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Packed capillary column supercritical fluid chromatography using SE-54 polymer encapsulated silica

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Abstract

A new method of preparing stationary phases for packed capillary column supercritical fluid chromatography (SFC) is presented. Surface deactivation of silica particles was carried out by dehydrocondensation of the silicon hydride groups in polymethylhydrosiloxane with the silanol groups on the silica surface. The deactivated particles were then coated with a thin film of SE-54 stationary phase. The coated layer was immobilized by a crosslinking reaction between the methyl groups of the surface-bonded polymethylhydrosiloxane and the SE-54 stationary phase using dicumyl peroxide as a free radical initiator. With these two reactions, the polar groups on the silica surface were more completely capped than with bonding only a monomolecular polymeric layer on the silica surface. The SFC performance of the newly developed packing materials was evaluated using a standard polarity mixture, a series of fatty acid methyl esters, a peppermint oil, and several high-molecular-mass and complex polymers.

1. Introduction

Packed capillary column supercritical fluid chromatography (SFC) has the advantages of larger sample capacity and higher plate number per unit time than open tubular column SFC [1,2]. The packing materials used in packed capillary column SFC are the key to obtaining the desirable separation performance. Currently, the most widely used packing materials are silica-based particles. This is because silica particles have good mechanical strength and a narrow size distribution. However, two factors should be considered when choosing particles for columns in SFC: the polarity and the porosity of the surface of the particles.

The adsorption activity of the silica surface due to silanol groups is a major concern in SFC. Various approaches have been followed to deactivate the packing materials. Using polar organic solvents as modifiers in the supercritical fluid mobile phase can reduce the polarity of the silica packing materials by interaction with the silanol groups. However, this method does not allow the use of flame ionization detection (FID) which is the most desirable detection method for quantitative analysis. A few selected polar compounds such as water or formic acid can be added to the carbon dioxide mobile phase to achieve SFC separation of polar analytes and still retain the use of FID [3,4].

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Deactivation of the silica surface is a common method to obtain less polar packing materials used for the separation of polar analytes. Deactivation can be carried out with a variety of monomeric or polymeric silylation reagents. Using small mono- or multifunctional silylation reagents, it is impossible to completely react with all silanol groups on the silica surface because of steric hindrance [5,6]. A number of reviews have described this in detail [7–9]. The residual silanol groups on the silica surface strongly interact with polar solutes and negatively affect the separation efficiency in SFC [10–12].

A polymer coating and crosslinking method was introduced by Figge et al. [13] for the preparation of packing materials used in reversed-phase high-performance liquid chromatography (LC). The coating was formed by crosslinking of the deposited polymers and by bonding the coating to the presilanized (trimethylsilylated or "pre-capped") silica particles via free radicals formed from the $Si-CH_3$ groups which were fixed to the silica surface. With this method, the silanol groups may be either partly eliminated by precapping, or remain unchanged on the surface underneath the coated layer. Schoenmakers et al. [14] and Ashraf-Khorassani et al. [15] compared the polymer-coated particles with other types of packings, and found that the polymer-coated packings had low activity and produced high efficiency in SFC.

Simultaneous deactivation and coating of the porous silica particles for micropacked column SFC was developed by Payne et al. [16]. This method was based on a dehydrocondensation reaction between a home-made polymeric silicon hydride reagent and the silanol groups on the silica surface. The results showed that the procedure generated a less active particle surface than that of a C_{18}-bonded stationary phase.

High particle porosity is detrimental in SFC because pores in the silica packing result in significant solute retention, and lead to long analysis times. Partially filling the pores with the polymer coating is a method to decrease the porosity. Recently, an excellent review on the reduction of the porosity of silica particles using polymers was published [17].

