Journal of Chromatography, 552 (1991) 635–647
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 2174

Spray jet assembly interface for the coupling of reversed-phase narrow-bore liquid chromatography and Fourier transform infrared spectrometry

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ABSTRACT

Liquid chromatography was coupled to Fourier transform infrared spectrometry via solvent elimination prior to infrared detection. The method allows the immobilization of analytes separated by reversed-phase liquid chromatography. Using a spray jet assembly, the effluent from a narrow-bore reversed-phase liquid chromatography column was continuously sprayed onto a linearly moving substrate suitable for infrared detection. The deposited compounds were analyzed by Fourier transform infrared microscopy. Transmission measurement using zinc selenide as substrate appears to be preferable to measurement in the reflection mode on an aluminum surface. With polycyclic hydrocarbons and quinones as model compounds, it was shown that the chromatographic separation is hardly affected during the immobilization process. The identification limits for these compounds were 10–20 ng. Aqueous methanol liquid chromatography eluents containing up to about 20% water can be handled.

INTRODUCTION

Hybrid techniques that combine chromatography with mass spectrometry (MS) and Fourier transform infrared spectrometry (FT-IR) have been primarily developed to permit unambiguous identification of sample constituents. Today MS is the most commonly applied technique for characterizing chromatographic peaks. In this respect FT-IR may be a useful alternative and complementary identification method, particularly for the analysis of structural isomers [1]. In gas chromatography (GC) the successful coupling to FT-IR [2] has led to the commercial availability of the equipment concerned. This is not the stage of development for liquid chromatography (LC) [3–5] in which, as in supercritical fluid chromatography (SFC) [6] and thin-layer chromatography (TLC) [7–9], interfacing to FT-IR has not yet reached an advanced state of sophistication.

0021-9673/91/$03.50

In the simples LC–FT-IR set-up, the column effluent is led directly through a flow cell, allowing continuous IR analysis [10]. However, this type of detection has several limitations imposed by the mobile phase. Most common LC solvents have intense absorption bands in the mid-IR region, necessitating flow-cell path lengths as short as 100 μm (or even less when the mobile phase is aqueous), which strongly limits the sensitivity of on-line IR detection. Furthermore, because these absorptions take up wide regions of the spectrum, the obtainable spectral information is reduced.

The preferred method to circumvent solvent interferences is the elimination of the solvent prior to the IR measurement of the solute. This involves an interface which evaporates the mobile phase and deposits the analytes onto a substrate suitable for IR detection. The LC chromatogram is stored and the deposited analytes can be examined over a longer period of time. For instance, after a rapid screening of the substrate applying low resolution and only a few scans per spectrum, high-resolution spectra with a high signal-to-noise ratio can be recorded for a few interesting parts of the chromatogram.

Applying normal-phase (NP) LC, the solvent elimination approach has been fairly successful [11,12], because of the high volatility of most organic solvents. Moreover, conventional IR sampling media can be used. In reversed-phase (RP) LC the use of aqueous eluents complicates depostion, because water has a relatively low volatility and it readily dissolves alkali halogenides. Unfortunately, in practice by far the most LC separations are based on RP-LC.

In the literature several solvent elimination interfaces for RP-LC–FT-IR, mostly modifications of NP-LC–FT-IR interfaces, are described. Conroy and co-workers [13,14] reported the on-line extraction of the RP-LC effluent with dichloromethane, which was subsequently deposited onto potassium chloride, kept in a train of sample cups suitable for diffuse reflection infrared detection (DRIFT). Kalasinsky and co-workers [15,16] used the acid-catalyzed reaction of 2,2-dimethoxypropane with water for the conversion of the non-volatile water in the mobile phase to the volatile methanol and acetone, thereby facilitating deposition on potassium chloride and analysis by DRIFT. The buffer memory technique was made appropriate for RP-LC by Fujimoto *et al.* [17], who used a stainless-steel wire net (SSWN) instead of a potassium bromide crystal as sampling medium. The aqueous effluent from a microcolumn is deposited onto the SSWN, from which the solvent is eliminated by a heated nitrogen gas flow. The sample components are partly suspended between the metal meshing, and analysis can be performed in the transmission mode. An impressive method for the coupling of narrow-bore RP-LC to FT-IR applicable for gradients containing up to 55% water in methanol is presented by Gagel and Biemann [18]. The column effluent is nebulized by nitrogen gas in a LC mixing tee and directed to a rotating reflective aluminum disk through a syringe needle which is enveloped by a heated nitrogen gas flow. After deposition, reflection–absortpion spectra are recorded. In their paper, the spectrum of a 31-ng injection of phenanthrenequinone is shown, indicating a considerable improvement in sensitivity over the RP-LC–FT-IR systems discussed above, which report detection limits in the (sub)microgram range. The MAGIC (monodisperse aerosol generation) interface, orginally developed for MS [19], has been applied by Robertson and co-workers [20,21] for FT-IR. Mobile phases containing between 0 and 100% water at a flow-rate of 0.3 ml/min could be handled without heating the effluent. Buffers can also be applied, although spectral subtrac-

tion remains necessary. This method seems promising, but until now only very few FT-IR chromatograms have been shown, and microgram amounts of compound have to be injected to obtain identifiable spectra.

