Journal of Chromatography, 506 (1990) 443–460
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 1728

Coupling of microcolumn high-performance liquid chromatography with Fourier transform infrared spectrometry

KIYOKATSU JINNO* and CHUZO FUJIMOTO
School of Materials Science, Toyohashi University of Technology, Toyohashi 440 (Japan)

SUMMARY

The coupling of high-performance liquid chromatography with Fourier transform infrared spectrometry is discussed, with emphasis on recent work by the authors' group.

INTRODUCTION

Infrared spectrometry has several important characteristics which make it a potentially ideal detection method for chromatography[1–4]. Most organic (and many inorganic) compounds have strong, relatively narrow absorption bands in the mid-infrared region that are highly specific and give detailed structural information about the compound. Even when an unequivocal identification is impossible, isomeric substitution pattern information can be obtained from the fingerprint region. Moreover, different functional groups have characteristic infrared bands, and hence the presence or absence of a class of compound can be quickly verified if the species are present in sufficient concentration. In this regard, IR spectrometry possesses many attributes expected of universal or selective detectors.

The combination of high-performance liquid chromatography (HPLC) with Fourier transform IR spectrometry (FT-IR) is not at as advanced state of development as that of gas chromatography (GC) with FT-IR. Although there are several reasons for the poor performance of these techniques, the most important is related to the IR absorbance of the mobile phase. All HPLC mobile phases absorb IR radiation at certain wavenumbers, and as the mobile phase is present at a much higher concentration than the sample components in HPLC, sample spectra can only be obtained in regions of the spectrum free from solvent absorption bands. Water and methanol, which are common polar solvents used in reversed-phase HPLC, completely obscure solute bands across the spectrum. Hence the observation and identification of trace compounds of interest in the presence of the HPLC mobile phase are major problems in using IR detection in HPLC. Therefore, two major approaches to HPLC–FT-IR has been proposed, namely flow-cell methods with using less conven-

0021-9673/90/$03.50

tional solvents[5–12] as the mobile phase and the solvent-elimination technique with more conventional solvents[13–23].

FLOW-CELL APPROACH IN MICROCOLUMN HPLC–FT-IR

In spite of the mobile-phase restriction, the flow-cell method provides the simplicity of interfacing and real-time monitoring of the chromatographic effluent and universal applicability to samples. If a solvent that has favourable transparency in regions separated from most of the analyte absorptions can be selected, the method offers the potential to serve as a universal or functional group-selective detector[5–12].

Perfluorinated solvents are typically such mobile phases and can be employed for the IR detection of sample components having C–H fragments in their structures[5,6]. Deuterated solvents are also reasonably transparent in certain narrow spectral regions, especially with very short path-length flow cells[7–12]. Further, there is an even greater range of choice of polarity with the latter than the former.

Microcolumns were easily incorporated into this combined technique because, although the high cost of such solvents precludes their use as mobile phases in conventional HPLC, microcolumn HPLC does provide opportunities for their use because of the low solvent consumption. The use of microcolumns has the further advantage that the effluent concentration is much increased over that with a conventional analytical column for a constant amount of sample injected. A higher analyte concentration means that short path-length flow cells can be employed, thereby allowing for wider IR window regions or the achievement of greater detector sensitivity for a fixed cell path length.

A laboratory-constructed flow cell for microcolumn HPLC–FT-IR is shown in Fig. 1[8]. A window cut out of a silver chloride pellet was employed, which allowed us

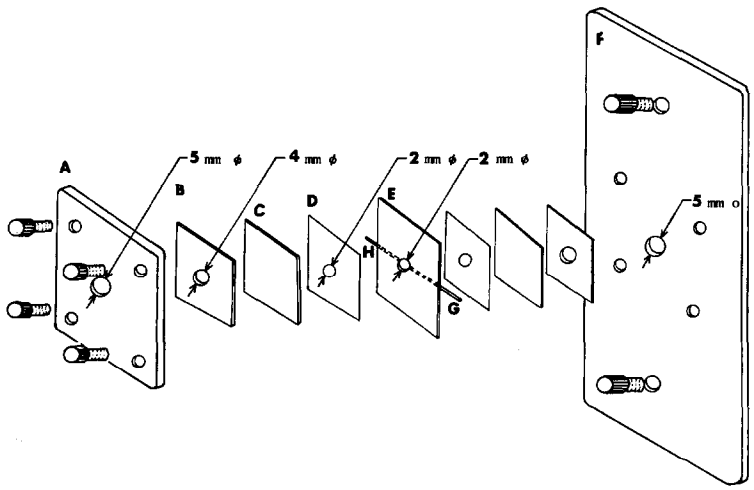

Fig. 1. Micro flow cell with minimized internal volume (1.3 μl) for microcolumn HPLC–FT-IR. A = Window holder; B = 1-mm PTFE pad; C = 0.5-mm AgCl window; D = 0.07-mm PTFE pad; E = 0.4-mm cell body; F = window holder for mounting; G = inlet tube (0.13 mm I.D.); H = outlet tube (0.31 mm I.D.). (From ref. 8 with permission.)

to observe several spectroscopically important regions, owing to its complete transparency across the entire spectrum. The assembly was mounted on the sample holder of a beam condenser in the sample compartment of a JEOL JIR 40X FT-IR spectrometer (JEOL, Tokyo, Japan). The total cell volume, including a 2-cm length of inlet tube for the cell, was estimated to be about 1.6 μl; this volume is still larger than that of a UV detector used in microcolumn HPLC. The absorption of the three most commonly used solvents for reversed-phase HPLC (water, methanol and acetonitrile) is much stronger than that of any of the solvents considered below. Although the spectrum of acetonitrile is less strongly absorbing than that of methanol, the presence of even 10–20% of water will make reversed-phase HPLC–FT-IR measurements extremely difficult and very short path-length cells should be employed, as shown in Fig. 2, where IR spectra of mixtures of acetonitrile, methanol and water and their deuterated counterparts are illustrated[2].

In terms of solute detection (rather than solute identification), it is essential to find solvents that are transparent to IR radiation at the wavenumbers of interest. There is no doubt that deuterated solvents satisfy this requirement, as shown in Fig. 2. A comparison of the FT-IR chromatograms of phthalates, separated by reversed-phase HPLC using deuterated and non-deuterated solvents, is shown in Fig. 3. By using the deuterated solvents, the chromatographic peaks measured at 2965 cm^{-1} (C–H stretching) appeared, which were not clearly distinguished from the baseline fluctuation when the non-deuterated solvents were used as the mobile phase. Also, more sensitive detection is achieved at 1300 and 1730 cm^{-1}, corresponding to C–O and C=O stretching vibrations, respectively: it seems likely that the reduced absorbance at these wavenumbers for the deuterated solvent accounts for the results[8].