In this paper, we report a new method for the preparation of packing materials used for packed column SFC. Simultaneous deactivation and coating of porous silica particles were carried out by dehydrocondensation of hydride groups on a commercial methylhydrosiloxane polymer and silanol groups on the silica surface. Then, a commercial polysiloxane stationary phase used in gas chromatography, SE-54 (5% diphenyl–94% dimethyl–1% vinyl siloxane), was coated and immobilized on the polymethylhydrosiloxane-deactivated silica surface via a free radical reaction. The effects of the polymer coating and surface deactivation on the polarity and porosity of the packing materials were examined by chromatographic measurements under SFC conditions. Efficient SFC separations of peppermint oil and various commercial high-molecular-mass and complex samples were obtained.

2. Experimental

2.1. Materials and instrumentation

Porous silica particles (10 μm diameter, 300 Å pore size) and SE-54 stationary phase were purchased from Alltech Associates (Deerfield, IL, USA). C_{18}-bonded particles (10 μm diameter, 300 Å pore size) were purchased from Phenomenex (Torrance, CA, USA). Polymeric silicon hydride reagents (polymethylhydrosiloxane and other methylhydrosiloxane polymer and copolymer samples) were purchased from Hüle (Bristol, PA, USA). Polydimethylsiloxane with a molecular mass of 3900 was purchased from Polysciences (Warrington, PA, USA). Fused-silica tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA). Column connections were made using Valco ZU.T zero dead volume unions (Valco Instruments, Houston, TX, USA). Packing of the capillary columns and the SFC separations were performed using a Lee Scientific Model 600 SFC instrument (Dionex, Salt Lake City, UT, USA).

Peppermint oil was purchased from Berje (Bloomfield, NJ, USA). All other chemicals were purchased from Aldrich (Milwaukee, WI, USA).

2.2. Deactivation of porous silica particles

A previously reported reaction vessel [16] was used for the deactivation reaction. Silica particles (0.5 g) were transferred into the reaction vessel and washed with 50 ml of HPLC grade water. The particles were dried with a vacuum pump connected to the vessel. The vessel was placed in a chromatographic oven and connected to an argon gas source (70 ml min^{-1}). The oven temperature was increased from ambient to 300°C at 5°C min^{-1}, and held at 300°C for 10 h. After cooling, 0.1 g of polymethylhydrosiloxane dissolved in 50 ml of HPLC grade CH_2Cl_2 was transferred into the reaction vessel. The particles were fluidized in the methylene chloride–polymethylhydrosiloxane solution with bubbling argon (70 ml min^{-1}) at ambient temperature. This facilitated uniform coating of the polymethylhydrosiloxane on the silica surface and filling of the pores in the silica particles with the polymer. After evaporating the methylene chloride, the temperature was increased from ambient to 270°C at 5°C min^{-1}, and held at 270°C for 20 h to carry out the dehydrocondensation reaction. After cooling, the particles were washed with 50 ml of HPLC grade CH_2Cl_2 and then dried in argon gas at ambient temperature.

2.3. Crosslinking reaction

SE-54 stationary phase (0.075 g) and 0.002 g of dicumyl peroxide were dissolved in 10 ml of HPLC grade CH_2Cl_2. The solution and the deactivated particles were introduced into the reaction vessel and fluidized with argon gas (70 ml min^{-1}) at ambient temperature. Evaporation of the solvent further covered the silica surface and filled the pores with polymer. The temperature was increased from ambient to 250°C at 2°C min^{-1} and held at 250°C for 10 h to carry out the crosslinking reaction, still under an argon gas purge (70 ml min^{-1}). After cooling, the product was washed with 50 ml of HPLC grade CH_2Cl_2 and dried with argon gas at ambient temperature.

2.4. Packing of capillary columns

Fused-silica capillary columns (40 cm × 320 μm I.D.) were packed according to the procedure described in Ref. [18].

3. Results and discussion

The conditions of the dehydrocondensation reaction were discussed in detail in Ref. [16]. The reaction temperature selected was 270°C. Free-radical crosslinking is widely used in the preparation of open tubular columns for gas chromatography and SFC [19,20]. Typical conditions were used in this study, and 250°C was selected as the highest temperature for crosslinking.

