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SUPERCRITICAL FLUID CHROMATOGRAPHY–INFRARED SPECTROSCOPY OF OLIGOMERS: USE OF BUFFER-MEMORY TECHNIQUE

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SUMMARY

The feasibility of using the buffer-memory technique for supercritical fluid chromatography–infrared spectroscopy (SFC–IR) is demonstrated. The effluent from a microtubular packed SFC column was deposited onto a crystal (or crystals) of potassium bromide as a continuous, narrow band (width about 1 mm) with instantaneous elimination of the mobile phase. This technique is compatible with IR detection of medium polarity oligomers, separated by SFC with 10% ethanol in hexane as the mobile phase. Chromatographic resolution is maintained, even for closely separated peaks, and identifiable spectra of the peaks can be obtained from the plate.

INTRODUCTION

Although supercritical fluid chromatography (SFC) was described as early as 1962 by Klesper *et al.*[1], it has experienced rather slow growth and limited acceptance as an analytical tool because of the technological difficulties in handling supercritical fluids in chromatographic systems. Recently, interest in SFC has revived. The previous technical limitations have been overcome through the availability of high-pressure instrumentation (solvent pumps and sample injection systems) developed for high-performance liquid chromatography (HPLC). Further, encouraging results have been obtained by the use of open microtubular columns[2,3] and microtubular columns packed with microparticles[4–7].

SFC has several advantages compared to gas chromatography (GC) and HPLC. As is well known, the properties of a supercritical fluid are intermediate between those of gases and liquids. Solute diffusivities are about 100 times higher in a supercritical fluid than in the corresponding liquid phase and viscosities are similar to those in the gas phase. Furthermore, the greater density of supercritical fluids compared with gases imbues the mobile phase with solvating powers, which can readily be controlled by application of pressure. As a result, these properties should enable greatly enhanced chromatographic efficiency compared to HPLC (although not as high as in GC), shorter analysis times than in HPLC and the possibility of separating high-molecular-weight and thermally labile compounds that cannot be separated by GC. Comprehensive reviews have been published[8–17].

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However, as in the case of HPLC, detection is currently the weakpoint of SFC. Most GC and HPLC detectors have been used with SFC, including flame ionization[10,12,18–22], thermal conductivity[21], UV-absorption[11–15], fluorescence[3,23] and refractive index[24] detectors, but a means of identifying the eluted components still exists. The ideal detector for SFC (and perhaps for all chromatographic separation techniques) is a mass spectrometer, because of its higher sensitivity compared with other detectors and its specificity in identifying unknown compounds or for confirming the presence of suspected compounds. However, the combination of SFC and mass spectrometry (MS) is in a relatively early stage of development[25–33].

An alternative possibility is infrared (IR) spectroscopy. This enables certain functional groups in the compounds to be analyzed. Besides, except for optical isomers, no two compounds having different structures have the same IR spectra. The sensitivity provided by IR instruments is poorer than that achieved by MS, yet as regards the available information, IR spectroscopy is more often complementary to mass spectrometry than competitive. Shafer and Griffiths[34] reported the first results of Fourier transform infrared (FTIR) spectroscopic detection in SFC, using a high-pressure resistant flow-cell and a mobile phase of carbon dioxide. More recently, a detailed discussion of this approach was provided by Novotny and co-workers[35], who had previously predicted the importance of SFC–FTIR[2]. However, as far as the flow-cell technique is concerned, the situation resembles that in HPLC. Measurements made by use of a flow-cell show severe spectroscopic interference from the intense absorption bands of the mobile phase. Moreover, when pressure programming (which is equivalent to temperature programming in GC or gradient elution in HPLC) is performed in order to vary the solvent strength, solvent compensation is essentially impossible since the absorptivities of the bands in the spectrum of a supercritical fluid increase with pressure. Shafer and Griffiths[34] and Novotny and coworkers[35] have found that carbon dioxide shows a few transparent regions just above the critical pressure, but some band intensities dramatically increase as the pressure is increased.