SOLVENT-ELIMINATION APPROACH IN MICROCOLUMN HPLC–FT-IR

It should be noted that the real utility of IR spectrometry is, most often, not in the availability of the chromatogram, but in that of the spectrum of the eluate. Obviously, the only way to avoid the solvent interference problem is to eliminate the solvent prior to the IR measurements. Here all of the spectrum could be observed, not just those bands in window regions of the mobile-phase solvent spectrum, and the concentration of the analyte in the IR beam could be increased. Therefore, various solute transport designs have been proposed to improve the identification capability of HPLC–FT-IR. Because of the reduced flow-rate, microcolumn HPLC again has great promise for this type of interfacing. The "buffer-memory" technique was developed to realize this advantage in HPLC–FT-IR[13–20]. The principle of the buffer-memory technique involves continuous deposition of the total column effluent on an IR-transparent medium such as a potassium bromide crystal plate, followed by the analysis of the eluates left on the collection medium as a function of the distance moved of the medium by transmission spectrometry. It is fairly easy to preserve a single substrate. Because the sample is left as a permanent record of the separation, more information can be effectively delivered to the analyst if the "memory" is accessed by other instrumental methods. In other words, because all information on the sample is stored in the memory, one can extract the desired information from it. In this sense, the substrate might be likened to a computer disk.

A typical example will be presented to demonstrate the potential of the proposed

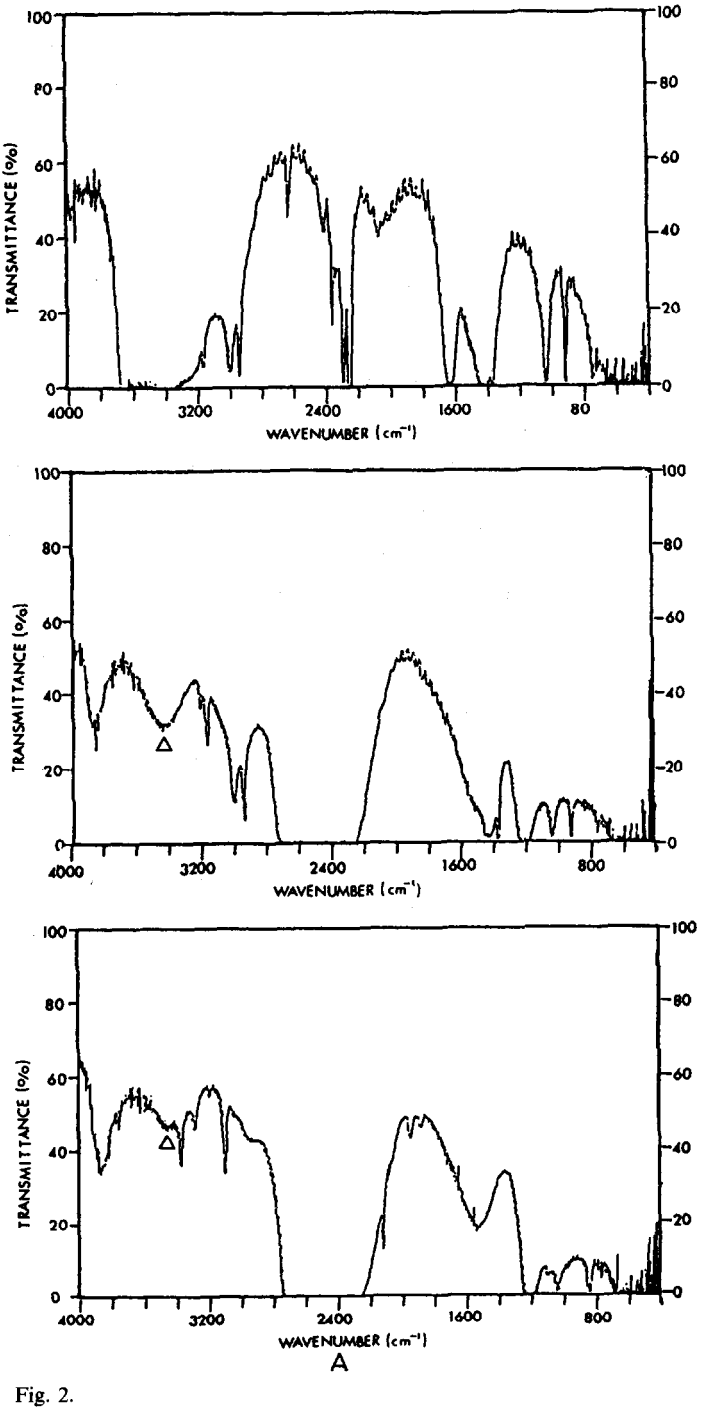

Fig. 2.

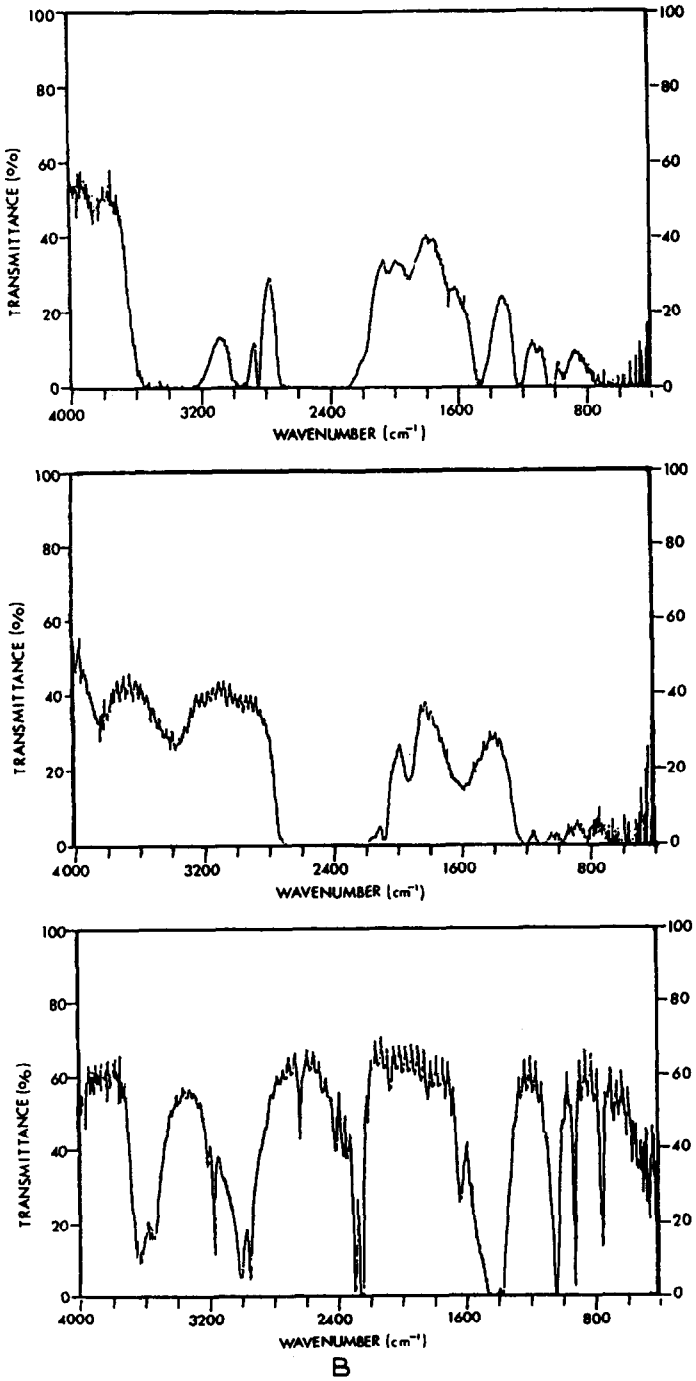

Fig. 2. IR spectra of deuterated and non-deuterated solvents used for reversed-phase chromatography. (A) Acetonitrile–water: top, CH_3CN–H_2O (90:10); middle, CH_3CN–^2H_2O (50:50); bottom, C^2H_3CN–^2H_2O (50:50). (B) Methanol–water: top, CH_3OH–^2H_2O (50:50); middle, $C^2H_3O^2H$–^2H_2O (50:50); bottom, CH_3CN–H_2O (99:1). (From ref. 2 with permission.)

