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Review

Coupling of column liquid chromatography and Fourier transform infrared spectrometry

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Abstract

This paper provides an extensive overview of the literature on the coupling of column liquid chromatography (LC) and Fourier transform infrared spectrometry (FT-IR). Flow-cell-based FT-IR detection and early solvent-elimination interfaces for LC–FT-IR are discussed in brief. A comprehensive description is given of interface systems which use spraying to induce rapid eluent evaporation, and which essentially represent the state-of-the-art in LC–FT-IR. Fundamental aspects of FT-IR detection of deposited compounds are considered with special attention to the use of FT-IR microscopy. Finally, the interface systems suitable for reversed-phase LC are summarized and evaluated. The overview shows that solvent-elimination techniques can provide good sensitivity and enhanced spectral quality, and suggests that LC–FT-IR may well become a widely applicable technique for the unambiguous identification of trace-level sample constituents.

Keywords: Reviews; Fourier transform infrared spectrometry; Infrared spectrometry; Liquid chromatography–Fourier transform infrared spectrometry; Detection, LC; Interfaces, LC–FT-IR; Instrumentation

Contents

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1. Introduction

Most organic compounds have a large number of relatively narrow absorption bands in the mid-infrared (IR) spectral region. These absorptions are highly specific and can give detailed structural information about a particular compound. By itself, the entire IR spectrum of an organic compound provides a unique fingerprint, which can be readily distinguished from the absorption patterns of other compounds. This means that when reference spectra are available, most compounds can be unambiguously identified on the basis of their IR spectra. These features make IR spectrometry a potentially strong technique for the characterization of chromatographic peaks. However, compared with UV–visible absorbance, extinction coefficients in IR are rather low, and the analyte concentration needed for IR detection therefore often is large with respect to the concentration usually injected into a gas or liquid chromatograph. Furthermore, the direct coupling of a chromatographic instrument and an IR spectrometer generally requires the construction of special flow cells or the development of suitable interfaces.

IR detection in chromatography became feasible in the 1970s with the introduction of the Fourier transform (FT) technique [1]. Compared with dispersive spectrometers, FT-IR instruments show an enhanced sensitivity and a complete IR spectrum can be recorded within 1 s. The latter feature enables IR detection to be performed in an on-line mode. Today, the combination of gas chromatography (GC) and FT-IR is a well established technique and various systems are commercially available. Three essentially different types of GC–FT-IR interfaces can be distinguished [2]. In the most commonly used set-up, the GC column effluent flows through a heated light-pipe and vapour-phase spectra are collected in real time at 1-s intervals. Light-pipe-based systems are instrumentally simple, but due to the short residence time in the flow cell components should be present in the 10–100 ng range for useful spectra to be generated. This inherent sensitivity limitation has been addressed by the use of interfaces in which the chromatogram is stored on a low-temperature substrate prior to IR detection. One type of storage interface involves the use of matrix isolation spectrometry. Each GC peak is trapped in an argon matrix which is condensed on a continuously rotated metallic substrate held at 13 K. After the separation is complete, the argon track is rotated into the IR beam and reflection–absorption measurements are made. Identifiable spectra can be recorded for sub-ng amounts of analytes. In the alternative ''cryo-trapping'' interface, the compounds separated by GC are directly deposited on a liquid-nitrogen-cooled IR-transparent window which moves through an IR beam which is focused by microscope optics. The IR transmission of the window is measured continuously so that the analyte spectra are obtained within seconds after deposition. Minimum identifiable quantities of about 100 pg have been reported using this

technique [3]. The latter method has been combined with on-line solid-phase extraction (SPE) for the IR-based identification of constituents present in aqueous samples at the sub-μg/l level [4].

Compared with GC–FT-IR, the development of coupled column liquid chromatography–FT-IR (LC–FT-IR) has proceeded much slower, and its viability has even been questioned [5,6]. However, progress in interfacing techniques during the past 5–10 years has brought LC–FT-IR to a stage of real analytical utility and the first commercial interfaces were introduced [7,8]. In the earliest combinations of LC and FT-IR [9,10], flow cells were used in a fashion analogous to LC with on-line UV absorption detection. In 1979, interfacing difficulties related to the IR absorptions of the eluent prompted Kuehl and Griffiths [11] to develop the first useful solvent-elimination based LC–FT-IR system in which the eluent is evaporated prior to IR detection. Since then two approaches can be discerned in LC–FT-IR, viz., the flow-cell (or on-line) approach and the solvent-elimination (or semi on-line) approach. In the latter case, an interface is used which effects evaporation of the eluent and deposition of the analytes on a substrate suitable for IR detection. In the contemporary practice of LC–FT-IR both approaches are applied, but since the flow-cell procedure cannot get around the limitations posed by the presence of the eluent, it has developed into a special-purpose method with restricted applicability. Solvent-elimination techniques have shown to be much more versatile and to yield interference-free spectral information for considerably smaller amounts of analytes. In other words, if the objective of LC–FT-IR is the unambiguous identification of low-level constituents of complex mixtures, semi on-line coupling obviously is ''the way to go''.

To clarify the rationale of semi on-line LC–FT-IR, the general characteristics of flow-cell LC–FT-IR will be discussed in the next section; further details can be found in several review papers [12–15].

2. Flow cell LC–FT-IR

The simplest way to couple LC and FT-IR is to let the column effluent pass directly through a flow cell with IR-transparent windows. The IR transmission of the LC eluent is continuously monitored, and spectral data are collected on the fly and stored throughout the entire chromatographic run. During or after the run the spectra and/or IR chromatogram are computed, and absorption due to the eluent is subtracted. Band broadening caused by detection is easily minimized in a flow cell design.

Unfortunately, the invariably significant absorption of the incident IR radiation by the LC eluent leads to serious limitations of the flow cell approach. Firstly, analyte absorption bands may be completely obscured by the most intense eluent absorptions. In other words, in flow cell LC–FT-IR the spectral information that can be obtained is limited and depends on the window provided by the eluent used. Ill-considered subtraction of strong solvent bands may even lead to the erroneous conclusion that there is no absorption of the analyte in the corresponding spectral regions. Secondly, gradient elution cannot be applied because accurate spectral subtraction is virtually impossible when the composition of the eluent is changing. Thirdly, the signal-to-noise ratio is reduced at any wavelength where solvent absorption is appreciable. Finally, the path length of the flow cell has to be limited in order to ensure that sufficient energy reaches the detector. For organic solvents the path length rarely exceeds 0.5 mm which, bearing in mind Beer's law, seriously reduces analyte detectability. For aqueous eluents the largest tolerable path length is even much shorter, i.e., about 30 μm, which implies that a practical combination of reversed-phase (RP) LC and FT-IR via a flow cell is quite unrealistic. Another drawback of flow cell measurements is that the use of signal averaging, which can be exploited to improve the signal-to-noise ratio, is limited due to the short analysis time available under dynamic conditions.

In order to minimize the problems associated with eluent absorption, the choice of solvents in flow cell LC–FT-IR is generally limited to chlorinated alkanes or deuterated solvents. These solvents leave relatively wide windows in the spectrum, although even these inevitably obscure part of the spectral fingerprint region (1200–700 cm^{-1}). The use of a small percentage of a more polar solvent in the eluent, as is quite common in normal-phase LC, may already prohibit effective detection. Due to the small optical path length, the absolute detection limits in on-line

LC–FT-IR are in the (high) μg range, which frequently implies that analyte concentrations of 1–10 g/l have to be injected to obtain identifiable spectra. On the one hand, such high analyte levels may lead to column overload – on the other, they are not frequently encountered in real-life samples.

Despite the described limitations and restrictions, flow cell LC–FT-IR has been and still is applied as a simple and low-cost method to obtain structural information about major constituents of mixtures [12,13]. In recent years, research in on-line FT-IR detection was mainly confined to size-exclusion chromatography (SEC) [16–19] and flow-injection analysis (FIA) [20–30], which generally are more suited to flow cell measurements than common LC. In SEC, the type of eluent often is not essential for the separation process so that a solvent appropriate for IR detection can be selected without detrimental effects on the chromatographic resolution. Also, column capacities and sample concentrations are usually high and low detection limits are often not required. Furthermore, SEC–FT-IR frequently serves to characterize and quantify compositional changes throughout a polymer mass distribution. For this purpose, information from one or two particular spectral windows is often sufficient and acquisition of full spectra is not necessary.

Flow cell FIA–FT-IR systems have been described for the rapid quantification of principal components of simple mixtures [20,23–26]. In FIA, the FT-IR spectrometer is used as a selective and quantitative detector which monitors one analyte-specific absorption band. This means that, like in SEC, the choice of carrier solvent is less demanding: the solvent should not spectrally interfere with the marker band of the analyte. In flow cell FIA–FT-IR interesting improvements in both analyte detectability and compatibility with aqueous samples have been accomplished by Garrigues and co-workers [21,22,30], who applied on-line solid-phase extraction (SPE). Large volumes (100–500 ml) of water containing the pesticide carbaryl were preconcentrated on an SPE cartridge containing C_{18}-modified silica. After drying, the cartridge was desorbed with dichloromethane which was on-line monitored by FT-IR. Detection limits of 50–100 μg/l were achieved for carbaryl which is good for an IR-based technique. A similar system was used for the determination of caffeine in soft drinks [30]. DiNunzio [31] used the on-line SPE concept to allow flow cell FT-IR detection of compounds separated by reversed-phase LC. In his automated LC–SPE–FT-IR system (Fig. 1) the analytes of interest were trapped on several small SPE columns filled with a hydrophobic sorbent, after on-line dilution of the column effluent. The SPE columns were dried with nitrogen, and sequentially eluted with tetrachloromethane into an FT-IR flow cell. Sub-μg quantities of analyte could be detected, while micrograms were required to obtain identifiable spectra. The system was used

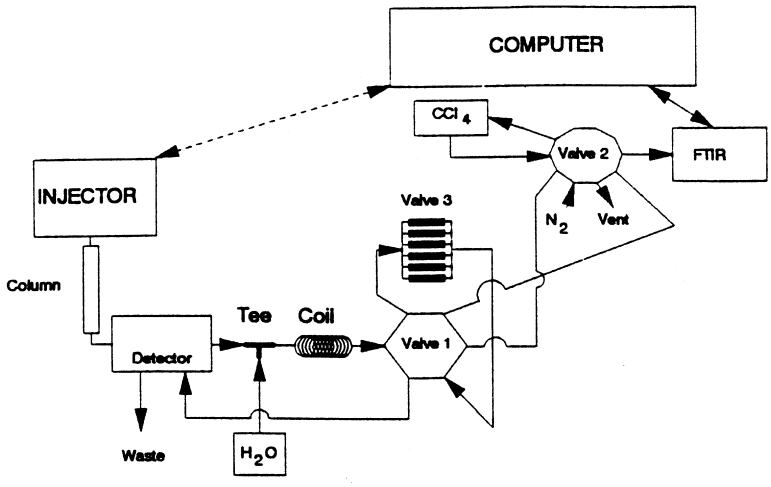

Fig. 1. Schematic of automated solid-phase extraction interface for flow cell LC–FT-IR [31]; valve 3 holds the SPE columns.

for the characterization of isomers and degradation products in pharmaceutical samples.

As has been outlined above, the major obstacle to the use of flow cell LC–FT-IR is the IR absorption of the eluent. An elegant solution to this problem would be the elimination of the eluent prior to the IR measurement of the analytes. This indirect approach involves the use of a solvent-evaporation interface that deposits the separated compounds on an IR-compatible substrate. Since the late 1970s, the semi on-line coupling of LC and FT-IR has been pursued by several research groups, which designed a variety of interface concepts. These systems will be discussed in the next sections, with a cursory description of the interfaces developed in an early stage.

The principal goal of FT-IR detection in LC is the identification (not quantification) of unknown compounds. Therefore, throughout this paper the analyte detectability of the various LC–FT-IR systems will be expressed as minimum identifiable mass and/or concentration (injected). That is, identification limits rather than detection limits will be given. Detection limits commonly are based on the most intense absorption band only, while an IR-based identification obviously requires several bands to be detected. According to the criterion formulated for residue analysis, at least six well-defined bands should be present in the IR spectrum in order to reliably identify a compound [32].

3. Early solvent-elimination interfaces

The aim of any solvent-elimination LC–FT-IR system is to sensitively acquire analyte spectra which are free from spectral interferences. This requires complete evaporation of the LC eluent, and deposition of the analytes in such a manner that proper IR detection is possible. After some less successful attempts [33], Kuehl and Griffiths [11,34] designed an adequately working interface for the coupling of conventional-size normal-phase (NP) LC and FT-IR. The interface consisted of a heated concentrator tube in which about 90% of the LC eluent were evaporated between the column outlet and a series of sample cups arranged in a carousel. The cups were filled with potassium chloride (KCl) powder which was used as substrate for diffuse reflectance IR analysis (DRIFT). The concentrated effluent was deposited (ca. 3 drops per cup) on the KCl surface and residual solvent was removed by passing nitrogen gas through the cups. The carousel rotated the cups into the FT-IR spectrometer where pure, identifiable spectra could be recorded for sub-μg amounts of analyte. Since with this system aqueous eluents could not be eliminated effectively and since water, of course, is not compatible with the KCl substrate, a modification was introduced to allow the use of RPLC [35]. Prior to entering the concentrator tube, the aqueous effluent was on-line extracted with dichloromethane. After on-line phase separation, the organic phase containing the extracted analytes was directed through the concentrator to the DRIFT carousel. The results obtained did not differ essentially from those obtained for NPLC. Conroy et al. [36] adopted the carousel-DRIFT method for use with narrow-bore NPLC by reducing the sample cup diameter from 4.5 to 2 mm. Because of the decreased flow-rate (20–50 μl/min), the concentrator could be omitted and the LC effluent dropped directly into the cups. Subsequent DRIFT analysis yielded identification limits in the 10–100 ng range.

Using a similar set-up, Kalasinsky et al. [37,38] coupled both narrow-bore NPLC and RPLC with DRIFT. The KCl deposition substrate was held either in a ''train'' of compartments or in a continuous trough. Aqueous eluents could be used by on-line conversion of the water into methanol and acetone via a reaction with 2,2′-dimethoxypropane (DMP) which was added post-column. To facilitate evaporation, the organic solvents were sprayed on the substrate with a simple nebulizer. The system could also be used for conventional-size LC by incorporating a flow splitter (Fig. 2) or a concentrator tube (cf. Ref. [11]). Typical identification limits obtained with these systems were 1–3 μg.