A solution to this problem is to eliminate the mobile phase, leaving the separated components for IR analysis. For HPLC–FTIR, at least two solvent-elimination techniques have been proposed, involving heating of an effluent containing the separated components and deposition of the concentrated sample onto a potassium bromide plate for absorption spectroscopy[36–39], or onto a potassium chloride powder for measurements by diffuse reflectance infrared Fourier transform spectroscopy (DRIFT)[40,41]. Preliminary results using SFC–DRIFT have recently been published[42].

This report describes the first practical demonstration of SFC combined with IR spectroscopy, using the buffer-memory technique, for the analysis of oligomers. In the present work, a ratio-recording IR spectrophotometer is used and the components are separated on a packed microcapillary column with a hexane-based solvent. An FTIR spectrophotometer is desirable because it has several distinct advantages over conventional dispersive instruments[43], but its use is often prohibited in many laboratories because of the high cost. The performance of the spectrometer used is essentially comparable to that of FTIR spectrophotometers except for the longer scan time required.

EXPERIMENTAL

Apparatus

The SFC–IR system used is shown schematically in Fig. 1. It includes a LC-840 pump (pump A) (Du Pont, Wilmington, DE, U.S.A.), a Twincle pump (pump B) (JASCO, Tokyo, Japan), a 7410 loop injector (volume 1.0 μl) (Rheodyne, Berkeley, CA, U.S.A.) a GC oven (Shimadzu, Kyoto, Japan) and a JASCO Uvidec 100-III UV detector. The mobile phase, *n*-hexane modified by ethanol, was delivered at room temperature into a fused-silica capillary column (100 cm × 0.5 mm I.D.) packed with Develosil ODS-10 (Nomura Chemical Co., Seto, Japan) which was placed in the oven. Constant-pressure operation was carried out solely by using pump A, which contained a stainless-steel coil partially filled with the mobile phase. The pressure programming was achieved by delivering the mobile phase of pump B to pump A at a constant flow-rate (about 1 ml/min), after setting the pressure of pump A and closing the valve located between a nitrogen cylinder and pump A, so that the pressure at the column inlet was allowed to increase with time. The flow-rate of the mobile phase fed into the column was varied between 7 and 10 μl/min. The programming rate can be determined by the preset pressure of pump B or the amount of mobile phase stored in pump A. The effluent from the column was transferred through a fused-silica capillary column, packed with Develosil ODS-5 (75 × 0.2 mm I.D.), onto a potassium bromide plate. This short column served as a pressure restrictor.

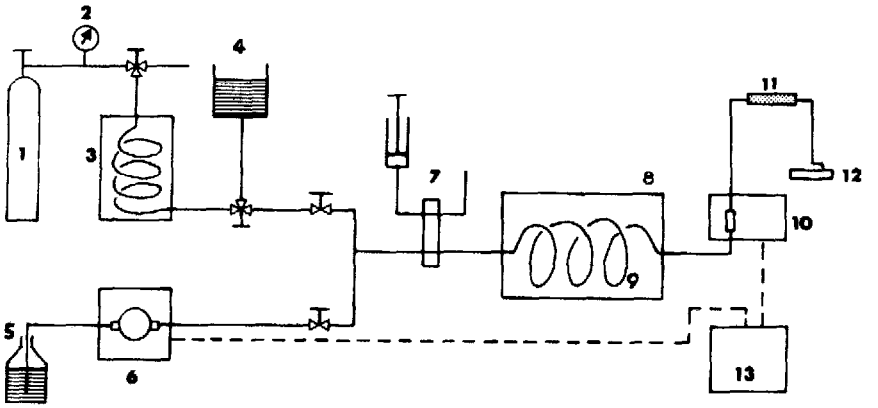


Fig. 1. Schematic diagram of the buffer-memory technique for SFC–IR. 1 = Nitrogen cylinder; 2 = pressure gauge; 3 = pump A; 4 and 5 = solvent reservoirs; 6 = pump B; 7 = sample injector; 8 = GC oven; 9 = packed capillary column (Develosil ODS-10, 100 cm × 0.5 mm I.D.); 10 = UV detector; 11 = restrictor (75 × 0.2 mm I.D. capillary column, packed with Develosil ODS-5); 12 = potassium bromide plate, mounted on the interface; 13 = recorder.