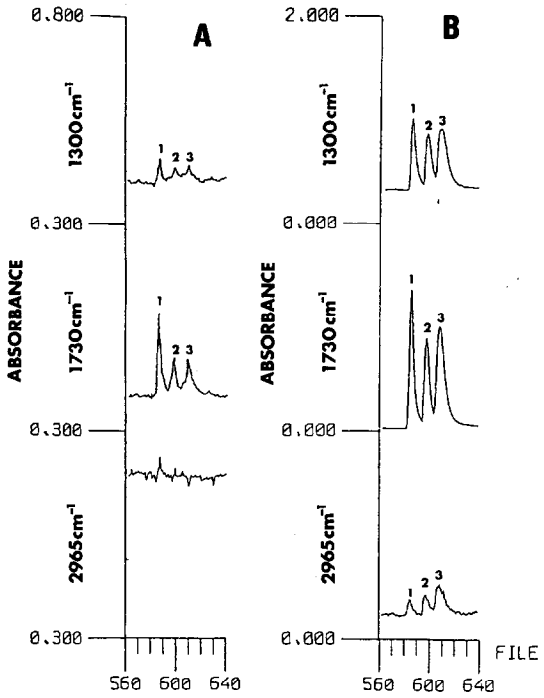

Fig. 3. Reversed-phase separation of phthalates: (A) with acetonitrile–water (90:10); (B) with the deuterated solvents (C^2H_3CN–^2H_2O) (90:10). Column, Chemcosorb ODS-H (14 cm × 0.5 mm I.D.); flow-rate, 4 μl/min; FT-IR accumulation, 10 times. Solutes: 1, dimethyl phthalate (14 μg); 2, di-*n*-butyl phthalate (13 μg); 3, di-*n*-pentyl phthalate (17 μg).

buffer-memory technique, which can be conveniently employed for the sequential analysis of chromatographically separated organic and organically bounded metal species by spectrometric methods. The concept includes the utilization of the proposed technique for *in situ* measurements by IR and X-ray fluorescence (XRF) spectrometry followed by mass spectrometric (MS) detection in order to elucidate the structures of unknown analytes.

To demonstrate the feasibility and usefulness of the proposed concept, a solution of metal diethyldithiocarbamate (DDTC) complexes was prepared. Three DDTC complexes, bis(diethyldithiocarbamato)copper(II), tris(diethyldithiocarbamato)chromium(III) and tris(diethyldithiocarbamato)cobalt(III), were dissolved in chloroform at concentrations of 1, 1.5 and 1.5% (w/v), respectively. Fig. 4 shows the IR transmittance chromatogram monitored at 1265 cm^{-1} after the eluent had been deposited and collected on a potassium bromide plate.

XRF spectra can then be measured without removing the HPLC peak of interest from the potassium bromide plate. The memorized plate is placed in a simple holder made of acrylic resin and moved manually through intervals as small as 1 mm in the Ge K X-ray beam to obtain the XRF spectra. The XRF spectrometer used was of the energy-dispersive type, therefore permitting simultaneous multi-element detection. One of the spectra obtained in this way is shown in Fig. 5. The fluorescence peaks of Cr,

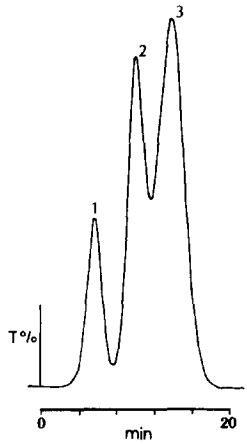

Fig. 4. IR chromatogram from a mixture of three DDTC complexes[16]. Mobile phase, benzene; flow-rate, 8 μl/min. The three complexes are (1) DDTC–Cu^{II}, (2) DDTC–Cr^{III} and (3) DDTC–Co^{III} (1.0, 1.5 and 1.5%, w/w, respectively). Monitoring at 1265 cm^{-1}.

Cu, Al and K, together with the Ge scattering peak, are clearly visible. The Al peak originates from the material of beam coursing.

The peak area of each analyte fluorescence was plotted against the retention time, where normalization has been performed by using the Al peak. Fig. 6 demonstrates the resulting chromatograms for each analyte metal. Although some peak broadening can be seen, which can be mainly attributed to the use of an X-ray beam mask which was larger than the width of the aperture used in the IR experiment.

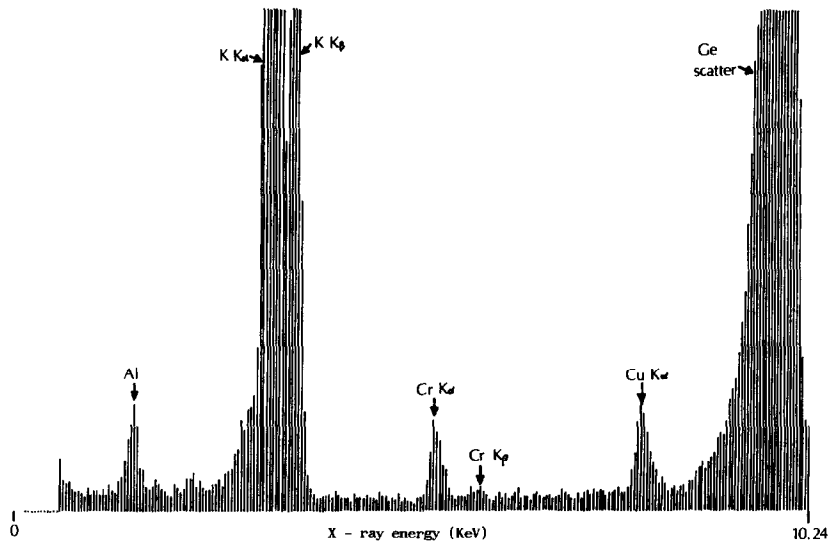

Fig. 5. XRF spectrum obtained through excitation of the species memorized on a potassium bromide plate with Ge K X-rays[16]. Counting time (live), 100 s.

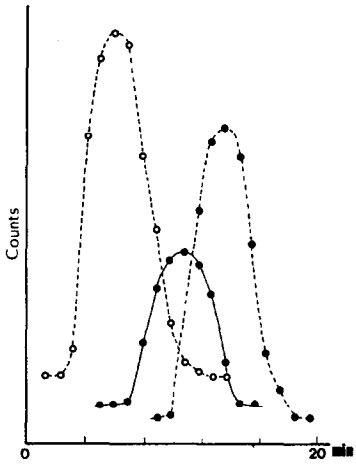

Fig. 6. Metal chromatograms obtained by XRF[16]. ○ = 8.05 keV; ● (solid line) = 5.42 keV; ● (dashed line) = 6.93 keV.

the peak maxima coincide in position with those of the IR chromatogram. A comparison between the two sets of chromatograms implies that the peaks labelled 1, 2 and 3 in Fig. 4 are due to copper-, chromium- and colbalt-containing species, respectively. Following the XRF measurements, the structure of the deposited components can be confirmed by mass spectrometry. The peaks were scraped off the plate with a clean blade and introduced into the ion source using a direct-insertion probe. As expected, the mass spectra were in excellent agreement with those of authentic samples.