To accomplish sensitive IR detection of deposited analytes, FT-IR microscopy is the technique of choice, as pointed out by Fraser *et al.* [22]. In the same paper a solvent elimination device for the coupling of NP-LC and FT-IR is described. This device is similar to the spray jet assembly recently used for the coupling of column LC to TLC [23,24]. In the present paper the spray jet assembly is presented as an interface for RP-LC–FT-IR. It allows the continuous deposition of the effluent from a narrow-bore RP-LC column onto the surface of a linearly moving substrate. After deposition, the immobilized chromatogram was analyzed by translating the substrate under an FT-IR microscope while spectra were being collected. The goal of this study was to demonstrate that chromatographic resolution is maintained during immobilization and that identifiable spectra of low amounts (10–20 ng injected) of model compounds can be obtained.

EXPERIMENTAL

A schematic of the LC interface set-up and the construction of the interface is shown in Fig. 1.

Chromatography

A laboratory-made syringe pump or an LDC/Milton Roy (Riviera Beach, FL, U.S.A.) microbore pump were used with a laboratory-made injection valve with an internal loop of 1.9 μl. During flow-injection experiments a Valco (Houston, TX, U.S.A.) injection valve with a 10-μl loop was used. Separations were performed on a

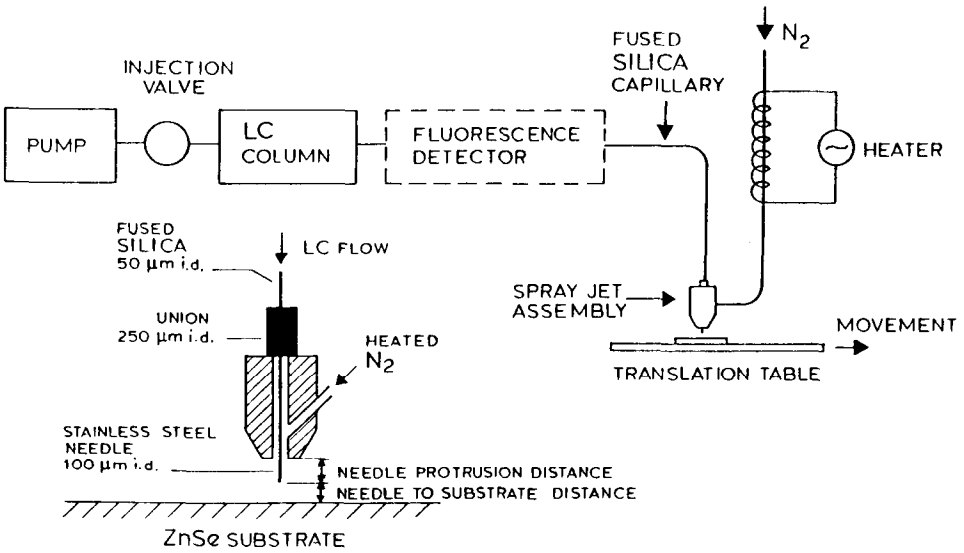

Fig. 1. Schematic of the chromatographic set-up and the interface. The insert shows the construction of the spray jet assembly.

170 mm × 1.1 mm I.D. column packed with 5-μm Rosil C_{18} (Research Separations Labs., Eke, Belgium) or a 250 mm × 1.0 mm I.D. column packed with 5-μm Adsorbosphere C_{18} (Alltech, Zwijndrecht, The Netherlands) at a flow-rate of 20 μl/min with methanol–water (95:5 or 80:20, v/v) as mobile phase.

Spectroscopy

IR data were obtained by using a Bruker (Karlsruhe, Germany) IFS-85 FT-IR spectrometer equipped with a Bruker A590 FT-IR microscope. The microscope, which contained a 16× Cassegrainian lens and a narrow-range mercury–cadmium–telluride (MCT) detector, was used in both the transmission and reflection modes. The aperture was rectangular and of adjustable size. Normally, 128 scans per spectrum were coadded at 8 cm^{-1} resolution. Background spectra were made using blank spots on the substrate surface in or adjacent to the deposition trace. Spectra were baseline-corrected.