The early DRIFT-based LC–FT-IR work clearly demonstrated that after solvent elimination complete IR spectra could be recorded for LC-separated compounds with considerably better sensitivity than obtained by flow cell FT-IR detection. However, the mechanically complex DRIFT systems have some drawbacks. DRIFT is intrinsically very sensitive, but the detection performance is easily affected by small disturbances of the surface of the powdered KCl substrate, which may be induced by the deposition

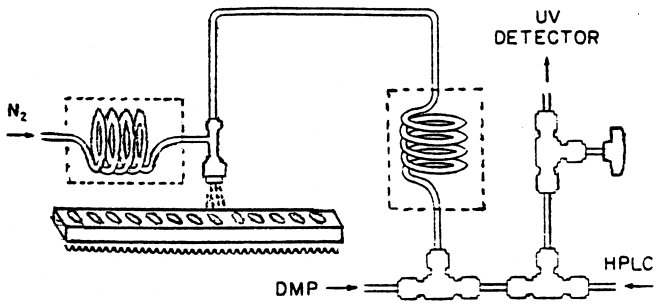

Fig. 2. Splitter-based LC–DRIFT system that uses 2,2-dimethoxypropane (DMP) for the handling of aqueous eluents [37,38].

process. KCl is very hygroscopic and the presence of water can therefore cause a major spectral interference. Furthermore, to avoid sample spreading, the substrate has to be held in (many) discrete cups which implies that the chromatogram is not continuously analysed but in fractions. The mandatory refilling of these cups after every chromatographic run was later denoted as ''excessively time-consuming'' and ''not acceptable'' [39].

In order to alleviate the problem of the evaporation of large eluent volumes, Jinno and co-workers [40–43] proposed the use of micro-LC columns in solvent-elimination LC–FT-IR. In their interface the effluent (typical flow-rate, 5 μl/min) from either a SEC or an NPLC column flowed down a stainless steel capillary to a continuously moving potassium bromide (KBr) plate. The small volume of volatile solvent was evaporated by a stream of heated nitrogen, leaving the analytes in a 2-mm narrow track on the plate. Subsequently the plate was scanned by FT-IR transmission spectroscopy using a 3× beam condenser. Jinno termed this method the ''buffer-memory'' technique which refers to the fact that the complete chromatogram is immobilized and stored on the substrate, allowing analysis over a longer period of time. The potential of this approach was illustrated by the analysis of one deposited chromatogram of a mixture of dithiocarbamate metal complexes by three spectroscopic techniques [43]. The analytes were separated on a silica micro-column, deposited on KBr and then first measured by FT-IR to obtain specific information on the ligands (Fig. 3). Subsequently, X-ray fluorescence (XRF) spectra of the complexes were recorded directly from the KBr plate by placing it in a sample holder of the XRF spectrometer, and moving it through the X-ray beam. In this way the metals could be selectively detected. Finally the analyte spots were scraped off the plate and directly inserted in the ion source of a mass spectrometer which yielded characteristic electron-impact spectra of the complexes.

A modification of the buffer-memory interface involved the replacement of the linearly moving KBr plate by a KBr disc that was rotated during deposition of the micro-LC effluent [44]. After the chromatographic run, the disc was transferred to the FT-IR sample compartment where IR transmission data were continuously acquired while rotating the disc (Fig. 4). This system has been reported to be commercially available from Jeol (Tokyo, Japan) [45], but no further experimental results have appeared in the literature. In order to permit the use of micro-RPLC, Fujimoto et al. [46] replaced the KBr plate by a stainless steel wire net. Because after deposition and drying the analytes were partly suspended in the metal meshes (15 μm), it was possible to record their transmission spectra, although intricate spectral anomalies were observed which appeared to depend on the eluent composition.

The buffer-memory technique showed the usefulness of the immobilization and storage of a continuous chromatogram on a flat substrate. Besides, it was considerably simpler than the DRIFT methods. However, in order to obtain good quality spectra, it was necessary to inject several micrograms of analyte. This often exceeded the sample capacity of the capillary columns and required unrealistically high analyte concentrations to be injected.

When using the buffer-memory manner of compound deposition on flat substrates, it is not possible

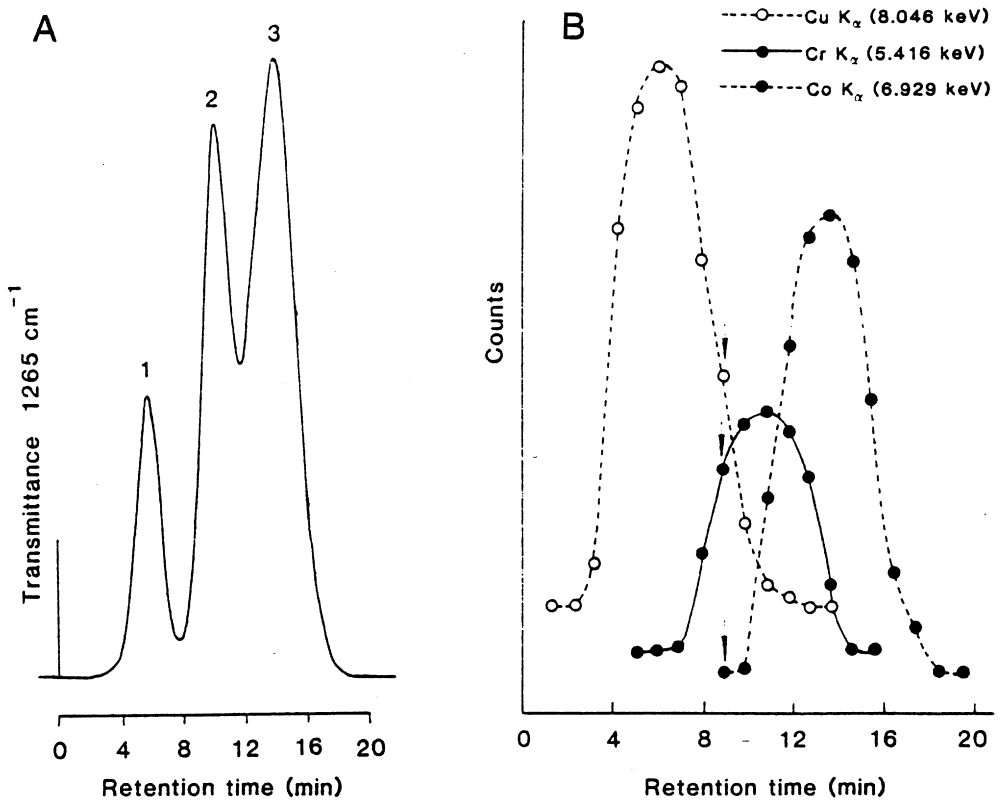

Fig. 3. (A) LC–FT-IR and (B) LC–XRF chromatograms of three dithiocarbamate complexes [45].

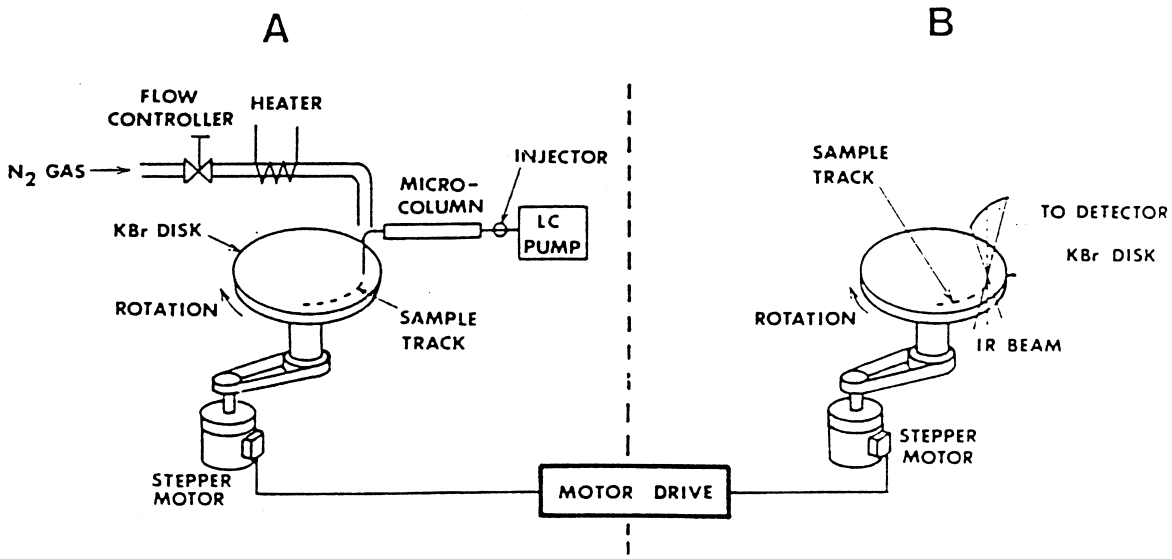

Fig. 4. Schematic of micro-LC–FT-IR system in which (A) the LC effluent is deposited on a rotating KBr disc and (B) IR spectral data are recorded from the deposited compounds [46].

to eliminate organic or aqueous eluents at flow-rates above 5 μl/min without the compounds becoming spread over too large an area of the substrate surface. To achieve a more viable coupling of LC and FT-IR, the use of solvent-elimination interfaces with enhanced evaporation power is essential. Ideally, the interface should be able to almost instantaneously evaporate the eluent, whether organic or aqueous, and to deposit the analytes as compact spots on a substrate that is easy to handle and clean, and can be used repeatedly. Since in on-line LC–MS the solvent-elimination problem is similar, it is not surprising that several LC–MS interface concepts have been applied to combine LC and FT-IR. At this point, however, a marked difference between the operation of MS and FT-IR interfaces should be noted. In an LC–MS interface the eluent is commonly nebulized into a divergent plume of small droplets in order to enhance solvent evaporation as much as possible. In LC–FT-IR, however, next to eluent evaporation the interface also should provide compound deposition into narrow spots, the latter aspect being extremely important because it largely determines the degree of extra band broadening and the IR sensitivity that can be achieved. Needless to say, there is a distinct challenge in the simultaneous execution of complete eluent evaporation and compact analyte deposition.

The solvent-elimination interfaces reported in the last decade all use some kind of spraying to induce rapid eluent evaporation. As will be described in the next section, LC–FT-IR systems have been developed that are able to directly eliminate aqueous eluents and some of these allow IR detection of (sub-) ng amounts of analyte.

4. Spray-type interfaces

In the more recent LC–FT-IR systems, interfaces are used that break up the LC eluent stream into small droplets to facilitate solvent evaporation. Some designs incorporate existing (commercial) equipment, while others have been built from scratch. Hitherto, two of these interfaces have been adapted by an instrument manufacturer and have appeared in the marketplace [7,8]. The following classification of the LC–FT-IR interfaces is primarily based on the applied method of solvent elimination and not on history, LC column dimensions [12,13] or substrate used.

4.1. Thermospray interface

In the thermospray interface (TSP) the LC eluent is led through a directly heated vaporizer tube. In the tube, part of the liquid evaporates to an expanding vapour which causes nebulization of the remaining effluent; as a result, a mist of desolvating droplets emerges from the end of the tube. When using the TSP in LC–MS, up to 2 ml/min of aqueous solvents can be introduced into the MS vacuum system. In the TSP-based LC–FT-IR systems reported so far, nebulization is performed at atmospheric pressure. Still, if the deposition substrate is heated, eluent flow-rates of 0.5–1 ml/min can be used.

In 1986, Griffiths and Conroy [47] reported preliminary results on the use of a TSP device for RPLC–FT-IR, but the interface was not described in detail. A mixture of phenol and three substituted phenols was separated on a C_{18}-bonded silica column with water–methanol (98:2, v/v) at 0.8 ml/min as eluent, and the analytes were deposited on diamond powder via a TSP. KCl powder could not be used as DRIFT substrate; because the TSP did not completely evaporate the eluent, the residual water affected the KCl and obscured a large part of the analyte spectra. Heating of the diamond powder allowed evaporation of the residual eluent, and satisfactory spectra of the three substituted phenols were obtained when μg amounts were injected. Phenol itself, however, could not be detected, probably because at the applied TSP temperature of ca. 150°C it evaporated together with the eluent. Improved analyte detectability was achieved by coupling the TSP to a narrow-bore LC column and using aqueous eluents with 15% methanol at a flow-rate of 20–50 μl/min [47]. With the earlier described DRIFT carousel [36] with microcups filled with diamond powder, identification limits of 10–20 ng (injected) were obtained for several substituted phenols, but, again, phenol could not be detected.

Jansen [48] used a TSP in combination with a moving-belt system to achieve FT-IR detection for SEC and RPLC. With a laboratory-made TSP, the SEC effluent was sprayed on a 13-mm wide stainless

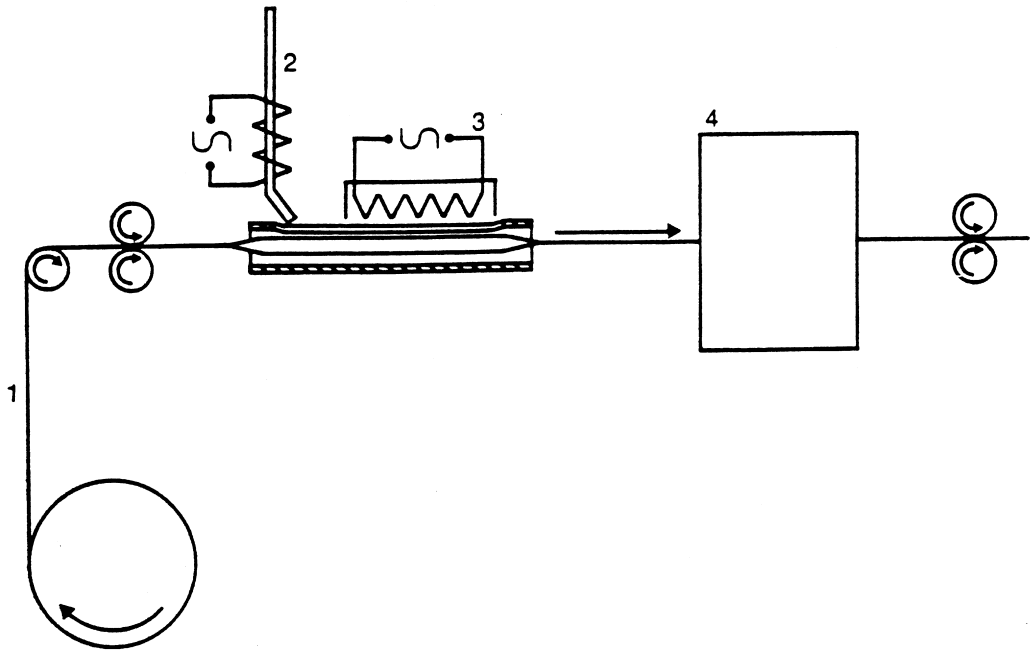

Fig. 5. Schematic of thermospray-moving belt interface for LC–FT-IR; 1=moving stainless steel tape; 2=thermospray interface; 3=infrared lamp; 4=diffuse reflectance cell mounted in the sample compartment of the FT-IR spectrometer [48].

steel tape which moves through an adapted IR diffuse-reflectance accessory mounted in the sample compartment of the FT-IR spectrometer (Fig. 5). It does not become clear from the paper whether pure diffuse-reflectance spectra were acquired, which seems unlikely, or reflection–absorption-type spectra. Most of the eluent was eliminated directly by the TSP and residual solvent, if any, was evaporated off the tape by an infrared lamp as the tape moved towards the spectrometer at a speed of 1.25 cm/min. The immobilized chromatogram was monitored continuously and solvent-interference-free spectra were recorded. The practicality of the system was demonstrated by analysing several simple polymer samples (20–80 μg injected) by SEC–FT-IR with dichloromethane or tetrahydrofurane as eluent at a flow-rate of 0.5–1 ml/min. Some low-molecular-mass monomers were not deposited (and thus could not be detected), because they were evaporated by the TSP. The characterization of two Irganox-type polymer additives which were separated by RPLC using an eluent with 30% (v/v) water, was also shown. The aqueous eluent (0.5 ml/min) could be handled efficiently, but as much as 100 μg of each additive had to be injected to obtain good quality spectra.

