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Review

Liquid chromatography–Fourier-transform infrared spectrometry

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Abstract

Over the past years the coupling of liquid chromatography (LC) and Fourier-transform infrared spectrometry (FT-IR) has been pursued primarily to achieve specific detection and/or identification of sample constituents. Two approaches can be discerned in the combination of LC and FT-IR. The first and simpler approach is to use a flow cell through which the effluent from the LC column is passed while the IR spectra are continuously recorded. The second approach involves elimination of the LC solvent prior to IR detection using an interface which evaporates the eluent and deposits the analytes onto a substrate. This paper provides a general overview of flow-cell based IR detection and briefly discusses early solvent-elimination interfaces for LC–FT-IR. A more comprehensive description is given of interface systems which use spraying to induce rapid eluent evaporation, and which basically represent the state-of-the-art in LC–FT-IR. Finally, the interface systems suitable for reversed-phase LC are summarized and the perspectives of LC–FT-IR are discussed. The overview indicates that flow-cell LC–FT-IR has rather poor detection limits but can be useful for the specific and quantitative detection of major constituents of mixtures. Solvent-elimination techniques, on the other hand, provide much better sensitivity and enhanced spectral quality which is essential when unambiguous identification of low-level constituents is required.

Keywords: Infrared spectrometry; Interfaces, LC–FT-IR; Solvent elimination; Fourier-transform infrared spectrometry; Reviews; Detectors, LC

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. Liquid chromatography (LC) is a powerful and versatile separation technique which can handle a wide range of sample types and compound classes. Because of the widespread use of LC and the (growing) need for analytical procedures that provide confirmation and/or identification of sample constituents, quite some effort has been – and still is – devoted to the coupling of LC and IR spectrometry. Today, with modern Fourier-transform (FT) IR instrumentation routinely available [1], spectra can be recorded from nanogram, or even picogram, amounts of pure substance so that IR detection, in principle, is suited for molecular recognition at analyte levels frequently met in LC. Unfortunately, because of the (spectral) characteristics of the mobile phase, the coupling of LC and FT-IR is not straightforward and often requires the construction of special flow cells or the development of rather complex interfaces. Therefore, compared with other LC detection modes such as UV diode-array or mass spectrometry (MS), the use of IR detection in LC is still rather limited.

FT-IR detection in chromatography became feasible in the 1970s and presently the combination of gas chromatography (GC) and FT-IR is a well established technique [2]. Compared with GC–FT-IR, the development of LC–FT-IR has proceeded much slower, and its viability has even been questioned [3,4]. However, progress in interfacing techniques during the past 5–10 years has brought LC–FT-IR to a stage of real analytical utility and commercial interfaces were introduced [5,6]. In the earliest combinations of LC and FT-IR [7,8], 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 [9] 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, although the detection limits and spectral information obtained with either approach may differ considerably. The sensitivity and applicability of flow-cell LC–FT-IR is restricted and solvent-elimination techniques have shown to be more versatile and to yield interference-free spectra 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 solvent-elimination 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 [10–13].

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 sensitivity of IR detectors is moderate when compared to detectors commonly used in LC, such as MS, UV absorbance and fluorescence spectroscopic detectors. Moreover, 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 1 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 the combination of reversed-phase (RP) LC and FT-IR via a flow cell is restricted to specific applications. 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.

2.1. Cell types and detection modes

A variety of flow cells, differing in optical material, path length and cell volume, is available for LC–IR detection purposes. In general these cells, including corresponding optics, fit in the standard optical bench of an FT-IR spectrometer. The choice for a specific IR-window material is mainly determined by the properties of the LC eluent and the spectral region that has to be monitored. For instance, a fully IR-transparent material such as potassium bromide cannot be used with RPLC. Instead, water-insoluble materials such as calcium fluoride (transparent above 1100 cm^{-1}) and zinc selenide (transparent above 450 cm^{-1}) have to be chosen. For quantitative analysis, the spectral window of the solvent can in principle be very small as the measurement of a single wavenumber is sufficient. In contrast, qualitative information requires IR transparency over a much wider spectral region in order to determine functional groups or to identify a compound using the fingerprint region (1300–600 cm^{-1}).