As described above, a new dimension has been added to the spectrometric detection and identification of HPLC-separated species. The buffer-memory technique provides some benefits with respect to both IR absorption detection and metal detection. To our knowledge, such an attempt has never been made in the area of chromatography. In most instances, chromatographic effluents are discarded after detection. Alternatively, the fractions may be taken in a fraction collector and subsequently analysed by a different technique. However, the method does have limitations, for example, one will have difficulty in storing the solute-containing vials as the number of isolated fractions increases, and spectroscopic retrieval at any point of the chromatogram is, in principle, impossible. In contrast, the substrate used in the buffer-memory technique also serves as a storage device.

Although the buffer-memory technique has been demonstrated to be a powerful interfacing technique between HPLC and FT-IR, it cannot tolerate the aqueous mobile phases used in reversed-phase chromatography because they are of comparatively low volatility and readily dissolve potassium bromide. Instead of the potassium bromide plate, a stainless-steel wire net can be utilized as a buffer-memory substrate[17]. The effluent from a reversed-phase microcolumn is deposited on the metal wire net from which the aqueous solvent is evaporated using a heated nitrogen flow, so that the deposit is suspended between the metal meshes. The separation of three cold medicine components is shown in Fig. 7. No interference from the mobile phase solvents, water and methanol, is seen in the three-dimensional chromatogram.

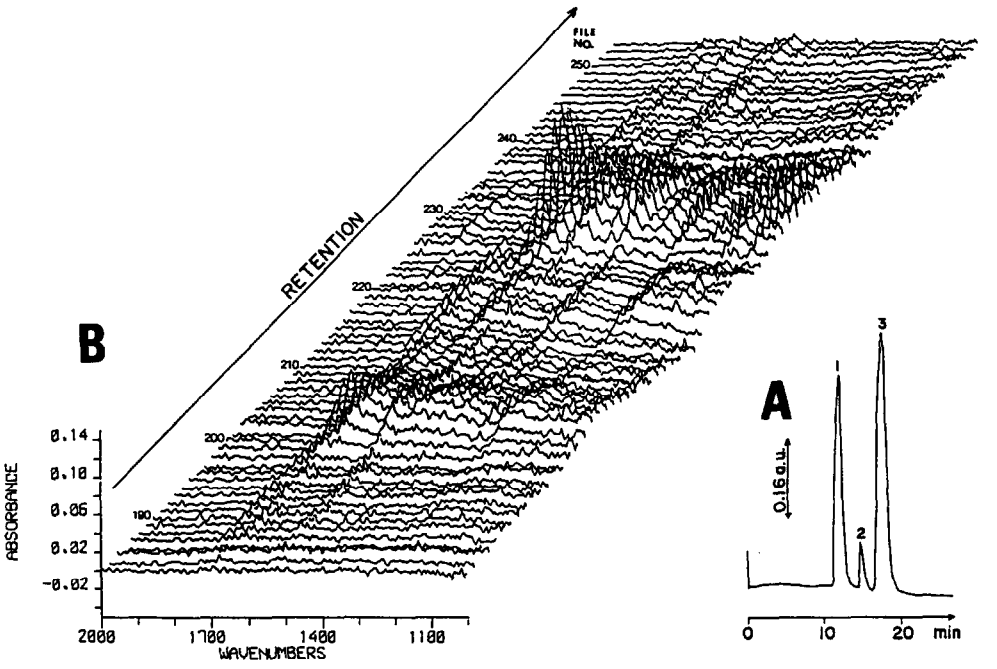

Fig. 7. Reversed-phase separation of caffeine, aspirin and phenacetin with (A) UV and (B) FT-IR detection via the buffer-memory technique[17]. Column, 25 cm × 0.5 mm I.D., Develosil ODS-10; mobile phase, methanol–water (3:2); flow-rate, 4 μl/min; accumulation, 100 times. Peaks: 1 = caffeine; 2 = aspirin; 3 = phenacetin.

The merit of the buffer-memory technique compared with several other approaches can be found in the combination of HPLC, thin-layer chromatography (TLC) and FT-IR[18,19]. The combination of HPLC with TLC utilizes two-dimensional development and hence gives a better analytical performance. In HPLC–TLC, a sample can be subjected to two separation processes at right-angles to each other. The total column effluent from microcolumn HPLC can be deposited on the TLC plate as a continuous line, and the plate developed by the usual process. After development, one can use diffuse-reflectance IR detection to measure the IR spectra of the sample spots on the TLC plate without the need for any chemical process.

It is well known that all adsorbents used for TLC show strong absorption of IR radiation. The diffuse-reflectance IR spectra of silica gel and alumina on TLC substrates are shown in Fig. 8. It is seen that the percentage of IR radiation diffusely reflected from both adsorbents is significantly less than that from potassium bromide powder. Alumina has a wider spectral window than silica gel, which means that more spectral information can be obtained with alumina than with silica gel. It is expected that diffuse-reflectance measurements will be impossible in the frequency range below 1200 cm^{-1} because of the strong absorptivity of alumina in this region. However, an alumina TLC plate was preferred in this example, as the remainder of the spectrum is transparent enough to allow spectral measurements of compounds on the plate.

A typical eight-component separation was performed by reversed-phase microcolumn HPLC and the components deposited on the alumina TLC plate were

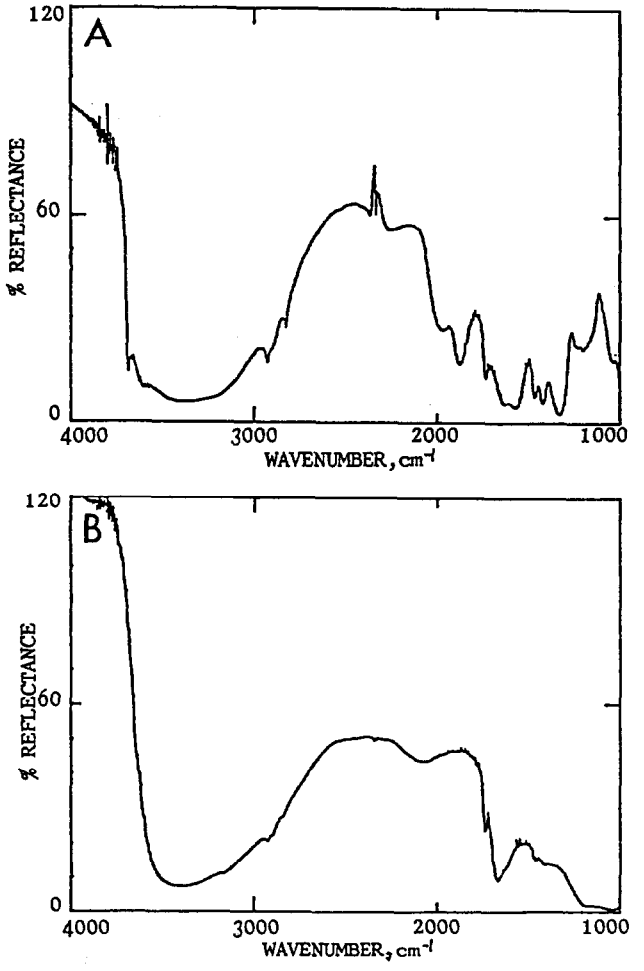

Fig. 8. Diffuse-reflectance IR spectra of (A) silica TLC plate and (B) alumina TLC plate measured by using potassium bromide powder as a reference.