Interface

The LC effluent was led to the spray jet assembly through a *ca.* 40 cm × 50 μm I.D. fused-silica capillary, which was connected with a union to a 100 μm I.D. and 475 μm O.D. stainless-steel syringe needle with a conically shaped tip. A nitrogen flow around the tip of the needle, which partially protruded from the assembly through a 600 or 900 μm I.D. nozzle, ensured deposition of the effluent and provided removal of the mobile phase. The evaporation was enhanced by an electric heater capable of heating the nitrogen up to 200°C. The distance between the needle tip and the deposition substrate and the protrusion distance of the needle from the assembly could both be varied. During the immobilization the substrate was moved by the translation table of a Camag (Muttenz, Switzerland) Linomat III applicator or a computer-controlled Bruker microscope X–Y stepper table. The latter was also used for the IR scanning of chromatograms.

Fluorescence detection

For on-line detection of the polycyclic aromatic hydrocarbons (PAHs), a Varian (Walnut Creek, CA, U.S.A.) Fluorichrom fluorescence detector adapted in the laboratory for use in micro LC was applied. Excitation was achieved with a deuterium lamp; the light was passed through a 10-mm path length cuvette filled with 25% (w/v) nickelsulphate and 40% (w/v) cobaltsulphate in water and a 2-mm Schott UG11 glass band filter. In this way a spectral window was obtained ranging from 255 to 368 nm. On the emission side there was a 4-mm Schott GG13 glass cut-off filter. Fluorescence scanning of the immobilized compounds was performed with a Carl Zeiss (Oberkochen, Germany) densitometer operating in the fluorescence mode. Excitation was achieved with a 48-W high-pressure mercury lamp at 313 nm using an M4Q-III prism monochromator. The emitted light of the compounds was passed through an optical filter, which rejected light with a wavelength shorter than 360 nm.

Materials

The model compounds fluoranthene (FLT), acenaphthenequinone, phenanthrenequinone (all from Aldrich, Milwaukee, WI, U.S.A.), pyrene, (PYR) (EGA-Chemie, Steinheim, Germany), benzo[*a*]anthracene (B[a]A) (Rütgerswerke, Castrop,

Germany) and benzo[*k*]fluoranthene (B[k]F) (Radiant Dyes Wermelskirchen, Germany) were used as received. Methanol (HPLC quality) was obtained from Baker (Deventer, The Netherlands). The water used was either dionized and distilled twice or of Milli-Q quality.

As deposition medium a 50 mm × 10 mm × 3 mm zinc selenide prism (Barnes Analytical, Stamford, CT, U.S.A.) or a 38 mm × 45 mm aluminum-coated mirror (Perkin–Elmer, Norwalk, CT, U.S.A.) was used.

FT-IR analysis

The IR substrate fitted in a rectangular hole in an aluminum plate (10 cm × 10 cm) supported at the edges. The plate fitted in a laboratory-made extension of the microscope table and protruded under a stand holding the spray jet assembly. Next, upon injection of a sample on the RP-LC column, the table started moving at a computer-controlled constant speed and the chromatogram was immobilized. After deposition, the entire aluminum plate, including the substrate with the immobilized chromatogram, was transferred to the microscope beam area and subsequently scanned, under computer control. Scanning parameters and the step width could be selected interactively and the scanning could be interrupted if refocusing or readjustment was required. After the collection of all chromatogram IR data was completed, the spectra were baseline-corrected and if required a three-dimensional stack plot of the chromatogram was produced.

RESULTS AND DISCUSSION

Interface parameters

The spray jet assembly previously developed to couple column LC to TLC [23,24] was used to deposit the RP-LC effluent onto materials suitable for IR analysis. Specific optimization of the device was needed to obtain sensitive IR detection and to ensure a minimum loss of chromatographic resolution during the deposition process. A high IR sensitivity is achieved by concentrating the analytes into an area which is as small as possible. Therefore, interface parameters affecting the deposition spot size were studied. PAHs, which can be very well detected by a densitometer operating in the fluorescence mode, were used as model compounds. Thus, information on depositions could be obtained relatively quickly and easily. Optimization of the interfacing was done by studying the deposition of a single PAH onto an aluminum-coated mirror using a flow-injection (no-column) set-up. The most important optimization aspects are discussed below.

Just before entering the spray jet assembly, the nitrogen gas could be heated by an electric heater. As expected, heating the nitrogen gas clearly enhanced the speed of evaporation of the mobile phase. For a mobile phase of methanol–water (95:5, v/v) efficient solvent removal was obtained at a nitrogen gas temperature of 100°C (range tested, 40–120°C). Sufficient evaporation of more aqueous eluents required higher nitrogen temperatures, *e.g.* for methanol containing 20% water 140°C was needed. Throughout the investigation no boiling of the effluent was observed, even when using nitrogen temperatures of 100°C or higher. This indicates that the real temperature at the needle tip was below 100°C, the cooling being primarily caused by the evaporation of mobile phase.