Chromatographic measurements were made using a test mixture containing four compounds of wide polarity. The polar compounds used were acetophenone, naphthol, benzo(f)-quinoline, and cholesterol. Chromatograms of the polar mixture are shown in Fig. 1. All four polar compounds could be eluted, and relatively good peak shapes were obtained using the column packed with SE-54-coated particles (Fig. 1A). Under the same experimental conditions, only acetophenone could be eluted using the column packed with untreated porous silica particles (Fig. 1C). This illustrates that there is very strong interaction between the polar groups on the silica surface and the -OH or -N= groups in the test solutes. From Figs. 1A and 1B, it can be seen that coating the SE-54 stationary phase on the surface of the polymethylhydrosiloxane-deactivated particles encapsulated the surface further and provided better peak shapes than could be obtained with only polymethylhydrosiloxane-deactivated particles. The different relative peak areas seen in Figs. 1A and 1B could result from irreversible adsorption or differences

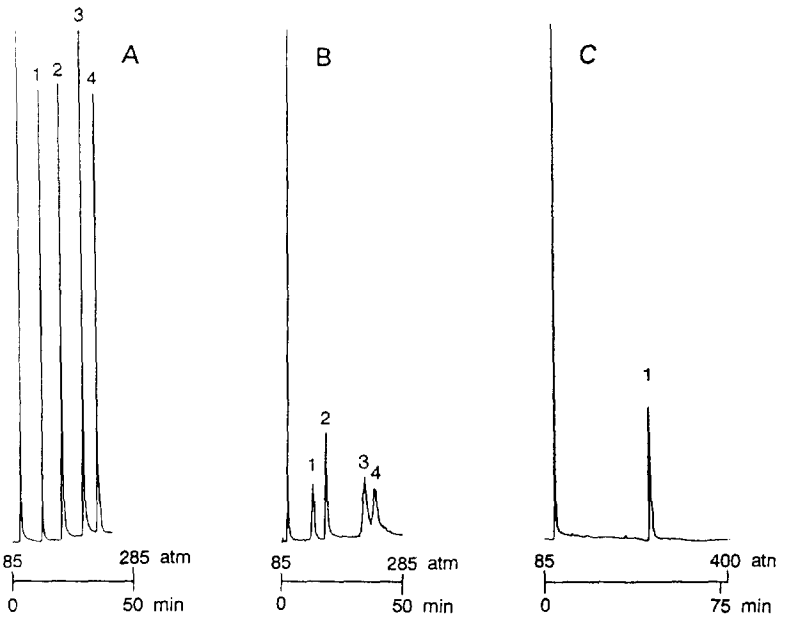

Fig. 1. SFC chromatograms of a mixture of four polar test compounds. Conditions: 40 cm × 320 μm I.D. fused-silica capillary columns packed with (A) silica particles deactivated with polymethylhydrosiloxane and coated and crosslinked with SE-54, (B) silica particles deactivated with polymethylhydrosiloxane only, and (C) untreated porous silica particles; neat CO_2; 90°C; linear pressure program from 85 atm to 285 atm at 4 atm min $^{-1}$. Peak identifications: 1 = acetophenone, 2 = naphthol, 3 = benzo[*f*]quinoline, 4 = cholesterol.

in split injection when the two columns were investigated.