The TSP-moving belt interface was also used by Robertson et al. [49–51], mainly for RPLC–FT-IR. In a preliminary study [49] four amino acids were separated using a purely aqueous eluent at 0.5 ml/min. When operating the laboratory-made TSP at 280°C, the integrity of the chromatographic separation could be maintained during deposition, and identifiable IR-reflectance spectra of the analytes were recorded. Next, the interface design was further optimized and the analyte–deposition efficiency and analytical potential were studied [50]. The TSP temperature (range tested, 150–190°C) and the TSP height above the moving tape (5–25 mm) were optimized in order to obtain deposited spots that matched the IR beam (ca. 2 mm) of the DRIFT accessory. In this way analyte identification could be achieved down to concentrations of 50 μg/ml or about 2.5 μg injected. The system was used for the separation, detection and characterization of non-UV-absorbing compounds such as saccharides and aliphatic carboxylic acids. However, with saccharides

the spectra showed significant band broadening in the fingerprint region due to thermal effects; this prohibited their use for identification purposes. The TSP–FT-IR system was also used for polymer analysis: to identify antioxidants (Fig. 6) [50] and to characterize polyester and polystyrene [51,52].

The main advantage of the TSP-based systems is that relatively high flow-rates (0.5–1 ml/min) of both organic and aqueous eluents can be handled and conventional-size LC can thus be used. Furthermore, in the TSP–moving belt system spectral data are acquired during the run, which gives the solvent-elimination FT-IR detector an essentially on-line character. On the other hand, the high temperature of the TSP may induce analyte losses by evaporation or thermal degradation and, despite optimization, the analyte spots on the moving tape are still quite large (minimum size, 3.5×2.5 mm [50]) which results in a moderate FT-IR sensitivity.

4.2. Particle beam interface

The particle beam (PB) interface, a solvent-elimination interface originally developed for LC–MS [53], was modified for LC–FT-IR by de Haseth and co-workers [54–60] and Wood [61]. In this interface the LC eluent is nebulized by helium and directed into a desolvation chamber where most of the liquid is vaporized. The mixture of gas, vapour and precipitated analyte molecules (i.e., particles) is accelerated into the momentum separator where the analytes (higher-momentum particles) travel straight through the skimmer cone, while the gas and vapour (lower-momentum particles) are pumped away by the vacuum system. When leaving the momentum separator, the analyte molecules would normally enter the MS ion source, but for FT-IR detection purposes an IR-transparent substrate is placed in the beam path to collect the analytes of interest (Fig. 7). After deposition, the substrate is removed from the vacuum chamber and transferred to the FT-IR spectrometer for analysis. Until now stationary substrates have been used in LC–PB-FT-IR, which implies that only fractions or individual peaks, and no complete chromatograms, were analysed.

A preliminary study [55] demonstrated that the PB-FT-IR interface can effect the elimination of aqueous eluents inclusive of pure water at flow-rates of up to 0.3 ml/min. As an example, a mixture of erythrosin B and *p*-nitroaniline was separated by gradient elution RPLC. Via the PB interface, the analytes (50 μg each) were deposited individually on a KBr disc with spot sizes of ca. 1 mm. Subsequently, identifiable transmission spectra were recorded for both compounds using a 5× beam condenser accessory (Fig. 8). Wood [61] studied LC–PB-FT-IR using dioctyldiphenylamine as model compound. With hexane as eluent an identifiable spectrum of 2

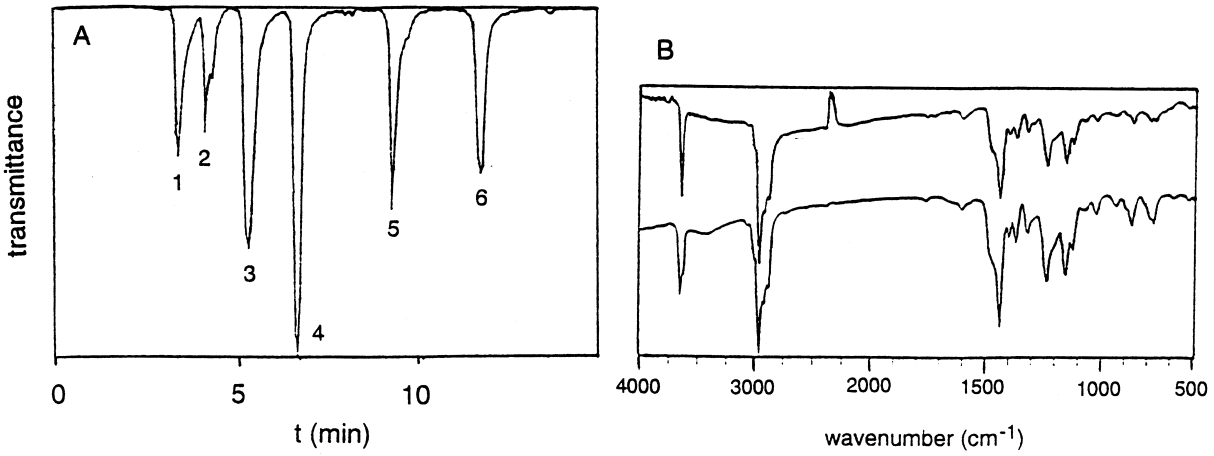

Fig. 6. (A) LC–TSP-FT-IR functional group (3100–2800 cm^{-1}) chromatogram of phenolic antioxidants. Peaks: 1=Irganox 3114 and 1035; 2=Irganox 1010; 3=Irganox 1330; 4=Irganox 565; 5=Irganox 1076; 6=Irgafos 168. (B) LC–TSP-FT-IR spectrum (top) and standard FT-IR spectrum (bottom) of Irganox 1330 [50].

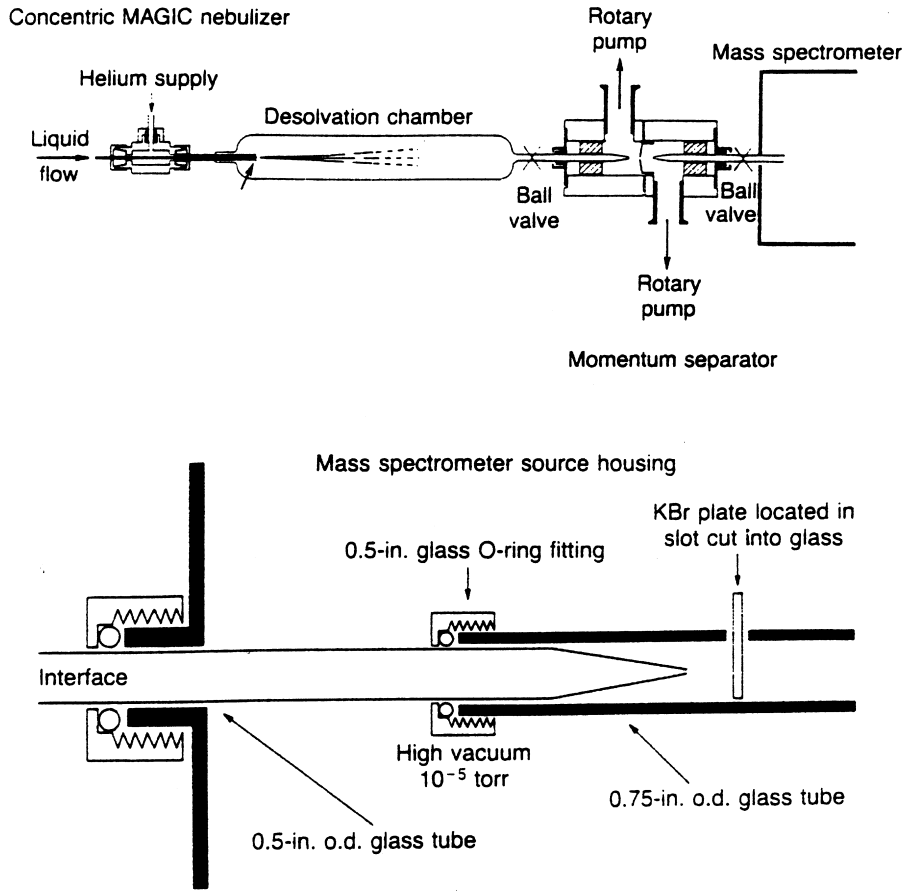

Fig. 7. Schematic of particle beam interface for LC–FT-IR as used by Wood [61]. 1 in. = 2.54 cm.

μg of the analyte was obtained after its deposition on KBr.

In solvent-elimination LC–FT-IR the presence of buffer salts in the eluent seriously disturbs the evaporation and deposition process. As a consequence the use of buffers is generally avoided. Since the PB interface has strong eluent-elimination capacities, it was believed that interferences caused by the buffer might be small in LC–PB-FT-IR. Therefore, de Haseth et al. [56] studied the deposition and IR detection of caffeine from several buffered solvent systems. The interface is indeed able to process an 0.3 ml/min flow of buffered eluent; however, the buffer salts are never completely eliminated so that it is hardly possible to obtain interference-free caffeine spectra from the KBr substrate. Best results were obtained with eluents buffered with ammonium acetate (which can be readily volatilized): a good quality spectrum was recorded after a 26-μg injection of caffeine, but buffer bands were clearly present in a 2.6-μg spectrum. When phthalate or phosphate buffers were used, the caffeine spectra were completely dominated by absorption bands of the buffer salts. Spectral subtraction procedures could be used to recover spectra from 130-μg caffeine depositions but were unsuccessful at the 13-μg level.

Turula and de Haseth [57–59] used the PB-FT-IR interface as a tool for the determination of protein structures. For β-lactoglobulin and lysozyme it was shown that their structural integrity is maintained during the PB desolvation process and the subsequent deposition on the substrate; i.e., the solution structure of the proteins can be studied by FT-IR. In

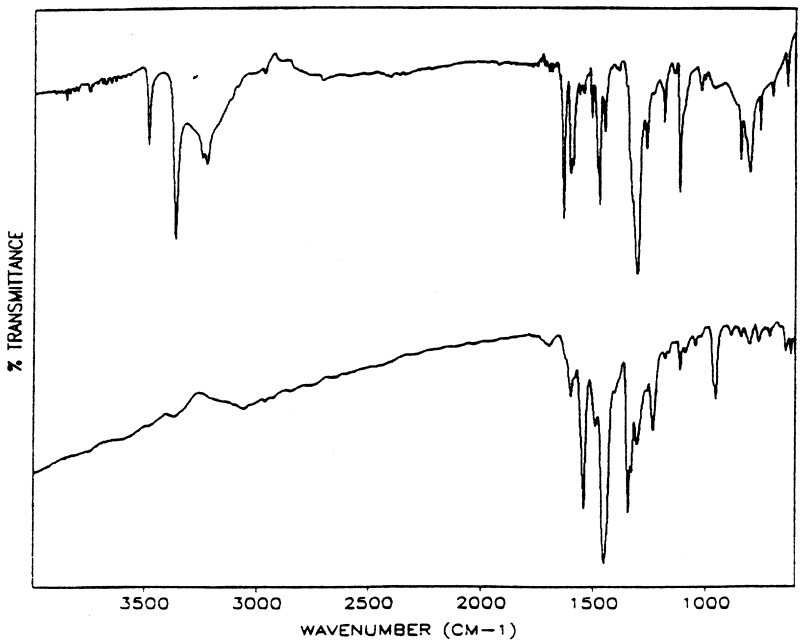

Fig. 8. LC–PB-FT-IR spectra of *p*-nitroaniline (A) and erythrosin (B) [55].

addition, lysozyme appeared to retain its biological activity. On the basis of major IR amide bands, structural changes of the protein caused by the stationary phase and the organic modifier were determined [59]. The sample loads in these experiments generally were quite high (5–500 μg). However, when using an FT-IR microscope an identifiable spectrum was obtained for 100 ng α-chymotrypsin, although some interfering bands from an eluent impurity were also present. Recently, Turula et al. [60] demonstrated the combined use of LC–PB-FT-IR and LC–electrospray (ESP)-MS for the structural characterization of tryptic digests of β-lactoglobulin.

The PB interface can effectively remove both organic and aqueous solvents and is, therefore, potentially useful for LC–FT-IR. Relevant applications in LC would, however, still require the construction of a device that allows the continuous deposition of a complete chromatogram on a moving substrate. The PB-FT-IR analysis of compounds at the ng level has been indicated [54], but the reported sample quantities mainly are in the (high) μg range. The modest analyte detectability no doubt is related to the fact that the efficiency of analyte transfer in the PB interface probably is 5–10% only.

4.3. Electrospray interface

The feasibility of ESP nebulization as a means of coupling micro-LC and FT-IR was studied by Raynor et al. [62]. A spray of charged droplets can be produced when a high electrical potential is applied between a capillary filled with a flowing liquid and a nearby plate. As a result of solvent evaporation and charge density, the initial droplets break up into smaller droplets which further facilitates solvent evaporation. For stable ESP operation low flow-rates (typically, 1–20 μl/min) are required so that the use of micro-LC is indicated.