The design and construction of the interfacing device used in this work is the same as one used previously in the HPLC–IR interface[37]. The species eluted from the SFC column were deposited onto a continuously moving potassium bromide plate. The mobile phase was evaporated on contact with the plate, leaving a permanent record of the solutes on the plate. When the chromatogram was complete, the plate was simply transferred to an IR spectrophotometer and the IR chromatogram recorded by monitoring the absorption at a preset wavenumber.

The chromatograms and spectra were obtained on a JASCO Model 810, double-beam ratio-recording IR spectrophotometer. It was fitted with a 3X beam condenser and a 4 × 1.7 mm aperture. The spectrum of each chromatographic peak recorded on the plate was obtained as follows. A reference spectrum was obtained from ten scans of a pure potassium bromide crystal and then stored in the data system. At each peak maximum on the IR chromatogram the plate was stopped and the IR spectrum was scanned ten times. The spectra of both sample and reference were measured with slit program W (spectral resolution: 5.4 cm^{-1} at 1000 cm^{-1}), and corrected for the absorption bands due to moisture on the beam condenser.

Chemicals

The styrene oligomer A-500 (nominal molecular weight 500) and the methylphenylsiloxane DC-710 (50% phenyl, nominal molecular weight 2600) were purchased from Toyo Soda Manufacturing Co. (Tokyo, Japan) and Gasukuro Kogyo Inc. (Tokyo, Japan), respectively. *n*-Hexane and ethanol were of reagent grade and based as the mobile phase without further purification. Potassium bromide crystals (40 × 10 × 3 mm) were obtained from JASCO.

RESULTS AND DISCUSSION

The previous studies of SFC–IR employed pure carbon dioxide[34,35] or carbon dioxide modified by methanol[42] as the mobile phase; carbon dioxide is the most convenient fluid for SFC, mainly because of its low critical temperature (T_c = 31.3°C). Throughout this work, *n*-hexane modified by 10% ethanol was used as the mobile phase. The critical temperature is 241.5°C, as found on linear interpolation[44] with T_c (*n*-hexane) = 234.2°C and T_c (ethanol) = 243.4°C. This mobile phase was chosen for several reasons. First, it is able to dissolve many oligomers even at room temperature, which enables the sample injection as well as the UV flow-cell detection to be performed at that temperature. Consequently, the chromatographic instrumentation is simplified. Secondly, because it is a liquid at room temperature, the deposition of the effluent onto a potassium bromide plate is easily accomplished. Thirdly, because it has a high vapour pressure under atmospheric conditions, the evaporation of the mobile phase is very easy. Finally, the utilization of the mobile phase prevents the generation of bubbles at the column exit; this is not the case when *n*-pentane modified by 10% ethanol is used. There may be a difference in selectivity between carbon dioxide and *n*-hexane, but we have no data which support this.

Before applying the buffer-memory technique to oligomers, it must be determined whether the separation efficiency achieved originally by the SFC column is decreased while the sample components are travelling through the connecting tube between the UV detector and the deposition point (see Fig. 1). In order to explore this problem, another UV detector was connected to the exit of the connecting tube, *i.e.*, at the position of the potassium bromide plate in Fig. 1. Fig. 2 illustrates the broadening due to the connecting tube. An oligostyrene sample, A-500, was used as a test solute; oligostyrenes have become almost "standard" samples for examining the quality of chromatographic systems. The sample (total amount 30 μg) was introduced as a solution in the mobile phase. The separation was carried out isobarically at 60 atm. The column temperature was held well above the critical temperature of

the mobile phase in order to reduce the effect of thermal variations upon the mobile phase. In Fig. 2 the degree of oligomerization is tentatively assigned above each peak. Calculation of the height equivalent to a theoretical plate (HETP) for the $n = 4$ peaks in the two chromatograms showed that the values differ only by 5%. This difference is smaller than expected; this may partly be due to the fact that the effluent enters the restrictor, which comprises a short slurry-packed capillary column. A 3-μg amount of A-500 was injected into the column, and it was demonstrated that the introduction of a large sample (as large as 30 μg) affects the resolution only slightly. Based upon the increased sample capacity, it is possible to inject a larger amount of oligostyrene for SFC than for HPLC, carried out on a column of the same size.