The distance which the needle protrudes from the jet influenced the formation of the spray and the efficiency of solvent removal. When the protrusion distance was too large (> 3 mm), the spray appeared as a narrow liquid stream which turned into fast-spreading droplets as soon as it hit the smooth substrate surface. At a small protrusion distance (< 0.5 mm) no well defined spray was formed and the effluent was sprinkled over a large area. Protrusion distances between 0.5 and 1 mm were found to give optimum deposition.

The influence of the distance between the needle tip and the deposition substrate on the peak width across and along the deposition trace is shown in Fig. 2. Upon decreasing the distance, the trace width was strongly reduced, which is favorable to sensitive IR analysis. However, decreasing the needle-to-substrate distance unfortunately caused some broadening of the deposition spot along the trace. Taking the two effects into account, a needle-to-substrate distance of 0.5 mm was chosen in further experiments.

The nitrogen flow causing nebulization of the effluent at the needle tip was controlled by the pressure at the nitrogen cylinder outlet. When the pressure was increased a distinct improvement of the trace width of the deposited spots was obtained, but simultaneously the peak width along the trace increased (Fig. 3). On the basis of the experimental data, 6 bar was selected as the best compromise, applying a mobile phase of methanol–water (95:5, v/v). It should be noted that, with a nitrogen pressure of below 4 bar, solvent removal was not sufficient. In this case too much of the effluent was still fluid when it reached the substrate surface, causing a poor deposition.

The results given so far were obtained with a 900 μm I.D. interface nozzle. Using a nozzle with an I.D. of 600 μm and leaving the other interface parameters unchanged, the width of the spots across the trace could be reduced to 100–300 μm. In further experiments the 600 μm I.D. nozzle was used.

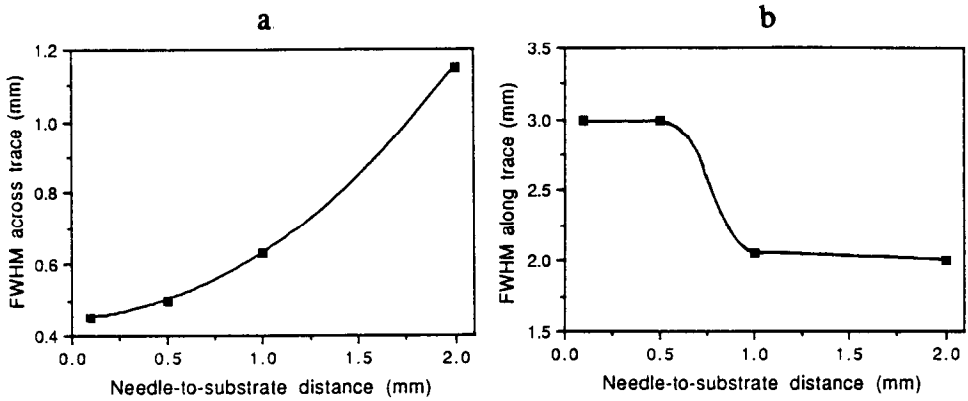

Fig. 2. Influence of the needle end-to-substrate distance on the peak width [(a) Across the trace and (b) along the trace] for the deposition of B[k]F onto an aluminum-coated mirror. FWHM is full width at half-maximum. Conditions: mobile phase, methanol–water (95:5, v/v); flow-rate, 20 μl/min; needle protrusion distance, 0.8 mm; nozzle I.D., 900 μm; nitrogen pressure, 8 bar; nitrogen temperature, 100°C; table speed, 2 mm/min.

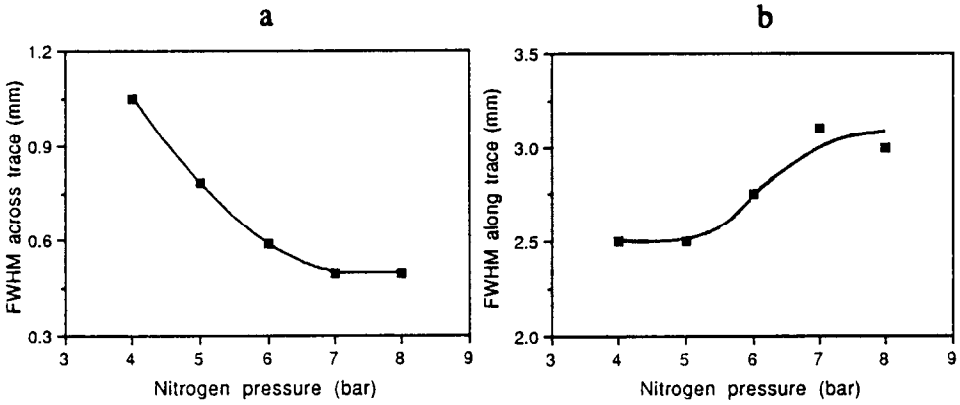

Fig. 3. Influence of the nitrogen gas pressure on the deposition of B[k]F onto an aluminum-coated mirror. (a) Scan across the trace; (b) scan along the trace. Conditions as in Fig. 2 except for needle-to-substrate distance, 0.5 mm.