subjected to diffuse-reflectance IR measurements. The collected track on the plate gave the reconstructed IR chromatogram shown in Fig. 9. As can be seen, four major peaks appear in the IR chromatogram (using UV detection, a similar chromatogram was obtained)[19]. By retrieving the filed spectra, one can obtain the IR spectra corresponding to the four separated peaks. One of the spectra is shown in Fig. 10, where the spectra of peak 3 in Fig. 9 were retrieved from the appropriate files. After the second development by normal-phase TLC, IR measurements were performed by scanning the plate and the reconstructed FT-IR chromatograms on the TLC plate are summarized in Fig. 11, which clearly indicates eight peaks. The spectrum of each peak can be easily called from the computer file. The spectra obtained are illustrated in Fig. 12, where identifiable spectra with even narrow wavenumber regions are seen. The spectrum of peak 8 is clearly consistent with that of peak 3 in Fig. 9. This means that

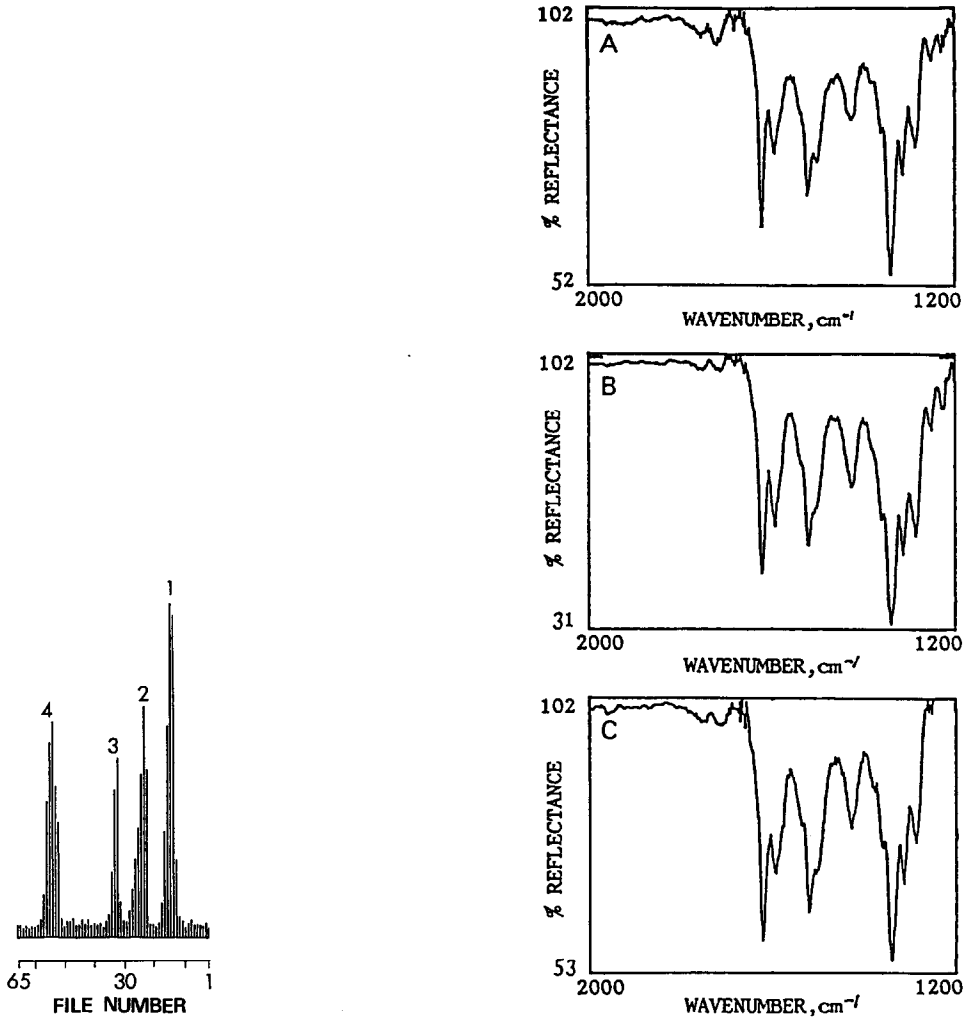

Fig. 9. FT-IR reconstructed chromatogram of the eight-component mixture after deposition of the microcolumn HPLC eluent on an alumina TLC strip at a speed of 1 mm/min. Column for HPLC: 30 cm × 0.53 mm I.D., Vydac 201 TPB5; mobile phase, methanol–water–acetone (60:35:5); flow-rate, 2.67 μl/min. (Reproduced from ref. 19 with permission.)

Fig. 10. File IR spectra from peak 3 in Fig. 9. (A) File No. 31; (B) file No. 32; (C) file No. 34.

TLC development did not lead to any problems in obtaining identifiable IR spectra. The detection limit of this microcolumn HPLC–TLC–FT-IR measurements has been calculated and the diffuse-reflectance IR spectrum of 50 ng of *o*-nitroaniline on an alumina TLC plate shown in Fig. 13 suggests that the limit is close to subnanogram amounts.

The buffer-memory interface has been adopted in a commercially available device (JEOL, Japan) with a rotating 50-mm diameter potassium bromide disk[20]. Fig.