Fig. 2 shows a chromatogram of weakly polar fatty acid methylesters (FAMEs). Even though the FAMEs are polar, the retention times and pressures needed for their elution can be reduced, and better peak shapes can be obtained, using the column packed with particles that were both polymethylhydrosiloxane-deactivated and SE-54-coated. An apolar sample was used to investigate the effect of porosity on chromatographic performance. The sample used in this test was a polydimethylsiloxane with mean molecular mass of 3900. Fig. 3 shows chromatograms obtained using columns packed with untreated porous silica particles and those encapsulated with SE-54 stationary phase. It can be seen that longer time and higher pressure is needed to elute the sample when the untreated porous silica particles are used. The porous silica particles have a certain range of pore size distribution and a large specific surface area which produce long retention of the sample. The polymethylhydrosiloxane deactivation reagent and SE-54 stationary phase fill and partially seal the small and deep pores in the silica particles, resulting in reduction of the specific surface area and the solute retention.

The deactivated and coated particles have reduced polarity as well as reduced porosity. A column packed with this packing material can be used to separate polar as well as high-molecular-mass, complex, nonpolar polymers using neat CO_2 as mobile phase. Fig. 4 shows the separation of peppermint oil using columns packed with both untreated porous silica particles and coated particles. A number of peaks seriously tail and few peaks can be obtained using the untreated silica particles as stationary phase. The separation can be greatly improved, leading to reduced analysis time and pressure, using the column packed with polymer-deactivated, SE-54-coated particles. Figs. 5 and 6 demonstrate the separation of a polymethylhydrosiloxane oligo-

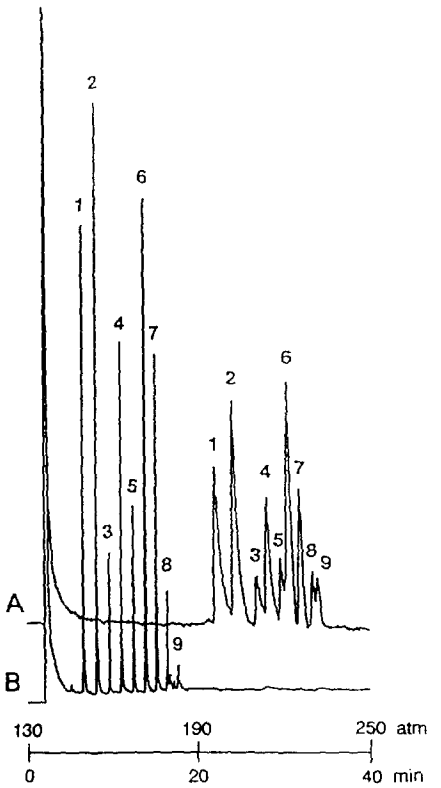

Fig. 2. SFC chromatograms of FAMEs. Conditions: 40 cm × 320 μm I.D. fused-silica capillary columns packed with (A) untreated porous silica particles and (B) silica particles deactivated with polymethylhydrosiloxane and coated and crosslinked with SE-54; neat CO_2; 85°C; linear pressure program from 130 atm to 280 atm at 3 atm min^{-1}. Peak identifications: 1 = capric acid methylester, 2 = lauric acid methylester, 3 = myristic acid methylester, 4 = palmitic acid methylester, 5 = stearic acid methylester, 6 = arachidic acid methylester, 7 = behenic acid methylester, 8 = lignoceric acid methylester, 9 = unknown.