Two types of flow cells can be distinguished for LC–IR: transmission cells and attenuated total reflection (ATR) cells. The basic part of a transmission cell consists of an IR-transparent cavity or two IR-transparent windows separated by a spacer. The LC effluent enters and exits the cell via capillary tubes and the IR beam perpendicularly passes the LC flow.

Zero-dead-volume (ZDV) IR flow cells have been developed to minimize the volumes needed to connect the cell to the column, which is very important when micro-LC is used. IR-transmission cells are often used in combination with a beam condenser to obtain a sufficiently high energy throughput and thus enhance the S/N of the recorded signal and/or spectra. According to Beer's law, the minimum identifiable concentration decreases when the path length of the transmission cell is increased. However, increasing the path length also results in an increase of the eluent absorbance, thus limiting the spectral window. In practice, the volume of the flow cell has to be minimized to (much) less than 1% of a typical LC-peak volume. Obviously, this puts a significant limitation on the obtainable sensitivity. Through the use of microbore LC columns, the volumes of flow cell and LC peak can be made more compatible and, for that reason, micro-LC–FT-IR is often preferred. It should be noted, however, that the loadability (both in mass and volume) of micro-LC columns is rather limited.

The second type of flow cell is based on the phenomenon of ATR and is called cylindrical internal reflectance cell or CIRCLE cell [14]. The cell consists of a cylindrically shaped IR-transparent rod-crystal with cone shaped ends which is incorporated in a flow cell. The effluent of the LC column flows around the optical crystal while the interrogating IR beam enters the crystal at one end, reflects off the internal surfaces of the crystal and then exits at the other end. Several crystal materials can be used but zinc selenide (ZnSe) is commonly preferred because of its high IR transparency, high refractive properties and insolubility in water. CIRCLE cells are available with an internal volume of 1–25 μl. The effective path length of a this type of cell is defined by the number of reflections in the optical element and, therefore, sensitivity can be enhanced by using longer crystals. These, however, also imply an increased cell volume and, thus, extra broadening of the LC peaks. CIRCLE cells cannot be used for quantification in a straightforward manner since the penetration depth of the IR radiation in the LC eluent is limited (typically 1–5 μm) and wavelength dependent. Special computer algorithms are used to compensate for this. The small optical penetration depth also implies that adsorption of small amounts of compounds to the crystal surface may have significant effects on the performance of the cell.

2.2. Use of flow-cell IR-detection

Despite the described limitations and restrictions, flow-cell IR detection has been and still is applied as a simple and low-cost method to obtain specific and/or quantitative information about major constituents of mixtures [10,11]. On-line LC–FT-IR, including automated subtraction of the solvent background, has already been described in the mid 1970s [7,8]. In subsequent studies, Taylor and co-workers [14,15] demonstrated that micro-LC offers improved IR-sensitivity compared to conventional (wide-bore) LC and that halogenated hydrocarbons can be useful eluents because of their relatively high IR-transparency. The strong IR absorption of water hampers the use of flow-cell IR detection in RPLC and therefore on-line extraction methods and deuterated solvents have been applied to circumvent the IR-opacity problems.

Johnson et al. [16] used on-line liquid–liquid extraction (LLE) to withdraw analytes from an aqueous eluent into an chlorinated organic solvent. After on-line phase separation, the organic phase was monitored in an IR-transmission flow cell. Based on an earlier proposed concept [17], DiNunzio used on-line solid-phase extraction (SPE) to allow flow-cell FT-IR detection in RPLC [18]. In an automated LC–SPE–FT-IR system (Fig. 1), the column effluent was diluted with water and the analytes of interest were trapped on several small SPE columns filled with a hydrophobic sorbent. 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.