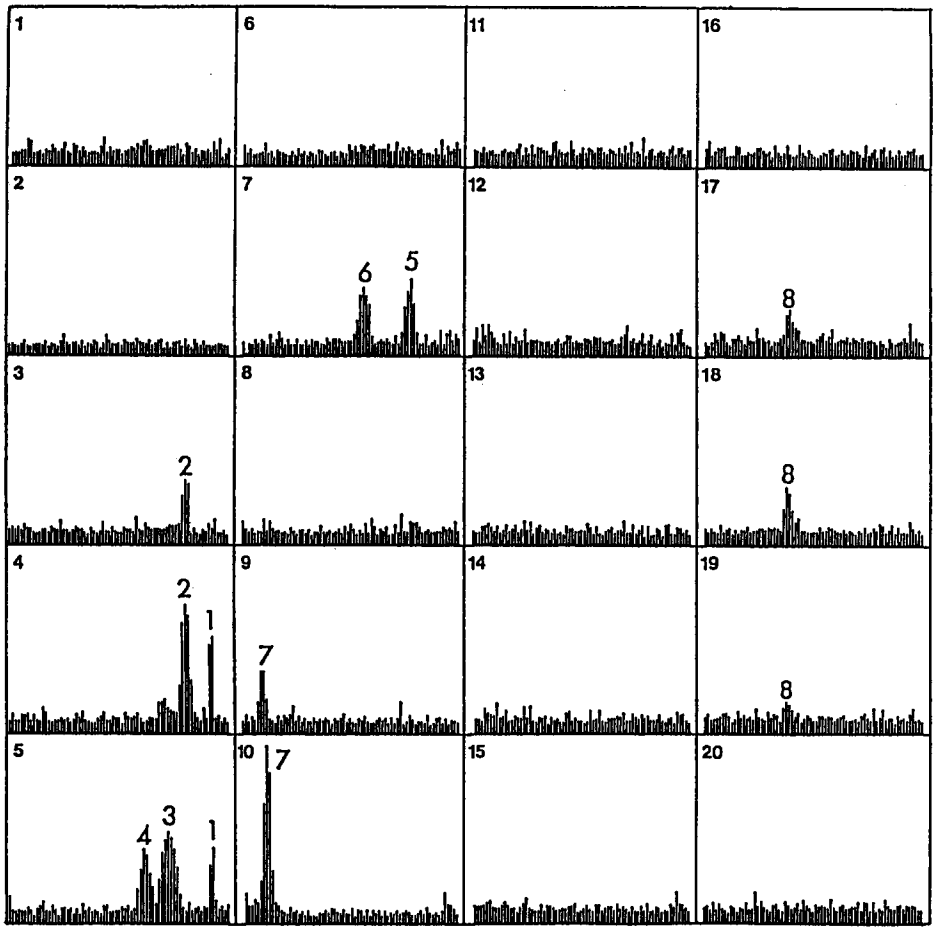

Fig. 11. FT-IR reconstructed chromatograms obtained after the HPLC deposits had been developed on the alumina TLC plate with chloroform–*n*-hexane (50:50). (From ref. 19 with permission).

14 shows the collection device. The disk is placed in a circular stainless-steel holder with a 5-mm high flange and locked by means of a constant spring at the side of the flange. The disk holder has an opening 8 mm wide, and is about 110 mm long near the edge at the base to allow an IR beam to pass through the holder with a crystal in place. The chromatographic effluent is brought onto the salt surface as the disk rotates. The turning speed of the disk can be changed between 2 and 198 min per revolution by the motor drive. After the chromatographic run, the salt disk, together with the holder, is removed from the holder mounting shaft and then transferred to a 3 × beam condenser that is equipped with a disk rotary assembly whose construction is the same as that outlined above. The disk is rotated once again with the identical drive unit and spectral data are continuously obtained by using GC–FT-IR software.

To illustrate the performance of this device, a separation of polymer additives was performed on a 20 cm × 0.53 mm I.D. fused-silica size-exclusion chromato-

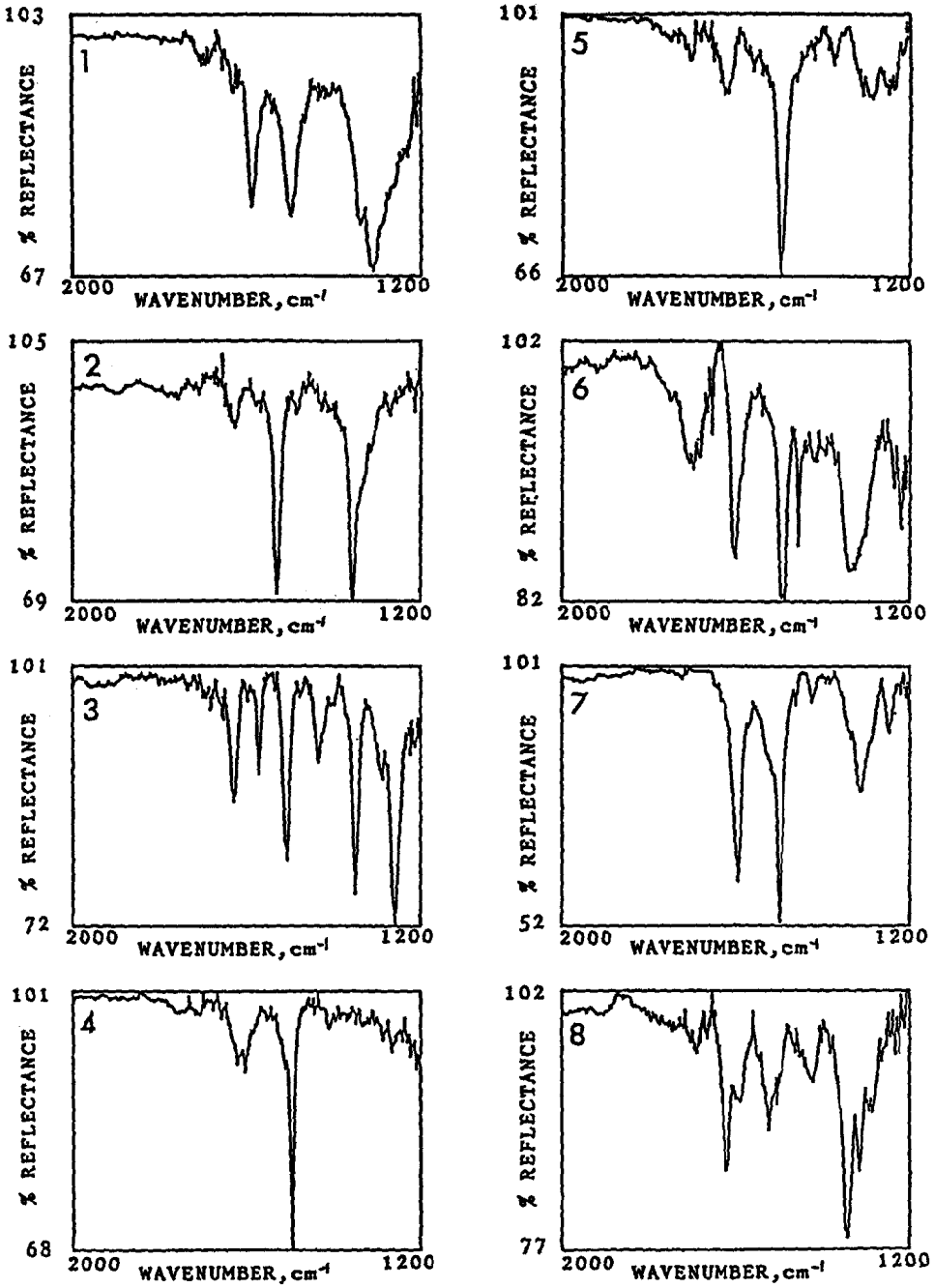

Fig. 12. Filed IR spectra of the components of the test mixture in Fig. 11. (From ref. 19 with permission.)

