlich, and O. R. Battaglia, in *Recent Advances in Capillary Gas Chromatography*, Vol. 2, W. Bertsch, W. G. Jennings, and R. E. Kaiser, Eds. (Hüthig, Heidelberg, Germany, 1982), p. 259.
6. D. M. Ottenstein, L. A. Witting, P. H. Silvis, D. J. Hometchko, and N. Pelick, *J. Am. Oil Chem. Soc.* **61,** 390 (1984).
7. D. M. Ottenstein, D. A., Bartley, and W. R. Supina, *J. Chromatogr.* **119,** 401 (1976).
8. M. I. Aveldano, M. Van Rollins, and L. A. Horrocks, *J. Lipid Res.* **24,** 83 (1983).
9. B. Vonach and G. Schomburg, *J. Chromatogr.* **149,** 417 (1978).
10. W. W. Christie, *J. High Resolut. Chromatogr./ Chromatogr. Commun.* **10,** 148 (1987).
11. E. W. Hammond, *Chromatography for the Analysis of Lipids* (CRC Press, Boca Raton, FL, 1993).
12. V. Ferraz and P. Sandra, *Fifteenth International Symposium on Capillary Chromatography*, Riva del Garda, Italy, 1993, p. 1181.
13. A. Stolyhwo, H. Colin, and G. Guiochon, *Anal. Chem.* **57,** 1342 (1985).
14. N. E. Hoffman and J. C. Liao, *Anal. Chem.* **48,** 1104 (1976).
15. L. M. Sidisky, P. L. Stormer, L. Nolan, M. J. Keeler, and R. J. Bartram, *J. Chromatogr. Sci.* **26,** 320 (1988).
16. K. E. Markides, S. M. Fields, and M. L. Lee, *J. Chromatogr. Sci.* **24,** 254 (1986).
17. B. E. Rossiter, S. L. Reese, S. Morgan, A. Malik, J. S. Bradshaw, and M. L. Lee, *J. Microcol. Sep.* **4,** 521 (1992).
18. A. Malik, I. Ostrovsky, S. R. Sumpter, S. L. Reese, S. Morgan, B. E. Rossiter, J. S. Bradshaw, and M. L. Lee, *J. Microcol. Sep.* **4,** 529 (1992).
19. Y. Shen, W. Li, A. Malik, S. L. Reese, B. E. Rossiter, and M. L. Lee, *J. Microcol. Sep.*, submitted.

20. R. M. Campbell, N. M. Djordjevic, K. E. Markides, and M. L. Lee, *Anal. Chem.* **60,** 356 (1988).
21. M. Demirbuker, I. Hagglund, and L. G. Blomberg, *J. Chromatogr.* **605,** 263 (1992).
22. Y. Shen, A. Malik, W. Li, and M. L. Lee, *J. Chromatogr.*, accepted.
23. K. E. Markides, B. J. Tarbet, C. M. Schregenberger, J. S. Bradshaw, and M. L. Lee, *J. High Resolut. Chromatogr./Chromatogr. Commun.* **8,** 741 (1985).
24. A. Malik, W. Li, and M. L. Lee, *J. Microcol. Sep.* **5,** 265 (1993).
25. W. W. Christie, *J. Lipid Res.* **23,** 1072 (1982).
26. B. N. Storhoff and H. G. Lewis, Jr., *Coord. Chem. Rev.* **23,** 1 (1977).
27. S. J. Bryan, P. G. Huggett, K. Wade, J. A. Daniels, and J. R. Jennings, *Coord. Chem. Rev.* **44,** 149 (1982).

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