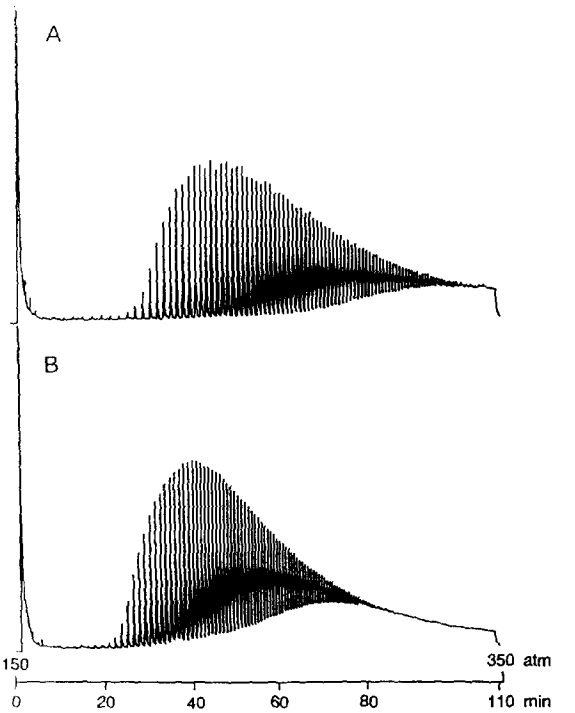

Fig. 3. SFC chromatograms of a polydimethylsiloxane sample (molecular mass 3900). Conditions: 40 cm × 320 μm I.D. fused-silica capillary columns packed with (A) untreated porous silica particles and (B) silica particles deactivated with polymethylhydrosiloxane and coated and crosslinked with SE-54; neat CO_2; 75°C; linear pressure program from 150 atm to 300 at 1.8 atm min^{-1}.

mer mixture. With increasing molecular mass, the composition of this polymer becomes very complicated. Fig. 7 shows the separation of a copolymer sample. Using the capillary column packed with the polymer-deactivated, SE-54-coated particles, impressive separations of these high-molecular-mass and complex nonpolar polymers were obtained using neat supercritical CO_2 as mobile phase at pressures lower than 300 atm.

C_{18}-bonded phases (ODS) are the most widely used packing materials in packed column SFC. Fig. 8 shows SFC chromatograms of different polar compounds on a capillary column packed with ODS particles. Comparing Fig. 8A with Fig. 1, it is clear that ODS particles have stronger polarity than either polymethylhydrosiloxane-deactivated or polymethylhydrosiloxane-deactivated and SE-54-coated particles. Comparing Figs. 8B and 8C with Figs. 2 and 3, it can be seen that for the separation of weakly polar esters and nonpolar polydimethylsiloxanes, there is no significant difference between the columns packed with ODS and SE-54-encapsulated particles except for the longer analysis time needed on the ODS column. These results suggest that ODS particles are suitable for the separation of non- or weakly polar compounds when neat supercritical CO_2 is used as mobile phase. Fig. 8D shows the separation of peppermint oil on a column packed with ODS particles.

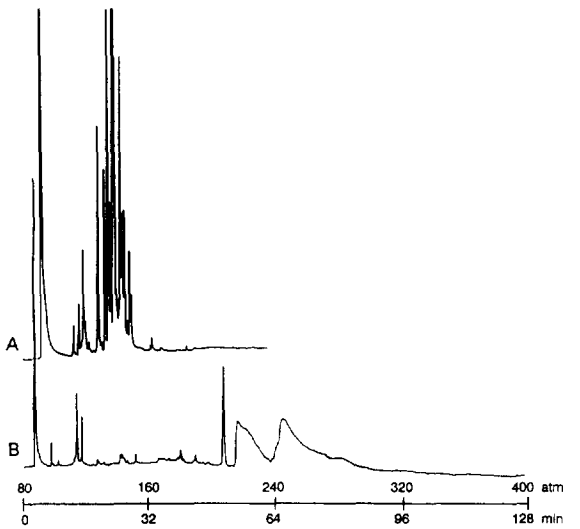

Fig. 4. SFC chromatograms of a peppermint oil. Conditions: 40 cm × 320 μm I.D. fused-silica capillary columns packed with (A) particles deactivated with polymethylhydrosiloxane and coated and crosslinked with SE-54 and (B) untreated porous silica particles; neat CO_2; 85°C; linear pressure program from 80 atm to 400 atm at 2.5 atm min^{-1}.