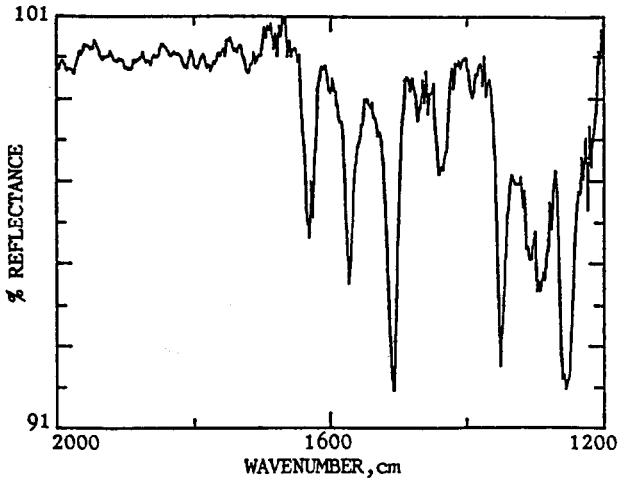

Fig. 13. Diffuse-reflectance IR spectrum of *o*-nitroaniline for a 50-ng amount collected on an alumina TLC plate.

graphic (SEC) column with tetrahydrofuran as the mobile phase[21]. The resulting Gram–Schmidt reconstructed chromatogram for 6 μg injected is shown in Fig. 15. The high signal-to-noise ratio confirms that the detection limit by this technique will be considerably lower than the amount injected (60 ng). Representative IR spectra are shown in Fig. 16.

OTHER APPROACHES IN MICROCOLUMN HPLC–FT-IR

Various other promising approaches in microcolumn HPLC–FT-IR can be considered.

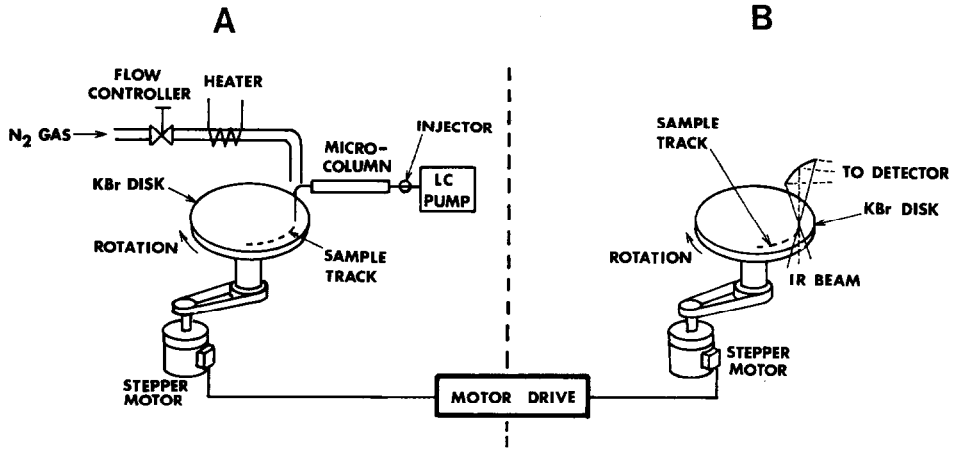

Fig. 14. Schematic diagram showing two operating modes of the interface. (A) Collection of the effluent as a continuous band on a potassium bromide disk; (B) measurements of spectral data with the device in the rear sample compartment of a JEOL FTIR spectrometer. (From ref. 20 with permission.)

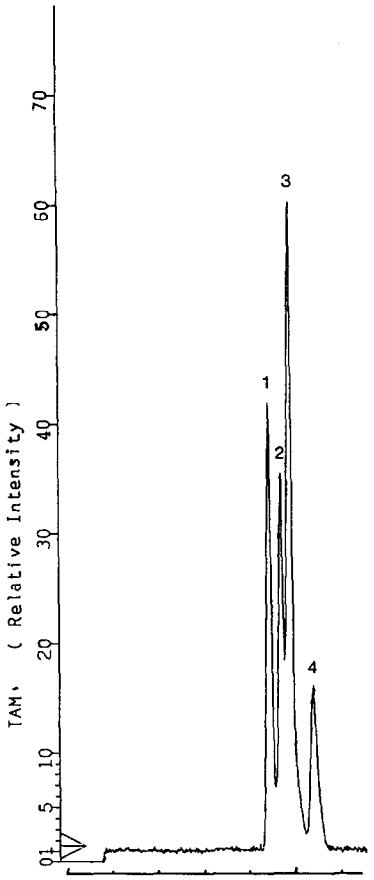

Fig. 15. Chromatogram of microcolumn HPLC–FT-IR separation of a synthetic polymer additive mixture[21]. Peaks: 1 = Irganox 1076; 2 = Santonox; 3 = Cyasorb UV-531; 4 = Cyasorb UV-9 (6 μg each). Column, 20 cm × 0.53 mm I.D. with SEC packing; mobile phase, tetrahydrofuran; flow-rate, 2.67 μl/min. From ref. 21.

An interface designed for the measurement of diffuse-reflectance spectra of peaks eluting from microcolumns was constructed by Conroy *et al.*[22]. This interface was based on the principle developed previously for conventional HPLC[24], and consists of a carousel containing 180 small cups filled with powdered potassium chloride. The effluent from a microcolumn (0.5 and 1 mm I.D.) was dropped into the cups. A drop monitor was mounted at the end of the transfer tube, which allowed only one drop to be collected in each cup. After each drop fell, the carousel was rotated using a stepper motor so that the next cup moved into place to receive the next drop. The carousel was preheated under an IR lamp so that the solvent evaporated rapidly and the solute was deposited. After deposition, the diffuse-reflectance spectrum of each deposit was measured. A reasonably high signal-to-noise ratio spectrum was obtained for 10 ng of 4-chloronitrobenzene eluted from 50 cm × 1 mm I.D. microcolumn packed with 10-μm silica by using 2% methanol in *n*-hexane.

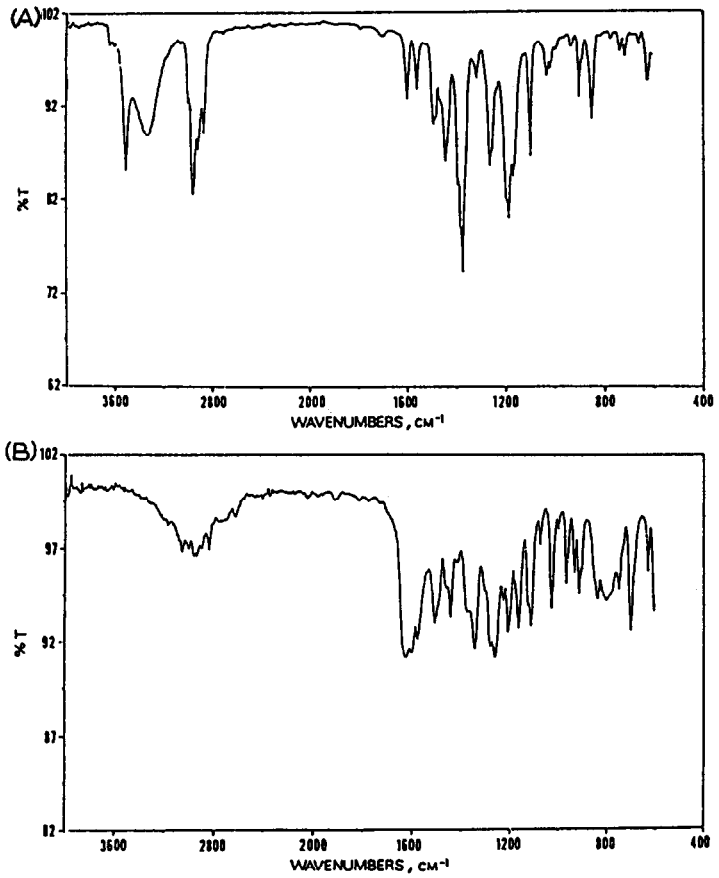

Fig. 16. IR spectra of (A) peak 2 in Fig. 15 and (B) peak 4 in Fig. 15. From ref. 21.