A-500 was chromatographed under the same conditions as described in Fig. 2 and the effluent was continuously deposited onto a single potassium bromide plate until the eighth peak had been eluted. A typical IR chromatogram at 698 cm^{-1}, which was generated from the plate, is shown in Fig. 3. It is seen that the resolution is somewhat diminished between almost all neighbouring peaks, but that the "chromatogram" is clearly recorded on the plate. The IR spectrum of the $n = 4$ peak (marked with an asterisk in Fig. 3) is shown in Fig. 4. Despite the fact that the amount corresponding to this chromatographic peak is very small, as expected from the response of the UV and IR chromatograms, this technique produces a high-quality spectrum, which is ideal for identification purposes.

The value of SFC is perhaps best realized when considering its applicability to a mixture of oligomers which span a wide range of molecular weights. We applied

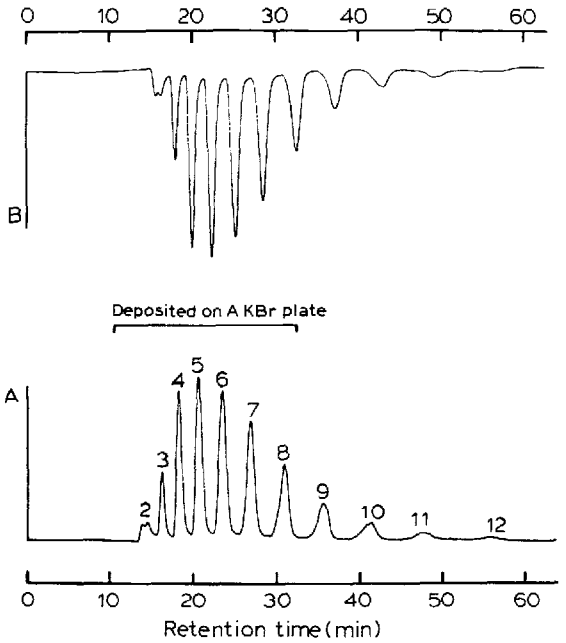


Fig. 2. Band broadening caused by the connecting tube between the column outlet and the deposition point: A, first UV detector; B, second UV detector. Column: 100 cm × 0.5 mm I.D., Develosil ODS-10. Mobile phase: 10% ethanol in *n*-hexane. Column temperature: 255°C. Column inlet pressure: 60 atm. Sample: 3% oligostyrene A-500 (1 μl). Detection: 225 nm.

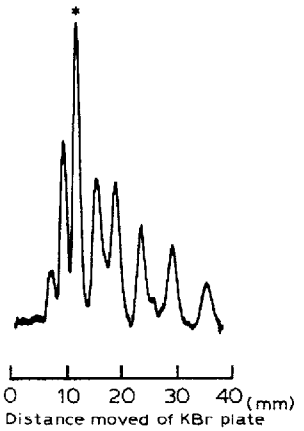


Fig. 3. IR chromatogram of oligostyrene A-500, obtained by using the buffer-memory technique. Detection wavenumber: 698 cm^{-1}.

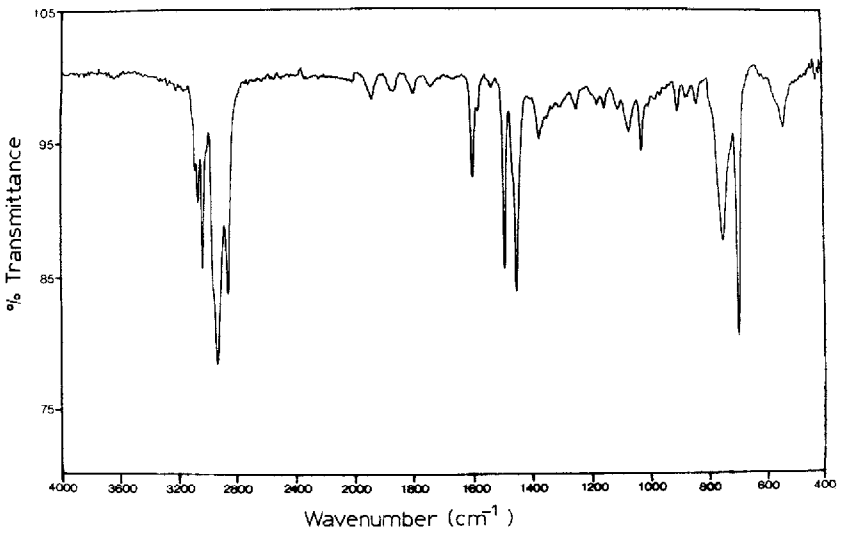
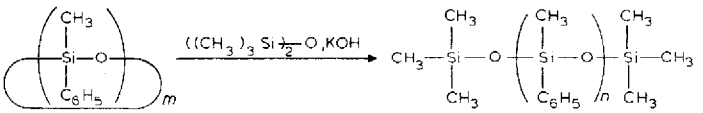