Raynor et al. [62] used an ESP interface to deposit the effluent from a micro-RPLC column (4 μl/min) on an IR-transparent zinc selenide (ZnSe) plate. The spray is formed under atmospheric conditions and a sheath flow of nitrogen gas is applied to enhance eluent evaporation and, as the authors claim, to prevent solvent being drawn back into the electrospray tip (Fig. 9). A mixture of caffeine and barbital

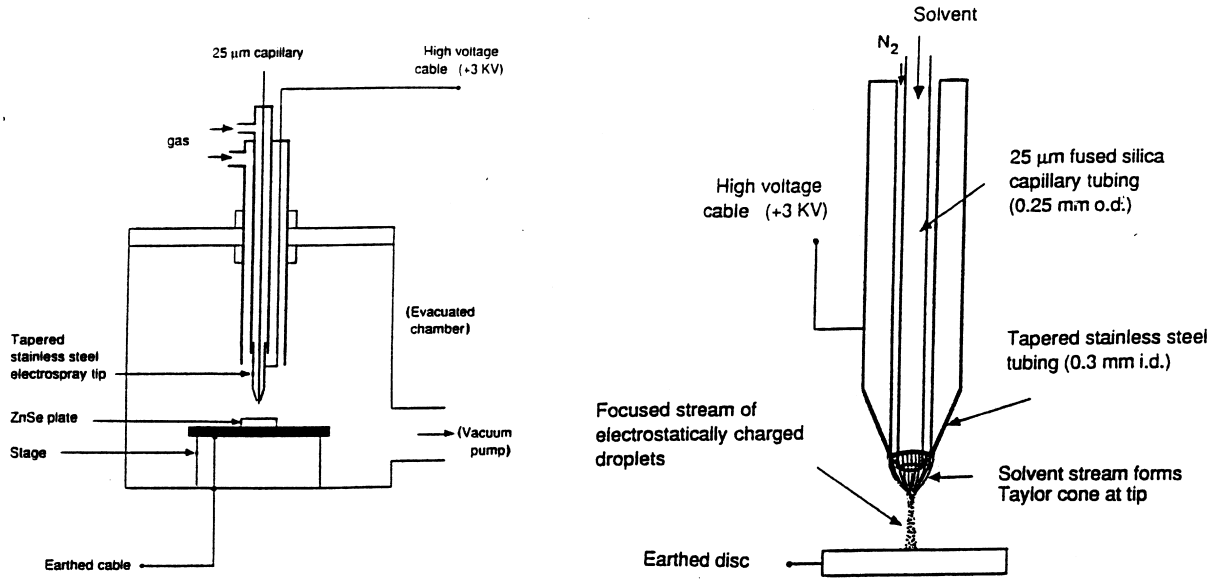

Fig. 9. Schematic of electrospray interface for micro-LC–FT-IR (left) with the electrospray tip in detail (right) [62].

(20 ng each) was separated with methanol–water (60:40, v/v) and deposited on ZnSe while moving the substrate manually. With an FT-IR microscope good-quality spectra were recorded from the 0.5-mm diameter analyte spots (Fig. 10). A spectrum obtained after flow injection of 2 ng caffeine could be positively identified by spectral library search, although subtraction of the interfering bands from a

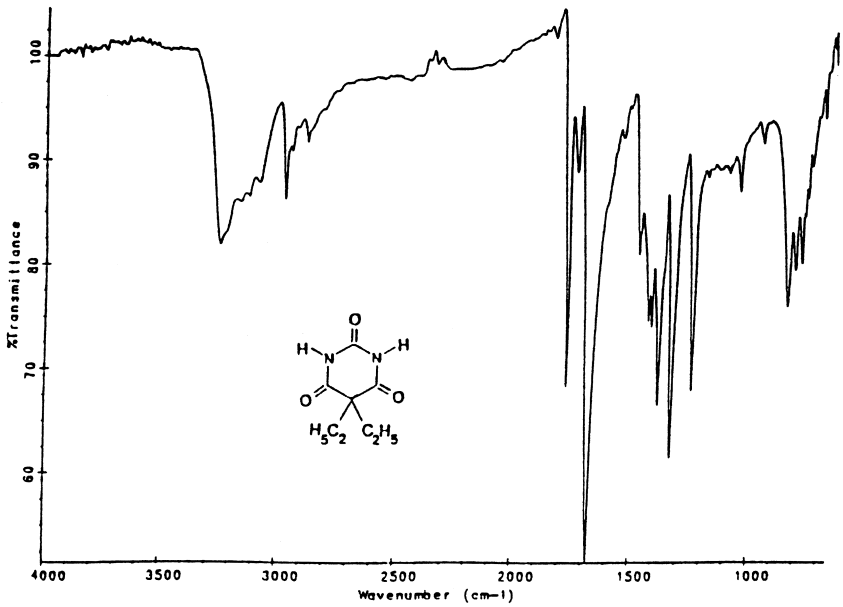

Fig. 10. LC–ESP-FT-IR spectrum of barbital [62].

siliceous impurity was required. Stable ESP conditions were achieved with hexane, dichloromethane, acetonitrile, methanol and several aqueous solvents, but problems were reported for pure water. Until now there have been no further studies on LC–ESP-FT-IR.

4.4. Pneumatic nebulizers

In a pneumatic nebulizer a high-speed gas flow is used to disrupt the liquid surface and to form small droplets which are dispersed by the gas. Organic solvents can be rapidly evaporated by pneumatic nebulization, while direct removal of aqueous solvents is possible when the nebulizer gas is heated. Pneumatic nebulization has been used in several solvent-elimination LC–FT-IR designs, among which are the most successful so far. The PB interface, which in principle is a pneumatic nebulizer as well, was discussed separately (Section 4.2) because the general design of this modified LC–MS interface is quite different from the custom-made LC–FT-IR interfaces discussed in this section.

Gagel and Biemann [63] reported a nebulizer-based LC–FT-IR method which involved continuous deposition of the effluent from a narrow-bore NPLC column on a rotating IR-reflective disc. The effluent was mixed with nitrogen gas and led into a syringe needle from which a fine spray emerged on to the surface of an aluminium mirror which resulted in a 1–2 mm wide deposition track (Fig. 11). The immobilized chromatogram was analysed by rotating the disc in the sample compartment of an FT-IR spectrometer equipped with a reflectance accessory with 3× beam condensing optics. Reflection–absorption (R–A) spectra of the analytes are continuously recorded and an FT-IR chromatogram can be constructed. The performance of the system was tested with a mixture of polycyclic aromatic compounds (PACs) (200–800 ng each) which were separated on a 1-mm I.D. silica column with hexane–dichloromethane (75:25, v/v) as eluent at 30 μl/min. The separation was nicely maintained during deposition and spectra of good intensity were obtained, although some differences with conventional KBr transmission spectra were observed. The authors attributed the deviations primarily to the Christiansen effect. The identification limit for anthracene was 125 ng injected on the column.

In order to accomplish elimination of aqueous solvents, Gagel and Biemann [64] improved the nebulizer design. The syringe needle was placed inside a nozzle through which heated nitrogen gas flowed at a temperature of 40–80°C. The extra nitrogen enhances solvent evaporation and focuses the spray onto a 0.5–1.5 mm region. With this set-up, eluents containing up to 55% water could be handled at 30 μl/min, and by programming the nitrogen gas temperature gradient elution could also be performed (Fig. 12). The RPLC separation and FT-IR identification of a number of isomeric naphthalenediols (500 ng each) was demonstrated and an identification limit of 31 ng (injected) was obtained for phenanthrenequinone. The recorded R–A spectra showed anomalies such as baseline curvature, distortions of the bands on the high-frequency side and excessive broadening of the O–H stretch bands. These spectral problems could be partially solved by replacing the

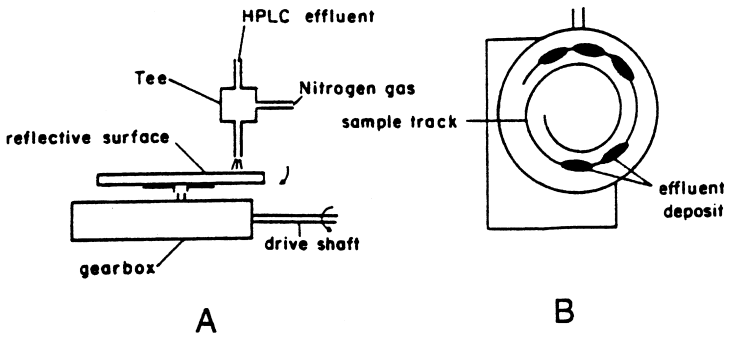

Fig. 11. Schematic of the narrow-bore LC–FT-IR system of Gagel and Biemann [63]; (A) side view during deposition; (B) top view of collection mirror.

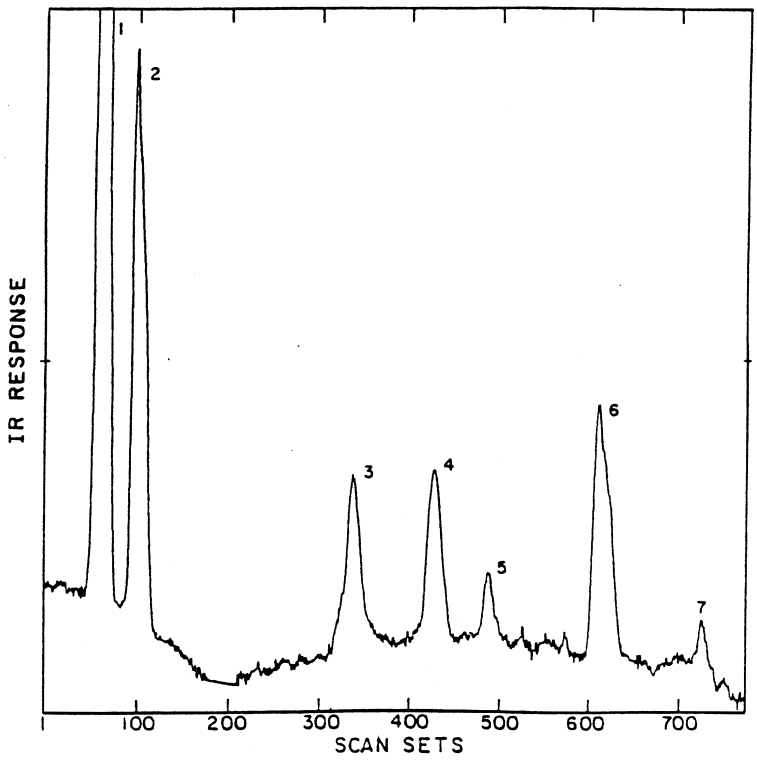

Fig. 12. LC–FT-IR chromatogram (Gram–Schmidt) recorded after deposition of a gradient elution separation of a mixture of (1) caffeine, (2) 2,7-dihydroxynaphthalene, (3) phenanthrenequinone, (4) carbazole, (5) anthrone, (6) 9-nitroanthracene and (7) anthracene [64].

original aluminium disc by a 2-mm thick IR-transparent germanium disc, the rear surface of which was coated with (IR-reflective) aluminium [65]. The authors claimed that the germanium layer prevents interference of the incident and reflected IR beam at the disc surface, thus minimizing spectral degrading effects.

A commercially available LC–FT-IR interface based on the pneumatic nebulizer design of Gagel and Biemann is produced by Lab Connections (Marlborough, MA, USA) under the name LC Transform (100 and 400 Series). The instrument consists of a sample collection module for deposition of the chromatogram and an optics module for R–A analysis of the collection disc. So far the commercial interface has been mainly applied in the field of SEC–FT-IR [66–69]. The essentials of these polymer-composition characterization studies are summarized in Table 1. Jordan et al. [70] used the LC Transform for the identification of triclosan, an antibacterial agent, in toothpaste. Samples were separated by conventional-size RPLC and 5% of the column effluent was immobilized on the disc. The identification limit for triclosan was at the low-μg level (on disc), i.e., 25–50 μg injected on-column.

Lange et al. [71] constructed a simple but effective concentric flow nebulizer (CFN) for the coupling of narrow-bore LC and FT-IR. It consists of two concentric fused-silica capillaries. The LC column effluent is led through the inner capillary (50 μm I.D.×150 μm O.D.) and helium gas through the outer capillary (1.7 mm I.D.) which is wrapped with heating wire [Fig. 13 (top)]. The hot gas serves to evaporate the solvent and to focus the spray emerging from the inner tube. In a preliminary study [72] this type of interface was used for NPLC with powdered KCl substrates, while in RPLC an IR-transparent ZnSe window was used. To enhance the elimination of aqueous eluents, the CFN was placed in a vacuum chamber [Fig. 13 (bottom)]. Finally,

Table 1
Use of the LC transform series 100 interface for SEC–FT-IR[a]

Sample	Concentration (mg/ml)	Injection volume (ml)	Eluent	Flow-rate (ml/min)		Ref.
				Column	Interface	
EP copolymer	3–5	0.2	TCB	0.5	0.15	[66]
Polystyrene–PMMA	1–6	0.2	THF	1	0.07	[67]
Styrene–butadiene rubber	–	–	THF	–	–	[68]
PC–ABS; HIPS	1	0.15	THF	–	0.1	[69]

[a] Abbreviations: EP = ethylene–propylene; PMMA = poly(methyl methacrylate); PC–ABS = polycarbonate–acrylonitrile–butadiene–styrene; HIPS = high-impact polystyrene rubber; TCB = trichlorobenzene; THF = tetrahydrofuran; – = not stated.

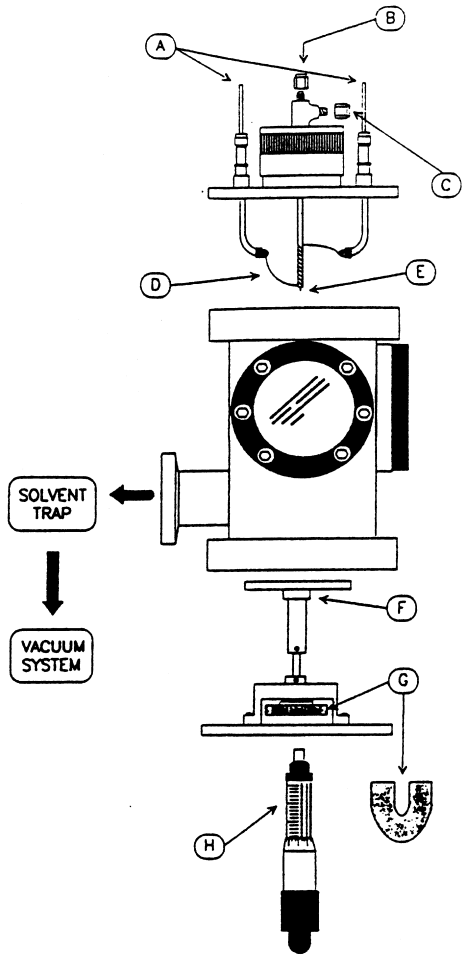

Fig. 13. Concentric flow nebulizer for narrow-bore LC–FT-IR (top) and interface chamber (bottom); A = electric connections; B = LC effluent inlet; C = helium gas inlet; D = heating wire; E = concentric fused-silica tubes; F = stage; G = magnet for stage rotation; H = vertical positioner [71].

since with this system the widths of the deposits are less than 200 μm, FT-IR microscopy was used for optimum detection. With the CFN, eluents up to pure water and with a flow-rate of 50 μl/min could be removed. Deposits of 60-ng amounts of model compounds on a stationary substrate yielded high quality absorption spectra (Fig. 14) indicating identification limits – in standard solutions – in the low-ng range. With smaller interface capillaries and a flow-rate of 2 μl/min, an identifiable spectrum was produced for 840 pg methyl violet 2B; in this case the spectrum of a co-deposited siliceous impurity had to be subtracted first.