Deposition substrate

A deposition substrate used in RP-LC–FT-IR should be composed of material which is compatible with aqueous mobile phases and which offers a large wave number area free of absorption bands. In our investigation two types of material were used, *viz.* an aluminum-coated mirror for reflectance–absorbance (R–A) measurements and a zinc selenide window for transmission measurements. Both materials have a hard and smooth surface which complicates deposition. Liquid that hits such a surface easily spreads as a result of the nitrogen flow. To prevent this spreading, solvents should be removed as fast as possible during the deposition. Apparently this requirement is met if the nitrogen gas temperature and pressure are high enough.

As regards deposition, the spots on the aluminum surface were larger than those on zinc selenide, and peaks as observed along the trace on the mirror showed irregularity in shape, which was not seen on zinc selenide. Apparently, the physical properties of the substrate affect the deposition. On the basis of these results zinc selenide is better suited as a deposition substrate than an aluminum-coated mirror. Even more important are the differences from an IR spectroscopic point of view. Transmission spectra of the compounds deposited onto zinc selenide showed better signal-to-noise ratio than the R–A spectra of the same amounts of material deposited onto aluminum. Moreover, zinc selenide allowed comparison with conventionally recorded potassium bromide spectra, whereas some of the R–A spectra were considerably distorted. This phenomenon is attributed to specular reflectance effects, probably the result of the limited film thickness and the very small particle size of the crystalline deposited material. During our study, Fuoco *et al.* [25] published a paper in which six different deposition substrates for SFC–FT-IR were compared. Using an aluminum mirror Fuoco *et al.* [25] also observed distortion of the spectra, depending on film thickness and microcrystallline nature of the deposited analytes. Furthermore, it was reported that best results were obtained with transmission measurements on zinc selenide. Considering this paper and our results, zinc selenide was chosen as the deposition substrate in further experiments.

Immobilization and detection of chromatograms

Fig. 4 shows the chromatogram of an LC separation recorded by fluorescence monitoring both on-line (Fig. 4a) and after immobilization zinc selenide (Fig. 4b); Table I gives the corresponding peak widths along the trace. The results show that the band broadening caused by the interfacing is acceptable. The widths of the spots across the trace of the immobilized analytes, determined visually by microscope, lay in the 100–150 μm range and tended to increase sligthly with retention time. These along trace–across trace dimensions of typically (10–15):1 indicate an elongated ellip-