Fig. 4. IR spectrum of the peak marked by the asterisk in Fig. 3.

the buffer-memory technique to Dow-Corning (DC-710), which is one of the most widely used GC stationary phases. DC-710 can be prepared by an equilibration reaction involving clearage and reformation of the siloxane bonds



where m and n are equal to the number of difunctional units in the oligomer. Obviously, in addition to a linear polysiloxane, some cyclic oligomers result from residual starting material[45]. Nieman and Rogers[46] reported the SFC separation of DC-710 with conventional packed columns. Fig. 5 shows the UV chromatogram of DC-710 where the separation was performed using essentially the same chromatographic conditions as those used for the separation shown in Fig. 2, except for the pressure applied. The pressure was maintained isobarically at 47.5 atm for 30 min after which it was programmed at 0.17 atm/min to 73.5 atm. It is seen that oligomers up to peak 33 are resolved, after which the trace returns to the baseline. On two sections of the potassium bromide plate a limited number of oligomers, from peak 1 to peak 11, were deposited. The resulting IR chromatogram is shown in Fig. 6; the spectrophotometer was set at 1126 cm^{-1}. The appearance of the chromatogram is similar to that in Fig. 5, except that the unresolved portion of the material is decreased. The IR spectra corresponding to each peak deposited on the plate were scanned, and are shown in Fig. 7 for peaks 1–5. All of the peaks following peak 5 showed identical spectra to that of peak 5. It is interesting that for peaks 1 and 3 the intensity of the band at 1050 cm^{-1} relative to that of both the bands at 1126 and 1026 cm^{-1} is greater than in the case of peaks 2, 4 and 5. Also, the band at 842 cm^{-1} found for peaks 2, 4 and 5 is not present in the spectra of peaks 1 and 3. Therefore, peaks 2, 4 and 5 represent homologous species which are different from peaks 1 and 3.

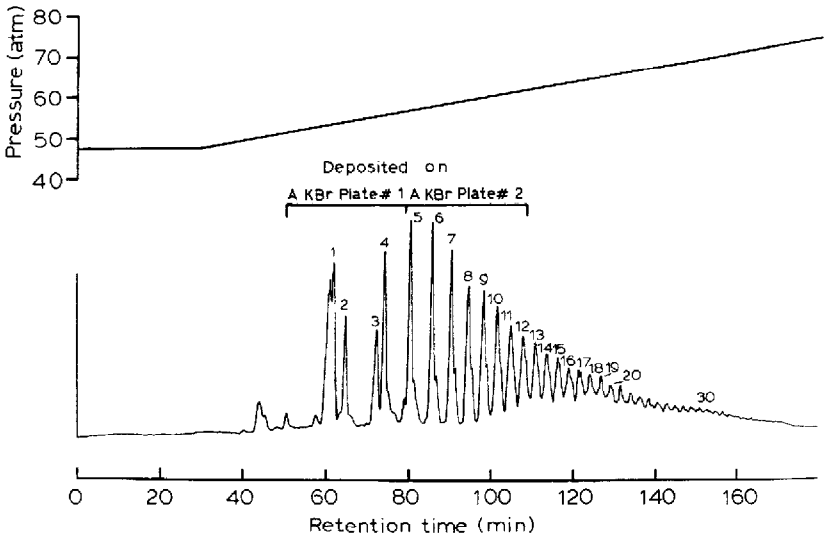


Fig. 5. UV chromatogram of methylphenylpolysiloxane DC-710. Column and mobile phase as in Fig. 2. Column temperature: 260°C. Sample load: 1 μl of 10% DC-710 in mobile phase. Detection: 254 nm.