In a further study [73] the CFN was installed in an evacuated compartment which included the IR-microscopic optics and a motor to translate the ZnSe window. With this system, an RPLC effluent (50 μl/min) could be continuously deposited on the moving substrate. After immobilization, it was possible to collect spectral data from the deposition track and to construct IR chromatograms without the need to transport the substrate from the bench to a spectrometer. The authors therefore presented their set-up as an on-line LC–FT-IR system. The performance of the system was illustrated by the repeated analysis of 60 ng theophylline. The band broadening caused by the interface was acceptable and the spectra were successfully searched against a library of conventional KBr spectra. To further improve the on-line character of the system, a modified CFN was installed on the optical bench of a Tracer (Bio-Rad, Dusseldorf, Germany) GC–FT-IR interface which allows spectral acquisition in real time. So far, only some preliminary results with this on-line LC–FT-IR system have been reported. These

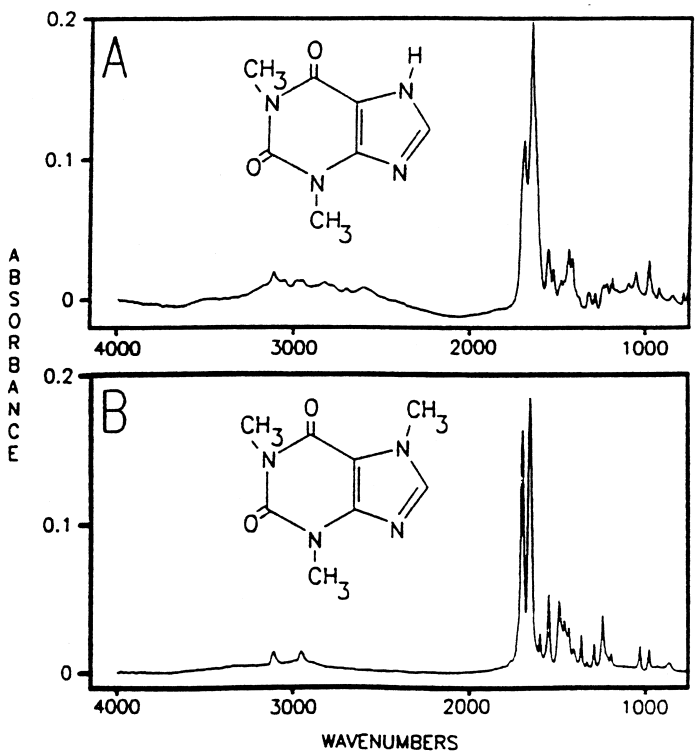

Fig. 14. LC–CFN-FT-IR spectra of (A) theophylline and (B) caffeine [71].

show reasonable IR peak shapes for theophylline (FIA of 140 ng) and real-time acquired spectra of 6–300 ng of the same compound [74].

The handling of buffered solvent systems by the CFN has also been studied [75]. Unfortunately, proper analyte spectra cannot be obtained when using a phosphate buffer (1 m*M*), because of strong co-deposition of buffer salts. However, if sufficient vacuum pump capacity is applied, a 1 m*M* ammonium acetate buffer can be completely eliminated. Higher ammonium acetate concentrations cause interferences and require subtraction of buffer bands from the analyte spectra.

Somsen et al. [76–79] modified a spray-jet interface which was originally developed for the on-line hyphenation of LC and thin-layer chromatography [80,81], for the coupling of narrow-bore RPLC and FT-IR. In this interface the column effluent (20 μl/min) is led through a stainless steel needle which protrudes through a spray nozzle. A heated nitrogen flow provides pneumatic nebulization and ensures eluent evaporation and deposition of the analytes on a moving substrate. In an interface optimization study [76] with quinones and PACs as model compounds, it was shown that deposits with a width of 100–300 μm can be obtained and that the chromatographic resolution is (essentially) maintained during the immobilization process. With ZnSe as substrate and FT-IR microscopy for detection in the transmission mode, identification limits in the 10–20 ng range were achieved. The narrow-bore RPLC–FT-IR system was used for the impurity profiling of a steroid drug [77] and for the characterization of a synthetic mixture of chlorinated pyrenes [78]. In the latter study three dichloropyrene isomers – which could not be distinguished by MS – were unambiguously identified on the basis of their FT-IR spectra. The usefulness of the spray-jet system in the identification of additives in polymer samples was also demonstrated [79]. For example, analysis of a poly-

(vinyl chloride) sample extract (Fig. 15) indicated the presence of monoesterified *N*,*N*-bis(hydroxyethyl)alkylamine, oleamide and Irganox 1076. The suitability of the interface for SEC–FT-IR was demonstrated by analysing a polystyrene standard mixture [79]. The oligomers were separated using pure dichloromethane (0.1–0.2 ml/min) as eluent and subsequently deposited on ZnSe for FT-IR detection. Representative FT-IR spectra were obtained which indicated that the system can be used to determine the compositional distribution of polymers.

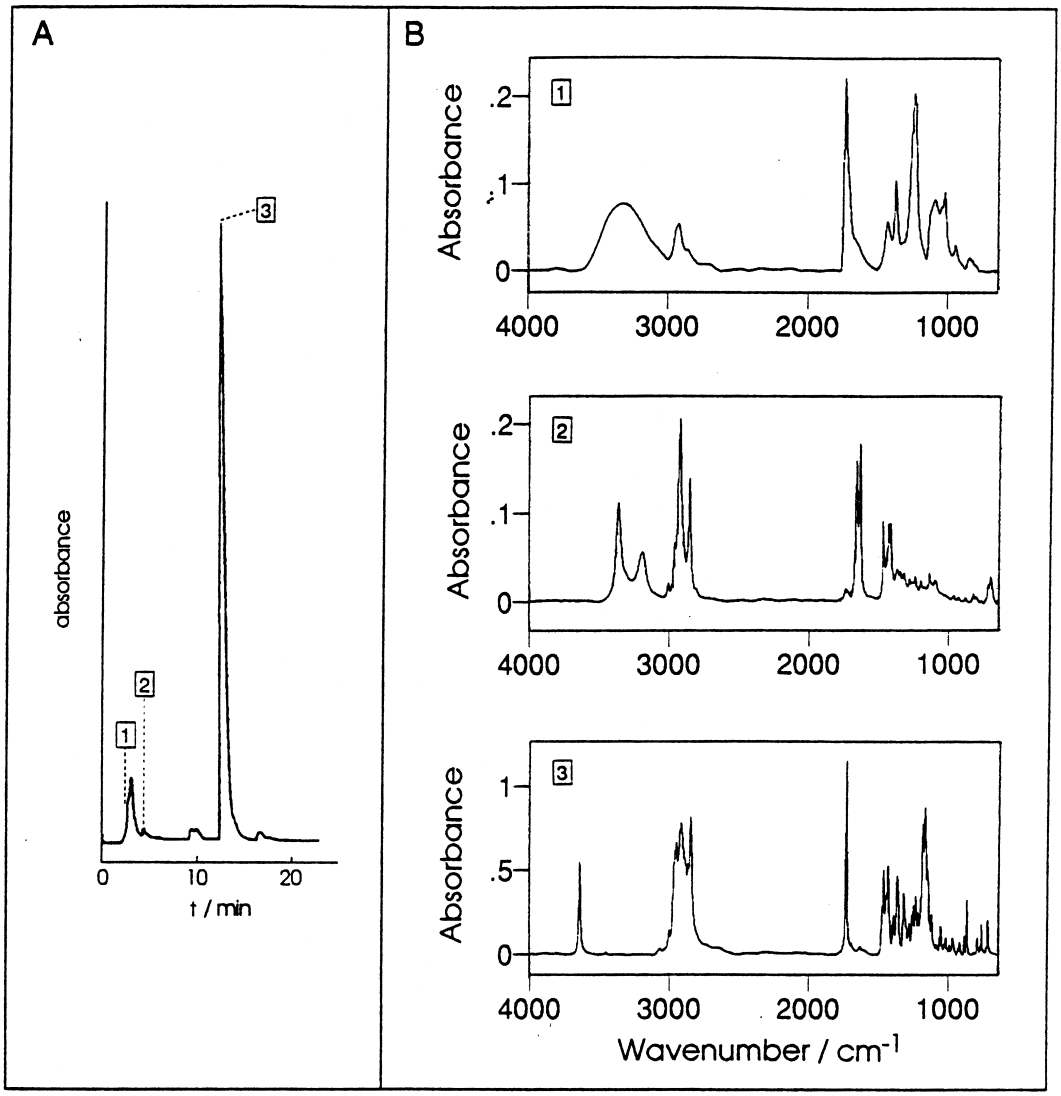

Fig. 15. (A) LC–UV chromatogram (275 nm) of a poly(vinyl chloride) sample extract. (B) FT-IR spectra of peaks 1–3. On the basis of library search and spectral interpretation the spectra were assigned to (1) a monoesterified *N*,*N*-bis(hydroxyethyl)alkylamine, (2) oleamide and (3) Irganox 1076 [79].

When RPLC is applied, the spray-jet LC–FT-IR system is limited with regard to the LC flow-rate (20–30 μl/min), the water content of the eluent (up to 20%, v/v) and the handling of buffered eluents. In order to take away these limitations, an on-line liquid–liquid extraction (LLE) module consisting of a phase segmentor, an extraction coil and a phase separator, was inserted between the LC column outlet and the spray-jet interface [82]. Dichloromethane, which can be effectively eliminated by the interface, was used as extraction solvent. The resulting LC–LLE–FT-IR system can handle eluents with high water percentages (20–100%, v/v) at flow-rates up to 0.2 ml/min so that 2-mm I.D. LC columns – a more common dimension in LC – can be used. Furthermore, the eluent may now contain non-volatile buffer salts which cannot be directly eliminated by an evaporation interface (cf. Sections 4.2 and 4.4). Since the salts are not extracted, phosphate-buffered eluents (0.01 *M*) can be used without causing interferences [82,83]. With large-volume injection, FT-IR detection of test compounds such as phenylureas and quinones was achieved at the sub-mg/l level. The detectability of the analytes expressed in concentration units, in the initial samples, was further improved by incorporation of on-line SPE for analyte enrichment. It was demonstrated that with SPE–LC–LLE–FT-IR triazine herbicides, including several isomers, can be identified at the low-μg/l level in river water (Fig. 16) [83].

In an alternative approach to improve the compatibility of the spray-jet interface with RPLC, the eluent flow-rate was reduced to 2 μl/min, i.e., micro-LC was applied [84,85]. Under these conditions, complete evaporation of aqueous eluents could be achieved, but to obtain a useful spray, the addition of excess make-up liquid (20 μl/min of methanol) to the micro-LC effluent was necessary. Because of the surplus of methanol, the performance of the interface becomes essentially independent of the water content of the eluent; that is, the system allows the use of gradient elution. The inherently moderate concentration sensitivity of micro-LC was overcome by using a micro-pre-column for on-line trace enrichment. The potential of the complete system was studied with triazines and pyrene as test compounds. With a 40-μl sample volume, good-quality FT-IR chromatograms and analyte spectra were recorded at the low-mg/l level (Fig. 17). When the sample volume was increased to 1.0 ml, the identification limits were improved to 10–160 μg/l.

4.5. Ultrasonic nebulizers

In an ultrasonic nebulizer a spray is formed by depositing the LC effluent on a transducer that is vibrating at ultrasonic frequencies. The vibrations cause the solvent to break up into small, desolvating droplets which are transported by a carrier gas towards a substrate. Such a system was used by Castles et al. [86] to continuously deposit the effluent from a narrow-bore RPLC column on diamond powder held in a moving trough. After deposition the through was translated through the optics of a DRIFT accessory. A two-component mixture was analysed and FT-IR chromatograms were constructed. Spectra of satisfactory quality were obtained for 3-μg injections, which indicates that identification limits will be on the order of 1 μg. In some instances, the complete and direct evaporation of the eluent by the ultrasonic nebulizer was not achieved because the vibrating surface was not uniformly effective and occasionally large droplets were formed which wetted the surface of the powder.

Dekmezian and Morioka [87] developed an interface for high-temperature SEC–FT-IR; in order to enhance solvent evaporation, an ultrasonic nebulizer was incorporated [88]. The nebulizer, which is placed in a vacuum chamber, was used to spray the SEC effluent on a fraction collector which holds a set of heated KBr discs. The discs were subsequently analysed by conventional FT-IR transmission spectrometry. With trichlorobenzene as SEC eluent, the system was applied to the determination of compositional changes of ethylene–propylene rubbers through changes of the molecular mass distribution. A block polymer reaction product was also analysed.

An interface comprising an ultrasonic nebulizer in a vacuum chamber is used by Lab Connections in their LC Transform 300 Series [7]. This commercial device sprays the chromatographic effluent on a rotating germanium collection disc, which is then evaluated by FT-IR using an R–A optics module (cf. Section 4.4). The system has been used for the

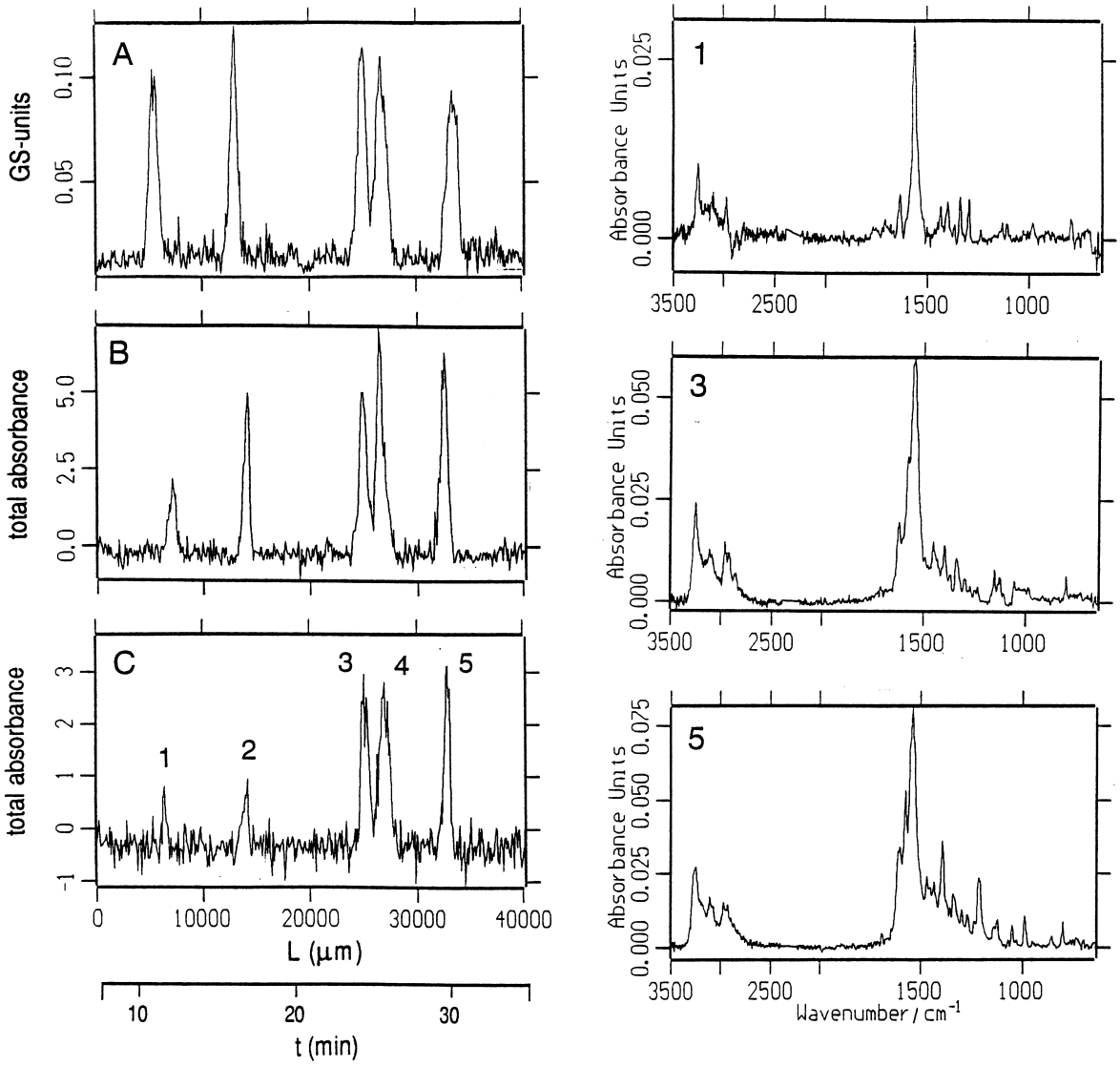

Fig. 16. (Left) SPE–LC–LLE–FT-IR chromatograms of river Meuse water samples spiked with five triazines: (A) 20 ml (30 μg/l), (B) 50 ml (6 μg/l) and (C) 100 ml (2 μg/l). (Right) FT-IR spectra of peaks 1, 3 and 5. Chromatogram representation, (A) Gram–Schmidt or (B and C) spectral window (1650–1500 cm^{-1}). Peaks: 1=simazine; 2=atrazine; 3=sebutylazine; 4=propazine and 5=terbutylazine [83].