Kalasinsky *et al.*[23] developed an interface that incorporates a post-column reaction to obtain diffuse-reflectance spectra of aqueous reversed-phase HPLC eluates. Water was removed from aqueous solvents by an acid-catalysed reaction with 2,2-methoxypropane to produce methanol and acetone, which can be readily evaporated before deposition on powered potassium chloride. This interface worked well for mobile phases containing as much as 80% water.

Castles *et al.*[25] adopted an ultrasonic nebulization technique combined with a vacuum collection station as an interface between the microcolumn and the diffuse-reflectance IR spectrometer. The inherent desolvation that occurs on nebulization of the reversed-phase eluent from microcolumn HPLC, together with the vacuum collection system and the diamond powder substrate, made possible the continuous collection of the solutes on the substrate held on a linear transport device. IR spectra of the compounds separated by reversed-phase HPLC were measured after deposition. The detection limits were relatively higher, however.

More recently, Gagel and Biemann[26,27] reported an impressive and promising

method for the continuous recording of IR spectra of components separated by microcolumn HPLC. By means of a heated gas nebulizer, the effluent from a microcolumn was sprayed onto and evaporated from a rotating aluminum disk that had a reflective surface. This is a similar concept to the thermospray interface developed for HPLC–MS interfacing. After deposition, the solutes were analysed by rotating the disk in the sample compartment of an FT-IR spectrometer, reflection–absorption spectra being continuously collected. The analysis of closely eluting isomers and the measurement of a spectrum from a 31-ng injection of phenanthroquinone separated by reversed-phase HPLC were demonstrated.

Although microcolumn HPLC separations were not tried in the interface evaluation, two promising methods in reversed-phase HPLC–FT-IR have recently been reported by Taylor and co-workers[28,29] and Robertson *et al.*[30]. The former used the flow-cell approach and the latter the solvent-elimination approach.

In the former approach, an interface device based on the post-column extraction of solutes into a suitable analyte fluid was constructed. Solutes from reversed-phase HPLC separations are extracted into carbon tetrachloride as an IR-transparent solvent and then an FT-IR spectrometer measures the spectra in an efficient, real-time fashion. In this concept, the combined method involves solute extraction in an aqueous–organic segmented stream, separation of the aqueous and organic phases and inspection of the organic effluent through a flow-cell interface. Greater potential may be possible if this concept can be applied to a microcolumn HPLC–FT-IR interface.

MAGIC ("monodispersive aerosol generation interface combining") was originally developed by Willoughby and Browner[31] and the interface technique has recently become commercially available for HPLC–MS interfacing. Robertson *et al.*[30] first reported the use of this MAGIC interface for conventional reversed-phase HPLC–FT-IR and demonstrated the potential of this technique for the further development of the HPLC–FT-IR solvent-elimination approach. If one could use microcolumn HPLC instead of conventional HPLC for this MAGIC interface evaluation, the results could be excellent.

It is interesting at this point to re-emphasize that the interface techniques which are commercially available for HPLC–MS seems to be applicable to HPLC–FT-IR, so in the future we should have a unified interface device for combined chromatography–spectrometry in a similar fashion to that proposed by Haefner *et al.*[32] for combination with FT-IR.

REFERENCES

1 P. R. Griffiths, S. L. Pentoney, Jr., A. Giorgetti and K. H. Shafer, *Anal. Chem.*, 58 (1986) 1394A.
2 K. Jinno, in E. S. Yeung (Editor), *Detectors for Liquid Chromatography*, Wiley, New York, 1986, Ch. 3.
3 P. R. Griffiths and C. M. Conroy, *Adv. Chromatogr.*, 25 (1986) 105.
4 K. Jinno, *Chromatographia*, 23 (1987) 55.
5 C. C. Johnson and L. T. Taylor, *Anal. Chem.*, 56 (1984) 2642.
6 K. Jinno, C. Fujimoto and S. Nakanishi, *Chromatographia*, 20 (1985) 279.
7 C. Fujimoto and K. Jinno, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 374.
8 C. Fujimoto, G. Uematsu and K. Jinno, *Chromatographia*, 20 (1985) 112.
9 K. Jinno, C. Fujimoto and G. Uematsu, *Am. Lab.*, 16, No. 2 (1984) 39.
10 K. Jinno and C. Fujimoto, *J. Liq. Chromatogr.*, 7 (1984) 2059.
11 C. Fujimoto and K. Jinno, *Chromatographia*, 17 (1983) 259.
12 L. T. Taylor, *J. Chromatogr. Sci.*, 23 (1985) 265.

13 K. Jinno, C. Fujimoto and Y. Hirata, *Appl. Spectrosc.*, 36 (1980) 313.
14 K. Jinno, C. Fujimoto and D. Ishii, *J. Chromatogr.*, 239 (1982) 625.
15 K. Jinno and C. Fujimoto, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 4 (1981) 532.
16 C. Fujimoto, K. Jinno and Y. Hirata, *J. Chromatogr.*, 258 (1983) 81.
17 C. Fujimoto, T. Oosuka and K. Jinno, *Anal. Chim. Acta*, 178 (1985) 159.
18 C. Fujimoto, T. Morita and K. Jinno, *J. Chromatogr.*, 438 (1988) 329.
19 C. Fujimoto, T. Morita, K. Jinno and K. Shafer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1988) 810.
20 C. Fujimoto, T. Morita, K. Jinno and S. Ochiai, *Chromatographia*, 23 (1987) 512.
21 C. Fujimoto and K. Jinno, *Trends Anal. Chem.*, 8 (1989) 90.
22 C. M. Conroy, P. R. Griffiths and K. Jinno, *Anal. Chem.*, 572 (1985) 822.
23 K. S. Kalasinsky, J. A. S. Smith and V. F. Kalasinsky, *Anal. Chem.*, 57 (1985) 1969.
24 D. T. Kuehl and P. R. Griffiths, *Anal. Chem.*, 52 (1980) 1394.
25 M. A. Castles, L.V. Azzaraga and L. A. Carreria, *Appl. Spectrosc.*, 40 (1986) 673.
26 J. J. Gagel and K. Biemann, *Anal. Chem.*, 58 (1986) 2184.
27 J. J. Gagel and K. Biemann, *Anal. Chem.*, 59 (1987) 1266.
28 J. W. Hellgeth and L. T. Taylor, *Anal. Chem.*, 59 (1987) 295.
29 S. Shah and L. T. Taylor, *LC·GC*, 7 (1989) 340.
30 R. M. Robertson, J. A. deHaseth, J. D. Kirk and R. F. Browner, *Appl. Spectrosc.*, 42 (1988) 1365.
31 R. C. Willoughby and R. F. Browner, *Anal. Chem.*, 56 (1984) 2626.
32 A. M. Haefner, K. L. Norton, P. R. Griffiths, S. Bourne and R. Gurbelo, *Anal. Chem.*, 60 (1988) 2441.