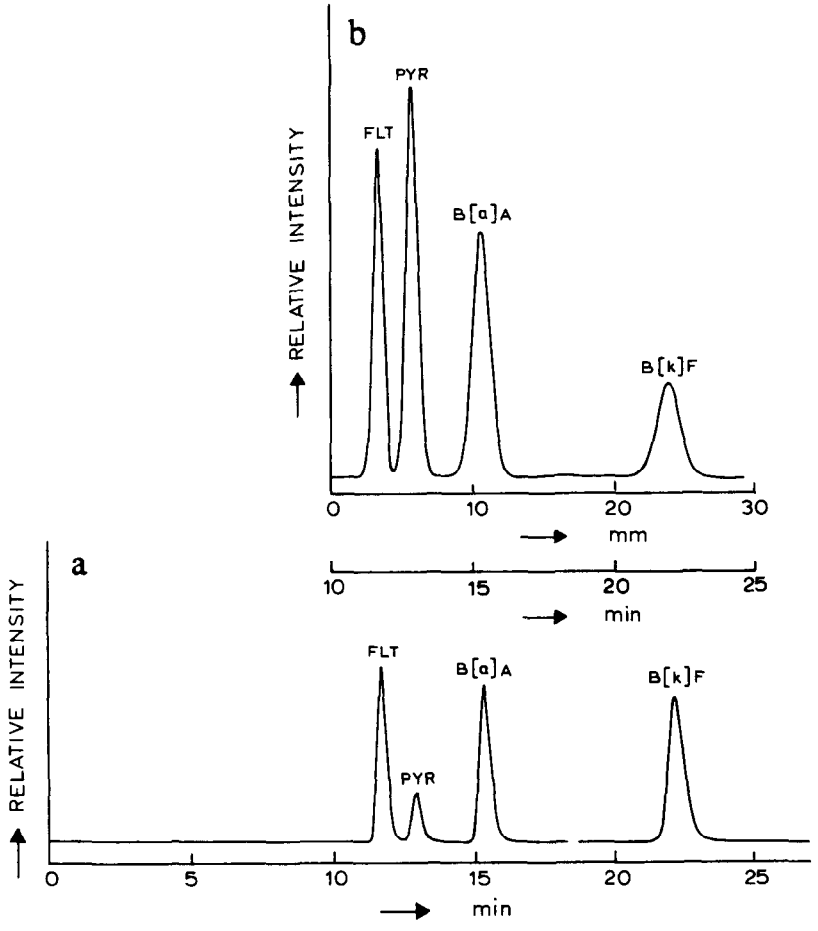

Fig. 4. Chromatogram of a test mixture of FLT, PYR, B[a]A and B[k]F (about 100 ng each; injection volume 1.9 μl). (a) On-line fluorescence detection (detector sensitivity: attenuation 50, except for B[k]F, 500). (b) Off-line fluorescence detection with densitometry after deposition onto zinc selenide. Conditions: column, 170 mm × 1.1 mm I.D. packed with Rosil C_{18}, 5 μm; mobile phase, methanol–water (95:5, v/v); flow-rate, 20 μl/min; needle protrusion distance, 0.5 mm; needle-to-substrate distance, 0.5 mm; nozzle I.D., 600 μm; nitrogen pressure, 6 bar; nitrogen temperature, 100°C; table speed, 2 mm/min. Note that the relative intensities of the two depicted chromatograms cannot be compared because of differences in excitation and emission conditions between on-line and off-line fluorescence detection (see Experimental section).

TABLE I

CHROMATOGRAPHIC BAND WIDTHS BEFORE (ON-LINE) AND AFTER (OFF-LINE) INTERFACING

FWHM is full width at half-maximum. Data taken from Fig. 4.

Compound	FWHM on-line (s)	FWHM off-line (mm)	FWHM off-line (s)
FLT	22	0.82	25
PYR	24	0.98	29
B[a]A	27	1.42	43
B[k]F	37	1.80	54

tical rather than circular spot, making a rectangular microscope aperture more appropriate than a round one for scanning the chromatogram. Fig. 5 shows a three-dimensional RP-LC–FT-IR plot for the separation of the same PAHs as in Fig. 4. The chromatogram was measured by scanning the substrate under the microscope with steps of 200 μm using a rectangular aperture. Between 10.4 and 21.4 mm, scans of the trace were produced only every 2 mm. The resulting spectra showed blank baselines. The spectra of individual PAHs are clearly recognizable in the chromatogram. The LC separation is well conserved and allows identification of the analytes by FT-IR. All spectra match well with their corresponding potassium bromide transmission spectra. The signal-to-noise ratio of the spectra is illustrated by Fig. 6A, showing a single spectrum of pyrene taken from the chromatogram. To determine the sensitivity of the method, various amounts of pyrene were injected and deposited.

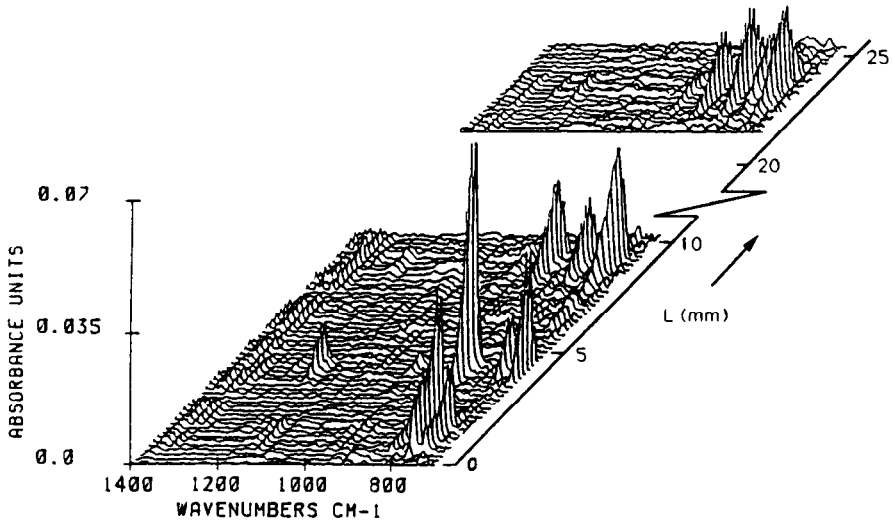

Fig. 5. Chromatogram of a test mixture (see Fig. 4) with IR detection. Conditions as in Fig. 4 except for column, 250 × 1.0 mm I.D. packed with Adsorbosphere C_{18} 5 μm; table speed, 1.8 mm/min. Spectroscopic conditions: distance between spectra, 200 μm; number of scans per spectrum, 128; resolution, 8 cm^{-1}; aperture size, 224 μm × 112 μm for FLT and PYR, 224 μm × 144 μm for B[a]A and B[k]F.