quantitative analysis of copolymers by SEC–FT-IR with tetrahydrofurane as eluent [89], and also for steroid analysis by RPLC–FT-IR [90]. The interface can handle 0.5 ml/min methanol–water (65:35, v/v). Good spectra of LC-separated anabolic steroids (injection of 5 μg each) were obtained and the limit of identification was claimed to be 100 ng.

5. FT-IR detection of deposited compounds

In solvent-elimination LC–FT-IR basically three types of substrates and corresponding IR modes can be discerned, viz., powder substrates for DRIFT, metallic mirrors for R–A spectrometry and IR-transparent windows for transmission measurements. The

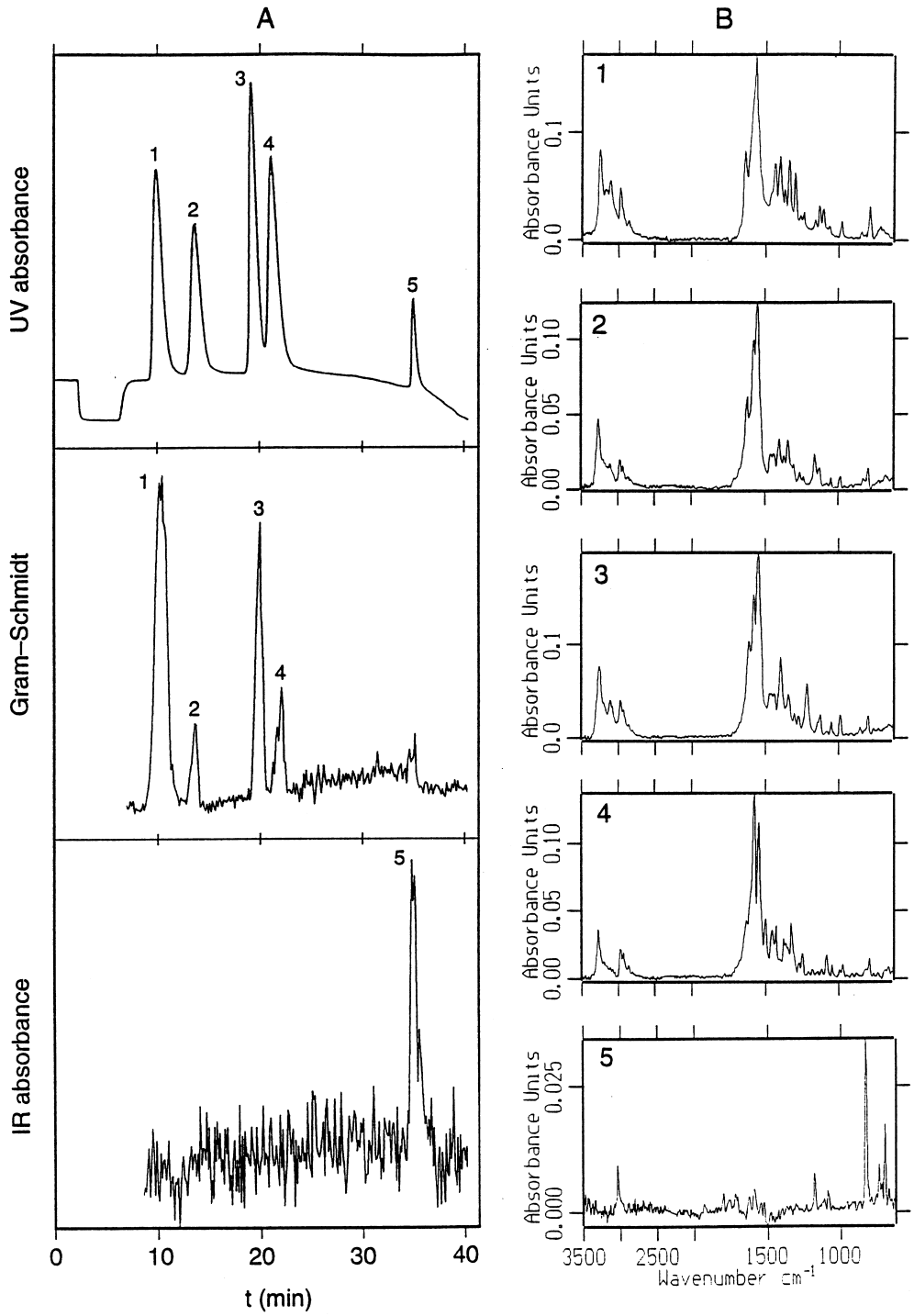

Fig. 17. (A) Gradient-LC–UV (top) and gradient-LC–FT-IR (centre and bottom) after on-line trace enrichment of a 40-μl mixture of test compounds (1 mg/l each). (B) FT-IR spectra recorded of peaks 1–5. FT-IR chromatogram representation, (centre) Gram–Schmidt or (bottom) spectral window (850–820 cm^{-1}). Peaks: 1=simazine; 2=atrazine; 3=terbutylazine; 4=trietazine and 5=pyrene [85].

analytical utility of these substrates is discussed in the next section, followed by a brief description of the influence of analyte morphology on spectral quality. The usefulness of FT-IR microscopy for sensitive IR detection of deposited analytes will be discussed in Section 5.3.

5.1. Substrates and IR modes

5.1.1. Diffuse reflectance

DRIFT detection of analytes on KCl powder was used in early solvent-elimination LC–FT-IR designs but as, in time, effective analyte deposition on flat and smooth substrates became feasible, other, more convenient detection modes were preferred. In the early interfaces, the eluent was not completely evaporated and a small part reached the substrate. KCl powders can tolerate some residual organic solvent without the analytes being spread over a large surface as would occur on a smooth substrate like a KBr plate. Because DRIFT as such is one of the most sensitive IR modes, sub-μg identification limits could be achieved when the residual solvent was evaporated quickly from the powder. If the eluent is not removed rapidly (e.g., when it is not highly volatile), it can draw analyte away from the KCl powder surface into the substrate. As a consequence part of the sample will escape detection, because the effective penetration depth of a DRIFT measurement is not more than 100 μm. To overcome this problem, Fraser et al. [72,91] successfully applied diffuse transmittance spectrometry instead of DRIFT, using a layer of KCl powder on an IR transparent substrate.

The main limitations of DRIFT detection in LC–FT-IR, however, only show up during application. Firstly, in DRIFT factors such as sample homogeneity, sample load and compactness of the powder layer, exert a significant influence on the (reproducibility of the) spectral data. These factors are not easy to control in LC–FT-IR. Secondly, to assure proper DRIFT detection, careful preparation of the powder substrate is a prerequisite; reorientation of the DRIFT matrix as a result of sample deposition may lead to a poor background compensation. Filling of cups or trays with the powder substrate may be very time-consuming and tedious and has to be repeated for every LC–FT-IR analysis. Finally, common DRIFT substrates such as KCl powder cannot be used in combination with aqueous eluents. In view of the overriding importance of RPLC, this is a very serious restriction. To allow the use of aqueous solvents, some authors have used diamond powder as DRIFT substrate, but it never has become really popular, because it is expensive (and thus not disposable) and not as easy to clean as flat substrates.

5.1.2. Reflection–absorption

Substrates suitable for FT-IR detection by R–A such as front-surface aluminium mirrors, are compatible with aqueous eluents and are easy to handle. However, analyte deposition on this type of substrate is not simple because solvent that hits the substrate will easily spread over the hard and smooth reflective surface. Therefore these substrates can only be used effectively in combination with an interface that has a strong solvent evaporation capacity like pneumatic nebulizers. As far as spectroscopic aspects are concerned, during the R–A measurement of a deposited analyte spot, the IR beam travels through the sample, reflects off the mirror surface and passes through the sample a second time on its way to the detector. The band intensities in the R–A spectrum therefore will be largely governed by a double-pass transmittance mechanism, so that data analogous to transmission data are obtained. Useful R–A spectra have been recorded for compounds separated by RPLC. However, several authors [63–65,76,92] have reported evidence of band asymmetry and spectral distortions. Fuoco et al. [92] indicated that aspects such as specular and diffuse reflection from the analyte, thickness and microcrystallinity of the spot, and optical characteristics of the substrate (may) affect the shape and intensity of R–A spectral bands obtained from analytes on metallic substrates. In order to reduce spectral distortions, Gagel and Biemann [65] proposed the use of an IR-transparent germanium disc with a reflective backing as deposition substrate for R–A measurements (see Section 4.4). This type of disc is applied by Lab Connections in their commercially available LC–FT-IR interfaces.

5.1.3. IR transmission

Until now, the most favourable results in LC–FT-IR have been obtained with IR-transparent deposition

substrates that allow straightforward transmission measurements. In principle a number of materials such as barium fluoride, zinc sulphide and silicon is useful as substrate, but so far only KBr and ZnSe windows have actually been applied. Since the LC eluent is rarely eliminated completely before the substrate is reached (deposition by the particle beam interface being the exception), KBr usually cannot be used in combination with RPLC. Instead, ZnSe is a water-resistant, inert material and is transparent over practically the complete mid-IR region. Since the ZnSe surface is both smooth and hard, solvent elimination has to be fast to achieve proper analyte deposition. From compounds deposited on ZnSe, good-quality transmission spectra can be recorded which exhibit symmetrical band profiles. In a comparative study on deposition substrates, Fuoco et al. [92] found that, when the size of the sample spot is small and microscopic optics are used for measurement, the sensitivity of transmission spectrometry is higher than that of diffuse reflectance measurements. The authors also concluded that the use of flat IR-transparent windows for transmission measurements causes fewer spectral artifacts than the use of flat metallic substrates for R–A measurements. This observation was experimentally confirmed by Somsen et al. [76] who used a ZnSe window and an aluminium mirror as deposition substrate.

As has been demonstrated in many studies [62,71,73,75–79,82,83], spectra obtained by solvent-elimination LC–FT-IR on ZnSe, generally closely resemble conventional KBr disc transmission spectra. This is very important, because existing spectral libraries can then be used for identification purposes. Commonly, commercially available library programmes are used to automatedly and quickly retrieve reference spectra that match or resemble the analyte spectra recorded on ZnSe. So far in LC–FT-IR, the choice of search algorithms for spectral retrieval has been quite arbitrary, and various algorithms have been used. However, in a study on the LC–FT-IR analysis of herbicides in river water, Somsen et al. [83] showed that the performance of such search procedures can be quite different. A search routine based on the matching of spectral peak frequencies only, was found to be most suitable to identify the analytes at the trace level. This conclusion was based on a data set of 45 spectra covering two herbicide classes (triazines and phenylureas). Further study on this subject is indicated, particularly because for the acceptance of FT-IR as a useful detection technique in LC, reliable (automated) identification of spectra is extremely important.

ZnSe windows and front-surface mirrors are relatively expensive and, therefore, the repeated use of one substrate is common practice in LC–FT-IR on flat substrates. In other words, the possibility to clean substrates effectively between analyses is of importance. ZnSe is inert and deposited compounds can be removed simply and quickly with e.g., water, alcohol, acetone and/or a wetted piece of soft tissue paper. The cleaning of aluminium mirrors is more delicate: the thin metal layer is fragile and can be damaged easily by rubbing. When the available substrate surface is used efficiently, several chromatograms can be deposited on a single substrate and the cleaning frequency can be minimized. The capacity of the substrate depends on its size and the width of the deposition trace. With optimum solvent-elimination interfaces narrow spot widths are obtained and in principle the chromatograms can be collected in parallel lines, spaced 1–2 mm apart. Using a typical 60×30 mm substrate, this implies that 14–29 lines (representing a total chromatographic time of 6–13 h) can be stored before the substrate has to be cleaned again.

5.2. Analyte morphology

The quality and appearance of the LC–FT-IR spectra will also be influenced by the morphology of the deposited analytes. The characteristics of the deposits will primarily depend on parameters such as eluent composition, evaporation rate, temperature and nature of the substrate and the analytes. During solvent elimination some compounds will form nice crystals while others will deposit as an amorphous layer. For instance, the decanyl and undecanyl esters of testosterone exhibit ‘‘amorphous’’ spectra (broadened bands) after deposition on ZnSe [77]. These spectra differ from ‘‘crystalline’’ spectra (sharp bands) which usually are present in spectral libraries. Also, some analytes will deposit as a smooth film, whereas others may form irregular clusters. This phenomenon has been observed during

the LC–FT-IR analysis of herbicides in river water [83]: triazines were deposited as homogeneous spots, while the spots of phenylureas revealed the presence of small, isolated domains. Despite these morphological differences, the recorded analyte spectra resembled the corresponding KBr spectra. When the spot thickness exceeds a certain level, the effect of scattering may become apparent; it usually leads to sloping of the spectral baseline since the scatter intensity depends on the IR frequency. A compound may also exhibit polymorphism so that mutually (slightly) different spectra can be obtained for the same compound. For example, some marked differences were observed in the LC–FT-IR spectra of the polymer additive Irganox 1076 [79], which appeared to depend on the time of recording the spectra, viz., immediately or one day after deposition on ZnSe. Lange et al. [71] showed that during the evaporation/deposition process, saccharin may be converted into its sodium salt, even though no salts are present in the eluent.