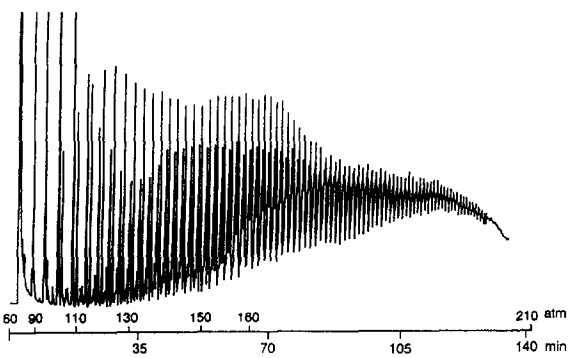

Fig. 6. SFC chromatogram of a polymethylhydrosiloxane sample (molecular mass 2270, PS120). Conditions: pressure program from 60 to 90 atm at 4 atm min^{-1}, 90 to 110 atm at 2 atm min^{-1}, 110 to 130 atm at 1.6 atm min^{-1}, 130 to 150 atm at 1.2 atm min^{-1}, and 150 to 300 atm at 1.0 atm min^{-1}. Other conditions are the same as in Fig. 5.

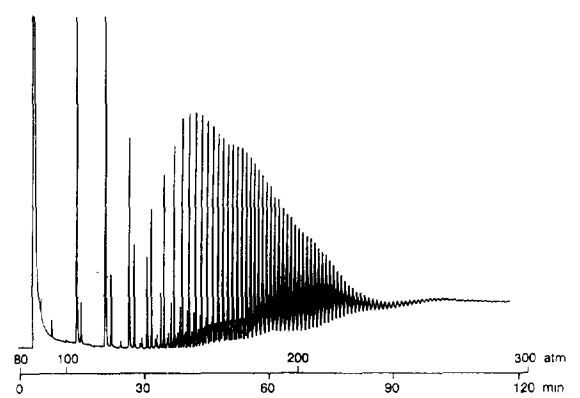

Fig. 5. SFC chromatogram of a polymethylhydrosiloxane sample (molecular mass 1500, PS119). Conditions: 40 cm × 320 μm I.D. fused-silica capillary column packed with particles deactivated with polymethylhydrosiloxane and coated and crosslinked with SE-54; neat CO_2; 75°C; linear pressure program from 80 atm to 300 atm at 1.8 atm min^{-1}.

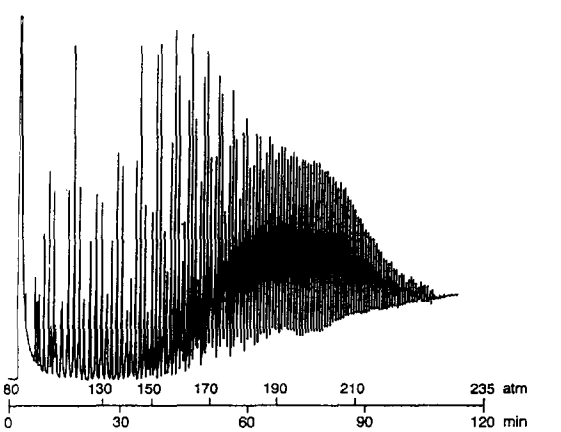

Fig. 7. SFC chromatogram of (30–50%)-methylhydro-(65–70%)-dimethylsiloxane copolymer (molecular mass 2000–2100, PS123). Conditions: 100°C; pressure program from 80 to 130 atm at 2 atm min^{-1}, 130 to 170 atm at 1.7 atm min^{-1}, 170 to 200 atm at 1.3 atm min^{-1}, and 200 to 300 atm at 1.0 atm min^{-1}. Other conditions are the same as in Fig. 5.