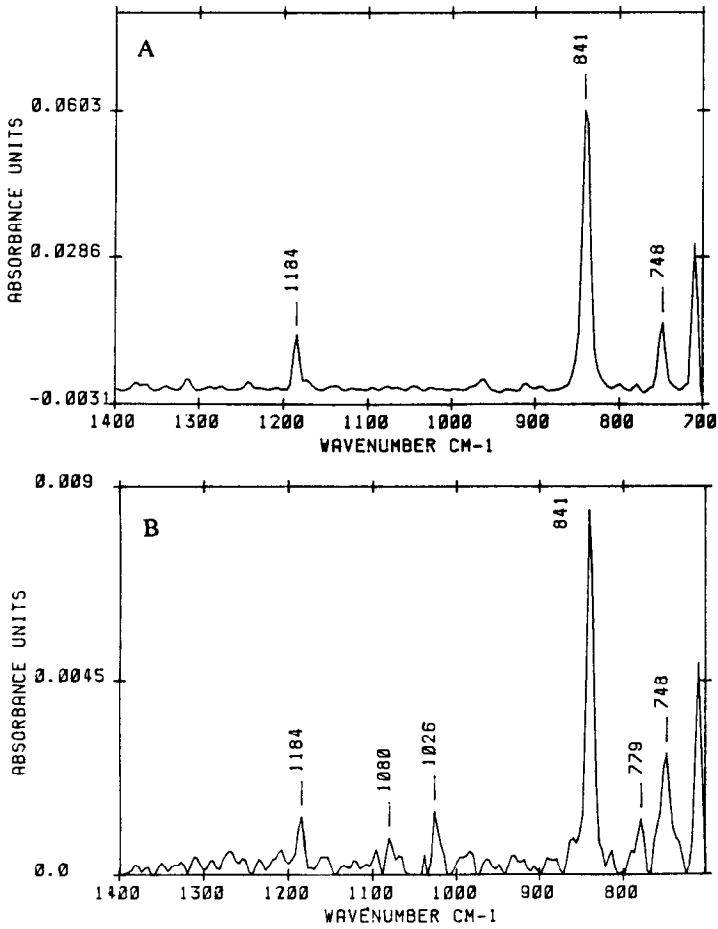

Fig. 6. RP-LC–FT-IR of pyrene. (A) Injection of 92 ng. (B) Injection of 13 ng. Conditions as in Fig. 5. except for aperture size, 200 μm × 80 μm, and number of scans, 1024 for (b).

Using 1024 scans, an identifiable spectrum of a 13-ng injection of pyrene was obtained (Fig. 6B).

To explore the potential of the interface, a mixture of acenaphthenequinone (AQ) and phenanthrenequinone (PQ) was separated on a C_{18} column with methanol–water (80:20, v/v) as mobile phase and the resulting chromatogram was immobilized on zinc selenide. AQ and PQ were selected because these quinones have been used as model compounds in several previously published studies on SFC–FT-IR and LC–FT-IR [18,22,25–27]. To obtain sufficient solvent removal the nitrogen gas temperature and pressure were raised to 140°C and 8 bar, respectively. No further optimization of the interface parameters was carried out for this mobile phase composition. The on-line recorded (not shown) baseline separation of AQ (t_R = 7.9 min) and PQ (t_R = 9.9 min) was retained on zinc selenide and the width of the spots across the trace was about 200 μm. Fig. 7 shows the spectra of deposited AQ and PQ after injecting