In general, FT-IR detection of deposited compounds on IR-transparent substrates does not pose serious problems. However, from the above it should be clear that analyte morphology and/or transformation should always be taken into consideration during the interpretation of spectra obtained by solvent-elimination LC–FT-IR.

5.3. Microscopic FT-IR detection

In order to increase the usefulness of FT-IR detection in LC, optimization of the analyte detectability is essential. In solvent-elimination LC–FT-IR the identification limits usually improve when the width of the analyte spots is decreased (see below for explanation). A prerequisite for this gain in detectability is the use of the appropriate detector and sampling optics. Optimum solvent-elimination interfaces can produce analyte spots with a width as small as 100–300 μm. For deposits of this size, the focus of a conventional beam condenser is too large and the use of an FT-IR microscope is indicated.

As an example, Fig. 18 shows the schematic optical diagram of the Bruker A590 FT-IR microscope. In the transmission mode of operation, the IR beam is focused onto a sample at the microscope stage (SS) by means of an off-axis condenser mirror (CM). The IR light transmitted by the sample is collected by a Cassegrain objective (MO1). The objective produces an image of the sample with a 15× magnification, and a circular or rectangular aperture (A) of variable size is placed in this image plane. The aperture permits selection of the sample region of interest. The radiation passing through the aperture is focused on a small area mercury–cadmium–telluride (MCT) detector (D) by another 15× Cassegrain lens (MO2), which yields a 1:1 projection of the sample region. The microscope can also be used with visible light. In this case, the light passing through the aperture is directed to a binocular viewer, so that the sample can be inspected visually. Moreover, the visible mode allows the desired region of the sample to be centred in the viewing field and to adjust the aperture to the appropriate size. This is an important advantage of a microscope over conventional beam condensing optics in which the sampling area cannot be checked visually. Another drawback of a beam condenser is that it is commonly used with the standard detector of the FT-IR spectrometer, i.e., the detector is not optimized for small samples.

The FT-IR microscope is generally applied to obtain structural information from tiny particles such as small flakes, single fibers or even single cells. FT-IR microscopy can also be used for the in situ characterization of inhomogeneities and/or various layers of macroscopic samples. Examples can be found in books devoted to the IR microspectrometry of small samples and sample areas [93–95]. In FT-IR microscopy, high spatial resolution is commonly essential in order to effectively isolate particles or sample regions of interest. This implies that diffraction effects caused by the aperture should be given careful consideration. The thickness of the sample also is a matter of concern, because it may seriously limit the optical throughput of the microscope. Fortunately, in solvent-elimination LC–FT-IR these potential difficulties are usually absent. In most cases, the width of the analyte deposition spots dictates an aperture diameter of 100 μm or more, so that aperture diffraction effects are negligible. The demands on spatial resolution are not severe either, since the spot length is normally large with respect to the aperture size. Furthermore, throughput losses caused by the sample are modest in LC–FT-IR,

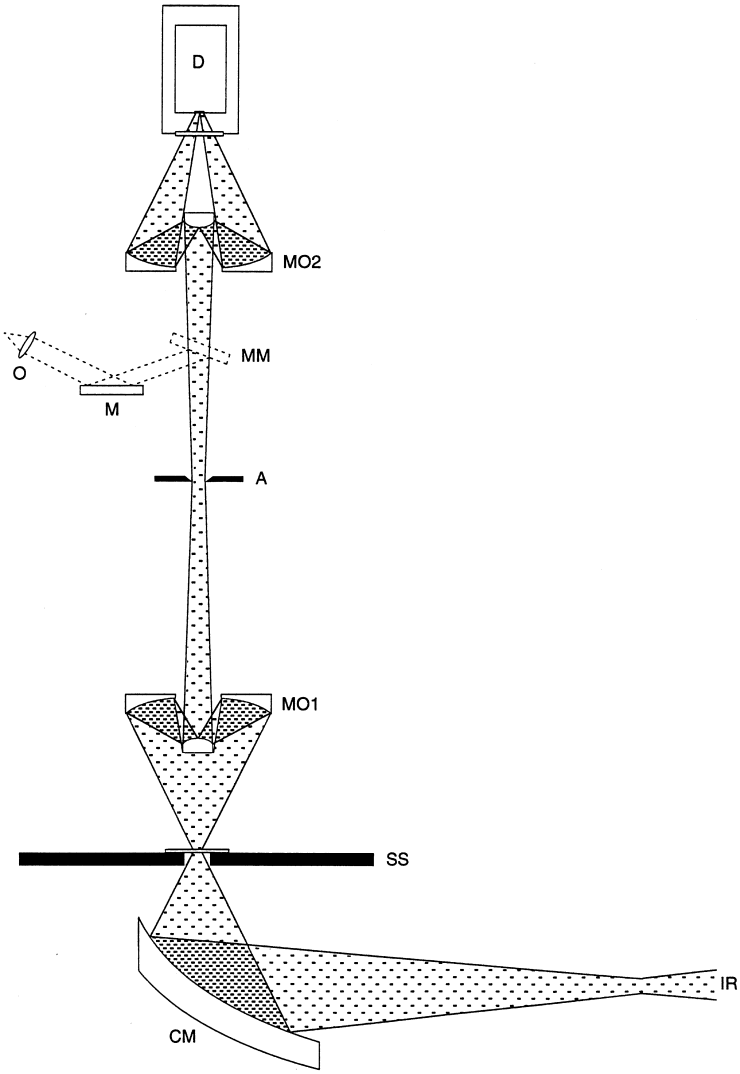

Fig. 18. Schematic of IR beam path of the Bruker A590 FT-IR microscope (transmission mode); IR: IR beam from FT-IR spectrometer; CM: condensor mirror; SS: sample stage; MO1, MO2: mirror objectives; A: aperture; MM: movable mirror; M: mirror; O: ocular; D: MCT detector.

because the absolute amounts analysed usually are relatively small, and the IR transmission of the spots therefore rarely is below 30%. The only problem that has to be dealt with is the fact that the refraction index and thickness of the substrate influence the focal plane of the microscope. The spectral background measurement should therefore be carried out on (a blank part of) the substrate with the IR beam focused on the surface of the substrate.

In principle, the sensitivity attainable in LC–FT-IR with solvent-elimination techniques can be enhanced by depositing the analytes in a small area with subsequent microscopic FT-IR detection. Frequently, this enhancement is rationalized by considering Beer's law only, but, as will be shown below, this approach is too simple. It is more appropriate to consider the factors which determine the signal-to-noise (S/N) ratio of the (microscopic) FT-IR measurement. In the following sub-sections this will be done by deriving a general expression for the IR

absorbance of an analyte spot and the S/N ratio of the absorbance measurement. Discussion of the latter parameters will show that the good sensitivity of FT-IR microscopic detection essentially results from the low noise level of the IR detectors in FT-IR microscopes.

5.3.1. Absorbance and spot size

Beer's law relates the absorbance, A, of a pure compound at a certain wavenumber, to its absorptivity, a (μl/mg mm), at that wavenumber, the optical thickness, b (mm), and the concentration (or density), c (mg/μl), according to:

$$A = abc \tag{1}$$

If it is assumed that a given mass, m (mg), of an analyte is deposited as a cylinder with diameter, d (mm), cross-sectional area, A_S (mm^2), height (i.e. optical thickness), b and volume, V (μl), A can be expressed as:

$$A = a \cdot \frac{V}{A_S} \cdot \frac{m}{V} = \frac{am}{A_S} = \frac{4am}{\pi d^2} \tag{2}$$

From Eq. (2) it follows that the analyte absorbance is inversely proportional to the sample area. Thus, in order to optimize the sensitivity, deposition of the analyte on as small an area as possible seems advantageous. However, in order to effect such an increase in absorbance, it is necessary that the diameter of the focused IR beam equals the diameter of the analyte spot, so that no blank substrate contributes to the measurement. With an FT-IR microscope, adjustment of the size of the interrogating beam is accomplished by use of apertures which reduce the IR beam to the appropriate size (see above). In other words, part of the IR radiance is rejected by the aperture, which implies that a decrease of the analyte spot diameter is accompanied by a decrease of the power received at the detector. This loss of energy will adversely affect the S/N ratio of the IR measurement [1]. Hence, the S/N ratio of the IR absorption experiment rather than the absorbance should be considered as a function of analyte spot diameter. This will be done below.

5.3.2. S/N ratio and spot size

In an actual FT-IR transmission measurement, the transmittance, T, and absorbance, A, at a certain wavenumber are obtained by measuring the intensity of the reference (or background) signal, E_R and the sample signal, E_S, and applying the well-known relationship:

$$A = -\log T = -\log\left(\frac{E_S}{E_R}\right) \tag{3}$$

If it is assumed that there is no uncertainty in measuring E_R, the S/N ratio or A/σ_A of the absorbance measurement is given by [96]:

$$\frac{A}{\sigma_A} = \frac{-E_R T \ln T}{\sigma_S} \tag{4}$$

where σ_A is the noise or uncertainty in absorbance, and σ_S the noise in E_S. Actually, A/σ_A is somewhat smaller because of the uncertainty in measuring E_R [96], but Eq. (4) gives a good approximation which suffices for the present discussion. In order to write A/σ_A as a function of analyte spot diameter d, E_R and T should be expressed in terms of d. From Eqs. (2) and (3) it readily follows that for a cylindrical spot of fixed mass:

$$T = 10^{-A} = 10^{-k/d^2} \tag{5}$$

where k (mm^2) is a constant. If it is assumed that the IR beam is uniform and that round apertures are used to adjust the beam size to the spot size, it follows that E_R is proportional to the spot area, A_S, or:

$$E_R = k' d^2 \tag{6}$$

where k' (mm^{-2}) is a constant for a given optical system. Substitution of Eqs. (5) and (6) in Eq. (4) yields:

$$\frac{A}{\sigma_A} = \frac{2.3kk'10^{-k/d^2}}{\sigma_S} \tag{7}$$

and thus

$$\log\left(\frac{A}{\sigma_A}\right) = 0.36 + \log(kk') - \log(\sigma_S) - \frac{k}{d^2} \tag{8}$$

In IR absorption spectrometry, the relatively high detector noise, σ_D, is dominant at all analytical signal levels, so that σ_S equals σ_D. Consequently, σ_D can be considered constant for a given detector regardless of the magnitude of E_R and T, so that Eq. (8) reduces to:

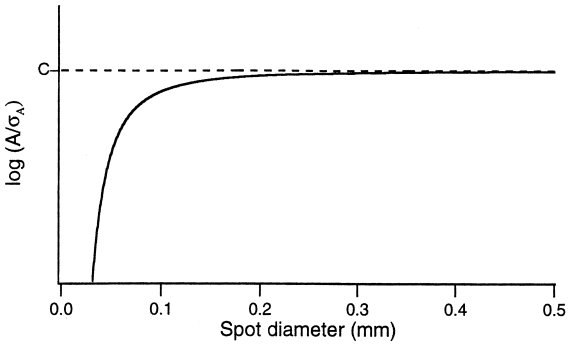

Fig. 19. Dependence of the S/N ratio (A/σ_A) on analyte spot diameter. The analyte mass is constant and $k=10^{-3}$ mm^2 which implies that a 0.1-mm spot exhibits an absorbance of 0.1 (Eq. (5)).

$$\log\left(\frac{A}{\sigma_A}\right) = C - \frac{k}{d^2} \tag{9}$$

where $C=\log(2.3kk'/\sigma_S)$. Fig. 19 which depicts the dependence of A/σ_A on the spot size, shows that for a given mass, optical system and detector, the S/N ratio of the IR absorbance measurement for all but very small spots is independent of the sample area. In other words, the increase in absorbance gained by reducing the spot size, is fully counterbalanced by the loss in energy caused by the use of beam diameter limiting apertures. Moreover, for very small spot sizes the S/N ratio rapidly deteriorates because the IR beam is reduced to such an extent that the absolute background and sample signal, E_R and E_S, respectively, become very low and therefore are dominated by detector noise.

5.3.3. Improvement of S/N ratio

The above indications suggest that no advantage will be obtained by depositing analytes in a small area. Nevertheless, compared with conventional FT-IR detection, FT-IR microscopy shows superior S/N ratio when small analyte amounts are measured. To understand this, one should consider C in Eq. (9) which largely determines the highest S/N ratio that can be obtained. In principle, C can be optimized by increasing k (analyte parameter) and k' (optics parameter), and by decreasing σ_S (detector parameter). Increasing k' is accomplished by appropriate focusing of the IR beam on the small sample and by effectively collecting the transmitted light, i.e., by using a strong beam condenser such as a microscope. The magnitude of σ_S is determined by the detector noise, σ_D, which is characterized by the noise equivalent power (NEP) of the detector. The NEP (W/Hz$^{1/2}$) depends on the area, A_D (mm^2), of the detector and its specific detectivity, D^* (mm Hz$^{1/2}$/W), according to [1]:

$$\text{NEP} = \frac{\sqrt{A_D}}{D^*} \tag{10}$$

Thus, σ_S can be minimized by using a small-area detector with a high detectivity. Since in FT-IR microscopy the size of the sample image on the detector surface usually equals the sample size (1:1 projection; see Fig. 18), FT-IR microscopes are equipped with detectors with a relatively small A_D (typically 0.01–0.04 mm^2). These detectors produce considerably less noise than, for example, a 4-mm^2 detector of a standard FT-IR spectrometer. In order to further reduce the noise of the microscope, liquid-nitrogen-cooled MCT detectors are commonly used, which exhibit a 10–100-times higher D^* than the conventional TGS detectors which operate at room temperature.

5.3.4. Optimum detection

From the above discussions it can be concluded that in order to fully benefit from the low noise of a small-sized detector, that is, from the optimum S/N ratio of FT-IR microscopy, the image of the analyte spot should just fill the detector area. In other words, to achieve the most sensitive FT-IR detection in LC, the width of the analyte deposits should have the same dimensions (typically, 100–200 μm) as the MCT detector used. For this reason, the potential of LC–FT-IR solvent-elimination interfaces to produce compact analyte spots is essential.

Here it should be noted that, as with any FT-IR experiment, the S/N ratio of the microscopic absorbance measurement can be improved by increasing the measurement time (signal averaging). Since in solvent-elimination LC–FT-IR the analytes are immobilized on the substrate, this advantage can be exploited to its full extent though, of course, at the cost of an increased time of analysis.