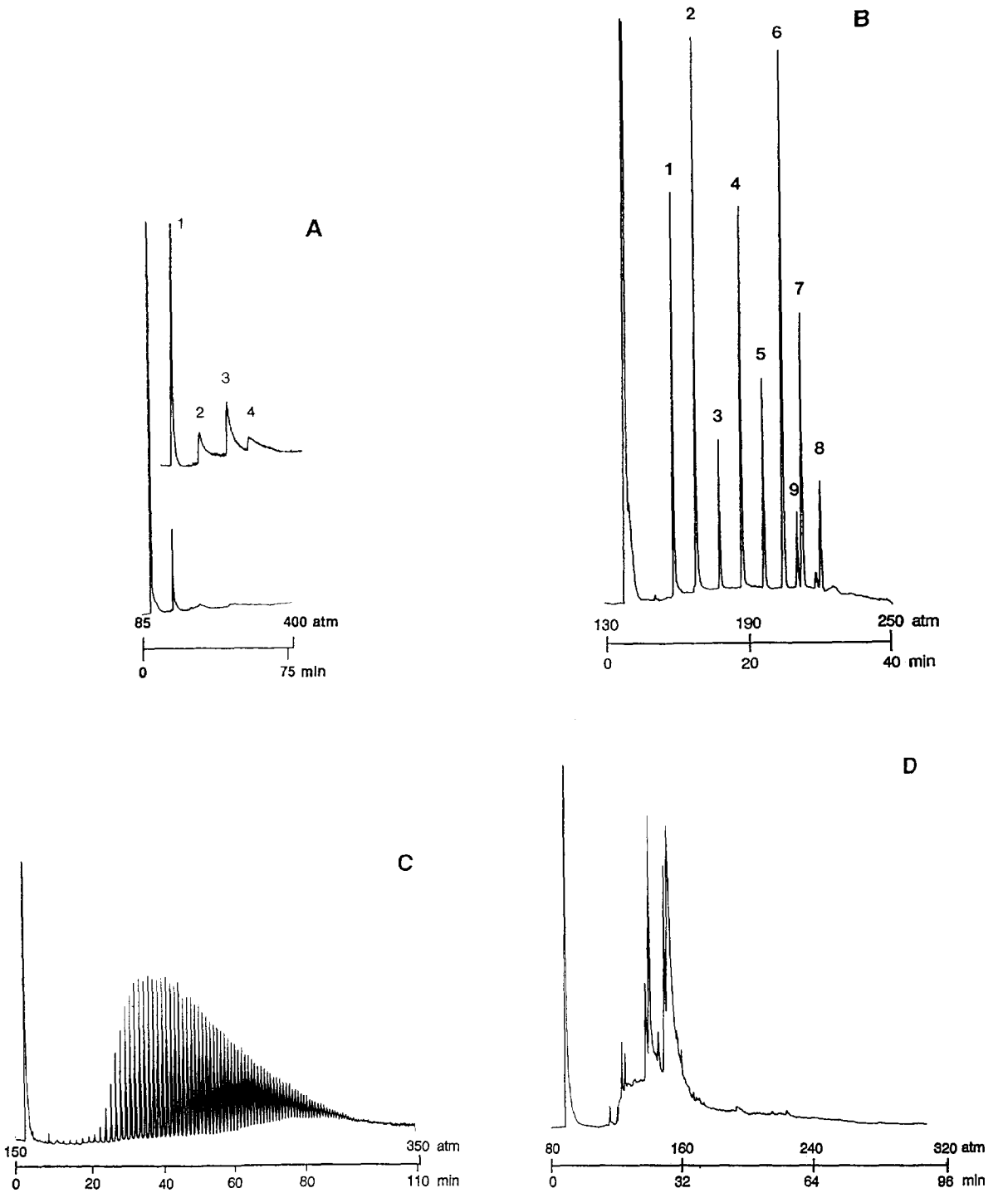

Fig. 8. SFC chromatograms of different polar compounds on a capillary column packed with commercial ODS particles. Conditions: 40 cm × 320 μm fused-silica capillary column packed with ODS particles. Other conditions and peak identifications for (A)–(D) are the same as those in Figs. 1–4, respectively.

Comparing Fig. 4 with Fig. 8D, it can be seen that the column packed with SE-54-encapsulated particles can give much better separation than when packed with ODS particles.

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