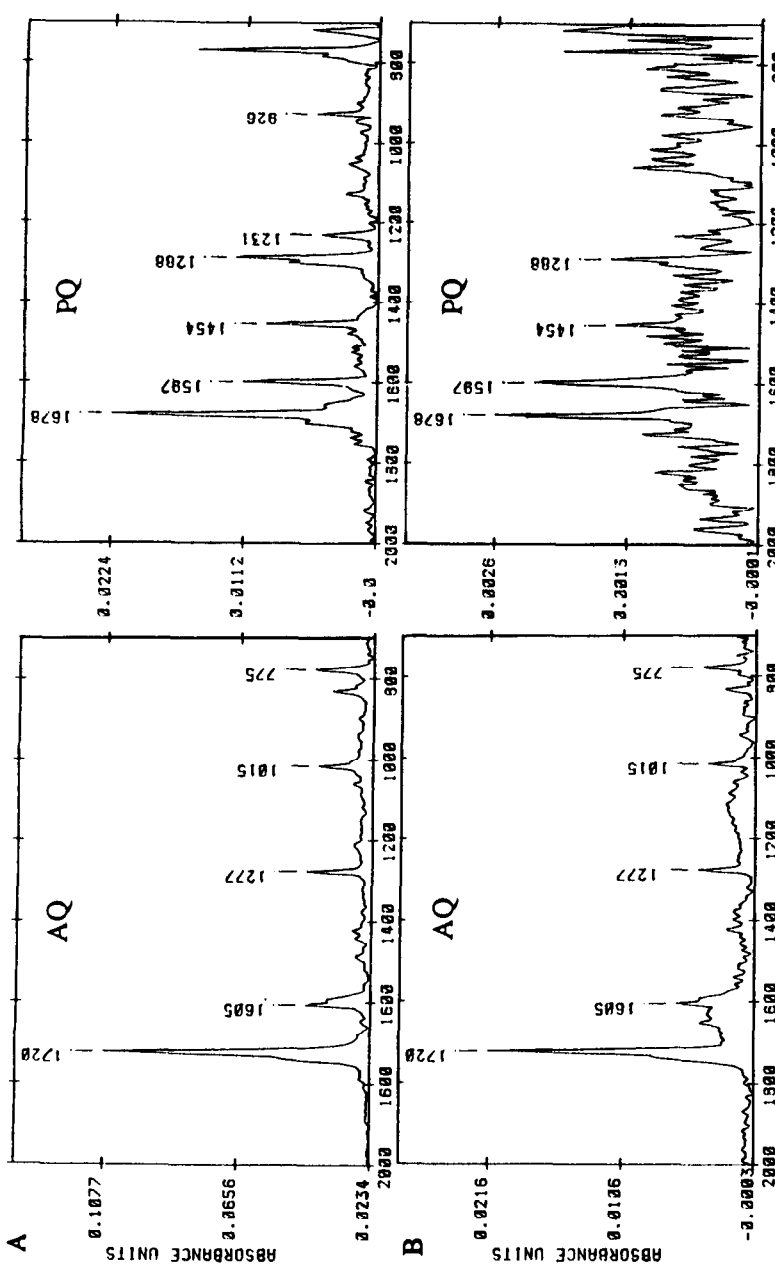

Fig. 7. RP-LC–FT-IR of AQ and PQ. (A) Injection of 154 ng AQ and 166 ng PQ. (B) Injection of 19 ng AQ and 21 ng PQ. Conditions: column, 250 mm × 1.0 mm I.D. packed with Adsorbosphere C_{18} 5 μm; mobile phase, methanol–water (80:20, v/v); flow-rate, 20 μl/min; needle protrusion distance, 0.5 mm; needle-to-substrate distance, 0.5 mm; nozzle I.D., 600 μm; nitrogen pressure, 8 bar; nitrogen temperature, 140°C. Spectroscopic conditions: resolution, 8 cm^{-1}. (A) Aperture size, 200 μm × 160 μm; number of scans, 128. (B) Aperture size, 200 μm × 65 μm; number of scans, 1024.

and separating two mixtures containing *ca.* 160 ng and *ca.* 20 ng per compound, respectively. It demonstrates that the interface is able to deposit quinones efficiently from methanol containing 20% water, and 20-ng amounts can still be identified.

CONCLUSION

The usefulness of a spray jet assembly as a relatively simple interface for RP-LC–FT-IR has been demonstrated using PAHs and quinones as model compounds. The chromatographic integrity of the LC separation is essentially maintained during the immobilization, and identifiable IR spectra of analytes can be obtained down to the 10–20 ng range. The main reasons for this good sensitivity are the deposition of the analytes as very narrow spots and the use of an IR microscope for beam condensing. The use of zinc selenide as IR substrate circumvents problems met in R–A FT-IR using aluminum mirrors and allows comparison with conventional potassium bromice transmission spectra.

Future research will concentrate on the handling of more polar compounds using mobile phases with a water content of over 20%. Quick evaporation of these more aqueous eluents will probably demand higher nitrogen gas temperatures. The higher polarity of the analytes could complicate deposition as well, because of the increased solubility of the compounds in an aqueous environment.

ACKNOWLEDGEMENT

The authors thank Dr. J. Gast of Bruker (Karlsruhe, Germany) for the loan of the microscopic X–Y stepper table and the rectangular microscope aperture used in this study.

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