Many reports have dealt with the spectra of polysiloxanes, but the vibrational assignments are by no means definite, even for simple oligomers such as dimethylpolysiloxanes[47]. The absorption characteristic of the phenyl group in combination with a silicon atom can be seen at 1429 and 1124 cm^{-1}. The strong bands lying in the 1087–1025 cm^{-1} region are assigned to the antisymmetric Si–O–Si stretching

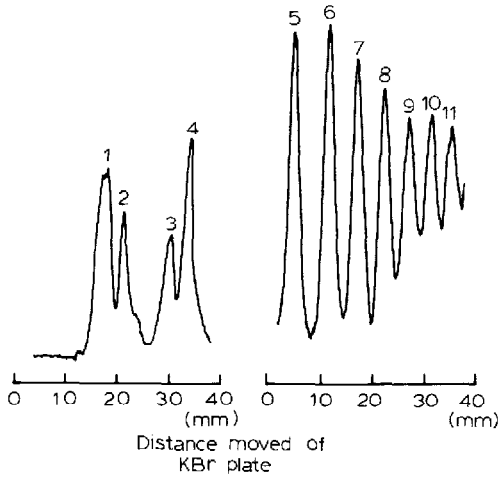


Fig. 6. IR chromatogram of DC-710, measured at 1126 cm^{-1}.

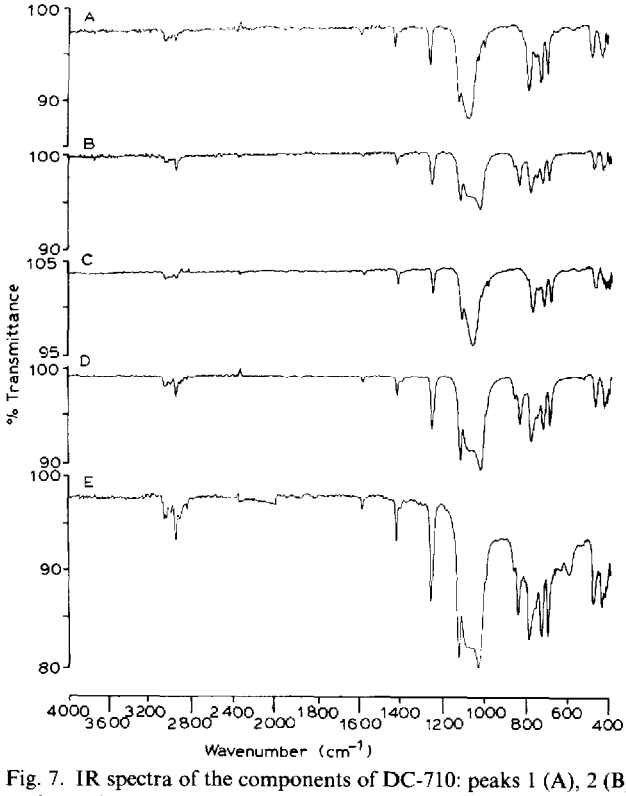


Fig. 7. IR spectra of the components of DC-710: peaks 1 (A), 2 (B), 3 (C), 4 (D) and 5 (E). See Fig. 6 for peak numbers.

mode. Less certain are the methyl rocking and Si–C stretching modes, found between 842 and 698 cm^{-1}, since there is a great possibility of mixing of the vibrations. The absorption at 1260 cm^{-1} may be due to the methyl symmetrical deformation. Taking into account the fact that the bulk of the material is a linear polysiloxane, it can be concluded that peaks 1 and 3 are due to cyclic polysiloxanes, whereas peaks 2, 4 and 5 are linear ones. Also, neither peak 1 nor 3 seems to be a six-membered methylphenylsiloxane, in that an intense band at 1020–1010 cm^{-1}, expected for the cyclic trimers of siloxane oligomers, is not found in their spectra.

It may be noted that IR spectroscopy does not give information about molecular weight, which is the most important item for identification purposes. If this need arises, the material may be scraped off the potassium bromide plate and than subjected to MS analysis[39]. In summary, the present work demonstrates that the buffer-memory technique is a powerful one for the SFC–IR analysis of oligomers. Unlike the flow-cell technique, it is compatible with a mobile phase containing a polar modifier.

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