6. Conclusions

In this paper, the literature on LC–FT-IR has been reviewed with emphasis on coupling techniques in which the eluent is eliminated and analytes are deposited on a substrate prior to IR detection. In principle, FT-IR detection can also be carried out in an on-line mode using flow cells, but in the past years it has convincingly been demonstrated that semi on-line coupling is the approach of choice in LC–FT-IR. Compared with flow cell techniques, FT-IR detection procedures based on analyte deposition offer a number of advantages. These include the possibility (i) to record spectra over the entire mid-IR region without interference from the eluent, (ii) to perform ''post-run'' signal averaging and (iii) to contain a relatively large part of the chromatographic peak within the IR beam. As a result, the solvent-elimination approach provides an analytical set-up which features increased sensitivity and enhanced spectral quality, two important conditions for the reliable identification of (low-level) sample constituents which is the primary objective of LC–FT-IR. Not surprisingly, the commercial LC–FT-IR systems which are presently available [7,8], are solvent-elimination devices.

Interestingly, also in GC–FT-IR and supercritical fluid chromatography (SFC)–FT-IR, analyte-deposition-based methods have proven to be more sensitive and versatile than flow cell-based techniques. This observation prompted Griffiths to propose a unified approach to chromatography–FT-IR interfacing, and the development of a single FT-IR detection system capable of handling effluents from different chromatographic techniques was announced [97–100]. Indeed, mutually similar interfaces have been developed for the coupling of GC [3], SFC [101] and LC [71,76] with FT-IR, in which the column effluent is deposited directly on a moving IR-transparent substrate and transmission spectra are recorded under an FT-IR microscope. However, the experimental conditions required to achieve elimination of gas, supercritical fluid or liquid, and to trap volatile or non-volatile analytes are evidently quite different. In other words, despite the analogy in approach, dedicated optimization of the interfaces for each type of chromatography will still be necessary.

Since the major part of LC analyses involves RPLC, the more recent research in the field of LC–FT-IR has concentrated on the development of interfaces which are suitable for the elimination of aqueous eluents. The characteristics of the RPLC–FT-IR systems described in the literature are summarized in Tables 2 and 3; they list systems in which the column effluent is sent directly to the solvent-elimination interface and systems in which – prior to solvent evaporation – a reagent, extractant or solvent is added on-line to the LC effluent to facilitate the elimination of the aqueous eluent, respectively. Basically, the RPLC–FT-IR systems based on thermospray, particle beam and ultrasonic nebulization can handle relatively high flows of aqueous eluents (0.3–1 ml/min) and allow the use of conventional-size LC, which evidently is an advantage. However, due to diffuse spray characteristics and/or a low efficiency of the analyte transfer to the substrate, these systems often exhibit identification limits (expressed as mass injected) which are at the best moderate (100 ng) and often unfavourable (1–10 μg); therefore, their analytical applicability is limited. The best results (0.5–5 ng injected) are obtained with pneumatic and electrospray nebulizers, especially in combination with ZnSe substrates. The electrospray interface appears to be promising for use in micro-LC–FT-IR, but until now its performance has been described in a single paper only. Considerably more attention has been devoted to LC–FT-IR systems based on pneumatic nebulizers. Hence, some of the important aspects of these systems, which represent the state-of-the-art, will be summarized below.

6.1. Spray and deposition characteristics

The pneumatic interfaces combine rapid solvent elimination with a relatively narrow spray. The latter aspect allows analytes to be deposited on ZnSe in a narrow trace (width, <300 μm), so that transmission detection by FT-IR microscope can be applied to achieve mass sensitivities in the low- or even sub-ng range. It is noteworthy that with a microscope (typical detection area, 150 μm$\times$150 μm), only part of the injected amount of analyte is actually analysed because the length of a deposited spot is dictated by the width of the chromatographic peak and therefore

Table 2
Characteristics of RPLC–FT-IR systems using direct eluent elimination[a]

Interface type	LC flow-rate[b] (μl/min)	Substrate	IR mode	Identification limit		Ref.
				Mass (ng)	Concentration (mg/l)	
Direct deposition	4	SSWN	trans	1000	10 000	[46]
Thermospray	800	Diamond powder	DRIFT	2000	–	[47]
	50	Diamond powder	DRIFT	10	–	[47]
	500	SS tape	R–A	10 000	–	[48]
	1000	SS tape	R–A	1000	25	[50]
Particle beam	300	KBr window	trans	1000	200	[55,56]
Electrospray	4	ZnSe window	trans-micr	1	10	[62]
Pneumatic nebulizer	30	Al mirror	R–A	30	30	[64]
	2	ZnSe window	trans-micr	0.5	8	[71]
	50	ZnSe window	trans-micr	1	17	[73]
	20	ZnSe window	trans-micr	5	3	[76,78]
Ultrasonic nebulizer	40	Diamond powder	DRIFT	1000	1000	[86]
	500	Ge disc	R–A	100	20	[90]

[a] Abbreviations: SS = stainless steel; WN = wire net; DRIFT = diffuse reflectance; R–A = reflection–absorption; trans = transmission; trans-micr = transmission with FT-IR microscope; – = concentration and injection volume not stated.
[b] Typical value.

commonly will exceed 1 mm. In other words, identifiable spectra are obtained from pg amounts of analyte. This implies that the mass detectability of the optimum solvent-elimination systems for LC–FT-IR approaches a level which is close to the minimum that can be identified by a modern FT-IR spectrometer [102].

6.2. Flow-rate and water content of the eluent

When RPLC is used, the systems based on pneumatic nebulization are limited with regard to the LC flow-rate and the water content of the eluent. The flow-rates that can be handled directly by these systems are 2–50 μl/min, which means that micro-

Table 3
Characteristics of RPLC–FT-IR systems using indirect eluent elimination[a]

Interfacing	LC flow-rate[b] (μl/min)	Substrate	IR mode	Identification limit		Ref.
				Mass (ng)	Concentration (mg/l)	
DMP/nebulizer	50	KCl powder	DRIFT	1000	1000	[37]
DMP/concentrator	500	KCl powder	DRIFT	5000	1000	[38]
LLE/concentrator	800	KCl powder	DRIFT	100	10	[35]
LLE/pneumatic nebulizer	200	ZnSe window	trans-micr	30	0.2	[82]
	200	ZnSe window	trans-micr	50	0.001[c]	[83]
Make-up/pneumatic nebulizer	2	ZnSe window	trans-micr	20	0.02[c]	[85]

[a] Abbreviations: DMP = reaction with dimethoxypropane; LLE = on-line liquid–liquid extraction; make-up = addition of excess methanol. For further abbreviations, see Table 2.
[b] Typical value.
[c] After trace enrichment by on-line SPE.

or narrow-bore LC (column I.D., 0.2–1 mm) has to be applied. The water content of the eluent that can be tolerated depends on the flow-rate. If flow-rates of 2–5 μl/min are used as in micro-LC, even pure water can be eliminated efficiently by a pneumatic nebulizer. However, if the flow-rate is in the 20–50 μl/min range, as in narrow-bore LC, rapid evaporation of highly aqueous eluents will cause problems. In these instances further enhancement of the solvent evaporation efficiency is required, e.g., by mixing the effluent with nitrogen gas before it enters the nebulizer [64] or by placing both the nebulizer and the deposition substrate inside a vacuum chamber [73]. With the latter set-up, 50 μl/min flows of pure water can be eliminated, although use of vacuum obviously restricts the applicability of the LC–FT-IR system to analytes which are distinctly non-volatile. The tedious evaporation of water can be circumvented by on-line LLE of the aqueous effluent with an organic solvent which subsequently is led to the evaporation interface. Because the extractant can be evaporated relatively easily, with an LLE–pneumatic nebulizer system much higher eluent flow-rates (0.2 ml/min) and percentages of water (up to 100%, v/v) can be handled. Of course, the required LLE module adds to the complexity of the system and the analytes must have a sufficiently high extraction efficiency.

6.3. Gradient elution

Gradient elution RPLC–FT-IR has not been studied thoroughly yet, but some solutions to the problem of efficiently evaporating an eluent with a changing water content have been proposed. One involves the gradual increase of the temperature of the nebulization gas during the gradient run in order to enhance the evaporation power of the interface [64]. As an alternative, excess methanol can be added to the effluent of a micro-LC column to partly mask the changes in its water content and, thus, eliminate the need to change the interface conditions during the gradient run [85].

6.4. Use of buffers

Since buffer salts may seriously affect the deposition and detection of the analytes, the use of buffered eluents is generally avoided in solvent–elimination LC–FT-IR. Even volatile buffer salts are not completely eliminated by a pneumatic nebulizer and will therefore cause interfering absorbances in the analyte spectra. Buffer salts can, however, be removed by using a phase-switching technique such as on-line LLE. In fact, until now the LLE–pneumatic nebulizer combination is the only LC–FT-IR system described in the literature which allows the use of non-volatile buffer salts without introducing interfacing disturbances and/or spectral interferences.

6.5. Analyte detectability

The injection volumes that can be handled with micro- and narrow-bore LC columns, are at most 1–2 μl; the concentration identification limits of the pneumatic nebulizer-based systems therefore are in the low-mg/l range. This is better than the detectability in concentration units encountered with most other LC–FT-IR techniques (Tables 2 and 3), and is certainly adequate for quite a number of analytical applications [77–79]. By using the LLE–pneumatic nebulizer combination, analyte detectability can be improved to sub-mg/l levels (Table 3) because 2-mm I.D. LC columns – and, thus, increased injection volumes – can be used. However, despite the low-ng identification limits, the detectability in concentration units of even the best LC–FT-IR systems will not be sufficient to meet current demands of, e.g., environmental and bioanalysis in which analyte levels are frequently in the low-μg/l range. Fortunately, the problem of insufficient sensitivity can largely be overcome by combining LC, preferably on-line, with an adequate trace-enrichment procedure such as SPE [103,104]. It has amply been demonstrated that on-line SPE of 10–100 ml of an aqueous sample can improve the concentration detectability in LC-based techniques by two to three orders of magnitude: by using SPE–LC–LLE–FT-IR compounds of environmental interest present in river water could be identified at the low-μg/l level [83]. It will be obvious that such a dramatic improvement of identification limits is unlikely to be obtained by optimization of the interfacing and/or IR detection only.

Similarly enhanced detectability can self-evidently be obtained in micro-LC–FT-IR where, due to small injection volumes, the concentration sensitivity inherently is extremely low and on-line trace enrich-

ment will be very rewarding. Today, efficient micro-pre-columns can be produced for the preconcentration of relatively large sample volumes (0.1–1 ml). Combined with micro-LC–FT-IR, such pre-columns allow analytes to be identified at the 10–100 μg/l level [85]. Although this still is less good than the results quoted above for 2-mm I.D. columns, it is much better than the high-mg/ml identification limits to be expected with conventional 50–100 nl injections. In other words, the gain in sensitivity considerably enhances the applicability of micro-LC–FT-IR.

6.6. Spectral quality

When a pneumatic nebulizer is combined with a ZnSe substrate, interference- and distortion-free transmission spectra can be obtained from the deposited compounds. The spectra commonly show symmetrical bands and are closely similar to conventional KBr disc transmission spectra. The latter aspect opens up the important possibility of searching the recorded spectra of (unknown) analytes against the currently available libraries of condensed-phase reference spectra, which considerably facilitates spectral recognition and identification.

6.7. Applicability

Compounds of various nature such as quinones, steroids, drugs, polymer additives and herbicides have been analysed successfully by pneumatic-nebulization-based LC–FT-IR. Like most solvent-elimination LC–FT-IR interfaces, pneumatic systems show no discrimination towards certain classes of compounds, that is, the interfaces can handle most types of analytes. In this respect the applicability of semi on-line LC–FT-IR can be called universal. However, too high a volatility of the analytes will hinder their detection. Volatile compounds will be evaporated by the nebulizer gas and, therefore, will not, or only poorly, be deposited on the substrate. For example, low-molecular-mass PACs such as naphthalene and anthracene could not be analysed by solvent-elimination LC–FT-IR [76]. Thermal degradation of analytes is commonly not observed during pneumatic nebulization, despite the fact that the nebulizer gas is heated to rather high temperatures (70–180°C). Probably, due to the rapid evaporation of the solvent, the spray droplets are cooled considerably, i.e., the analytes are not exposed to excessive heat. This is nicely illustrated by the LC–FT-IR analysis of thermolabile phenylurea herbicides for which a nebulization temperature of 150°C was used: good quality spectra of the parent compounds were obtained and no degradation products were observed [82,83].

Today, on-line LC–MS undoubtedly is one of the most important and versatile identification techniques, being suitable for routine applications, both in biomedical and environmental analysis. Quite a number of LC–MS interfaces have been developed and several of these have been commercialized. Nevertheless, even today there is no single ''universal'' LC–MS interface available: every interface has its specific limitations with regard to flow-rate and composition of the LC eluent, polarity and molecular mass of the analytes, and/or ionization technique(s) that can be used. Furthermore, with most interfaces the structural information that can be derived is limited because of insufficient molecular fragmentation, and generally discrimination between isomers is not possible with MS. Hence, even with adequate LC–MS techniques available, there often is a need for alternative and complementary detection techniques which independently confirm MS-based identifications and differentiate between structurally highly similar compounds.

In the last 15 years, LC–FT-IR has emerged as a potentially powerful identification tool with identification limits in the low-ng range. Unfortunately, most LC–FT-IR interfaces have been used only by their designers and there are very few publications on the application of LC–FT-IR to real-life problems. Still, applications such as are described in Refs. [77–79,83], indicate that LC–FT-IR can indeed provide relevant structural information and/or identification of unknowns. LC–FT-IR is particularly useful for the characterization of isomeric compounds [78,83,85], and, in this respect, is complementary to LC–MS.

In order to enhance the acceptance of LC–FT-IR, several items of interest should be considered. Amongst these are the further demonstration of the practicality of the technique for real-life samples, and the development and use of appropriate on-line

sample-treatment procedures to improve analyte detectability (see above). Another essential aspect is the availability and use of commercial interfaces. The LC-Transform (Lab Connections) interface has been available now for several years, but unfortunately only few applications have been reported. Because this solvent-elimination system uses a mirror substrate and standard FT-IR equipment, both the FT-IR sensitivity and spectral quality are limited. In a more viable approach an IR-transparent substrate should be used together with microscopic FT-IR detection. Such a configuration is used by the Infrared Chromatograph (Bourne Scientific) interface. In this commercial and automated design the LC column effluent is deposited on a moving ZnSe window which instantaneously passes through the focused beam of the IR spectrometer allowing spectra and IR chromatograms to be recorded in real time. The placement of the chromatograms on the substrate is controlled by computer which also keeps record of the position of deposited compounds. Previous deposits may be analysed again for example to improve the S/N ratio of spectra. The Infrared Chromatograph seems promising but, as it has been introduced only recently, it is still too early to assess its merits.

In LC–FT-IR, handling of the obtained spectral data also is a matter of concern. The identification of analytes on the basis of their IR spectra often is a difficult operation. Therefore, the automated retrieval of spectra in reference collections, and the computerization of decision-making and spectral interpretation appear to be essential. Several such procedures have already been introduced in the vibrational-spectroscopic field and high priority should be given to their implementation in the separation field.

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