In on-line LC–FT-IR deuterated solvents can be attractive substitutes for hydrogenated solvents. The elution properties of deuterated solvents are similar, but their absolute IR absorbance is smaller and their IR absorption bands are shifted to different spectral regions. A detailed study on the utility of deuterated solvents in flow-cell LC–FT-IR was carried out by Fujimoto et al. [19]. It follows that with respect to on-line LLE procedures the use of deuterated solvents has advantages in terms of simplicity, speed

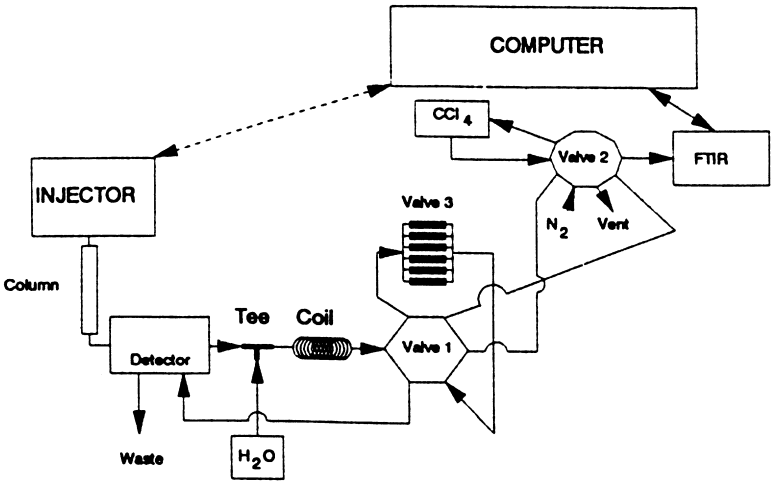

Fig. 1. Schematic of automated solid-phase extraction interface for flow-cell LC–FT-IR [18]; valve 3 holds the SPE columns.

and maintenance of chromatographic resolution. A major drawback, however, is their high price.

Despite the significant IR absorption of water, the direct detection of relatively high analyte concentrations (above 1 g/l) is possible in aqueous effluents, provided that the path length of the IR flow-cell is reduced to less than 30 μm. Recently this was demonstrated by Vonach et al. [20,21] who analyzed carbohydrates, alcohols and organic acids in soft drinks and wines by on-line LC–FT-IR using ion-exchange columns for analyte separation (Fig. 2).

In recent years, research in on-line FT-IR detection has been mainly confined to size-exclusion chromatography (SEC) [22–25] and flow-injection analysis (FIA) [26–39], 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 having

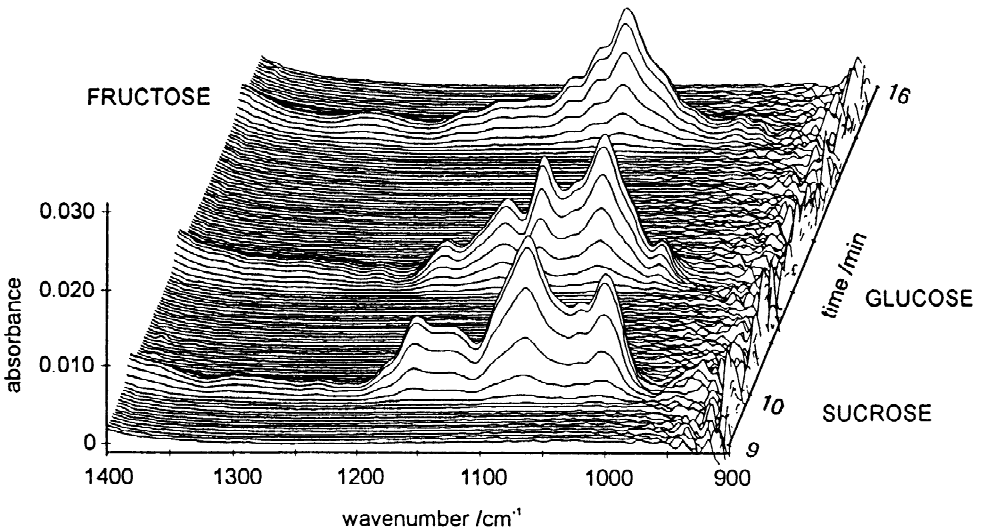

Fig. 2. Three-dimensional LC–FT-IR plot of a solution containing sucrose, glucose and fructose (10 g/l each) [20].

detrimental effects on the chromatographic performance. 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 (bio)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 [27,30–33]. 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, who applied on-line SPE [26,28,29]. 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 (Fig. 3). 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 [26]. Direct IR detection in aqueous matrices was accomplished by Kellner and co-workers [35–39] who used an optical path length of 25–30 μm for the FIA-based determination of sugars, phosphates and enzyme activities.

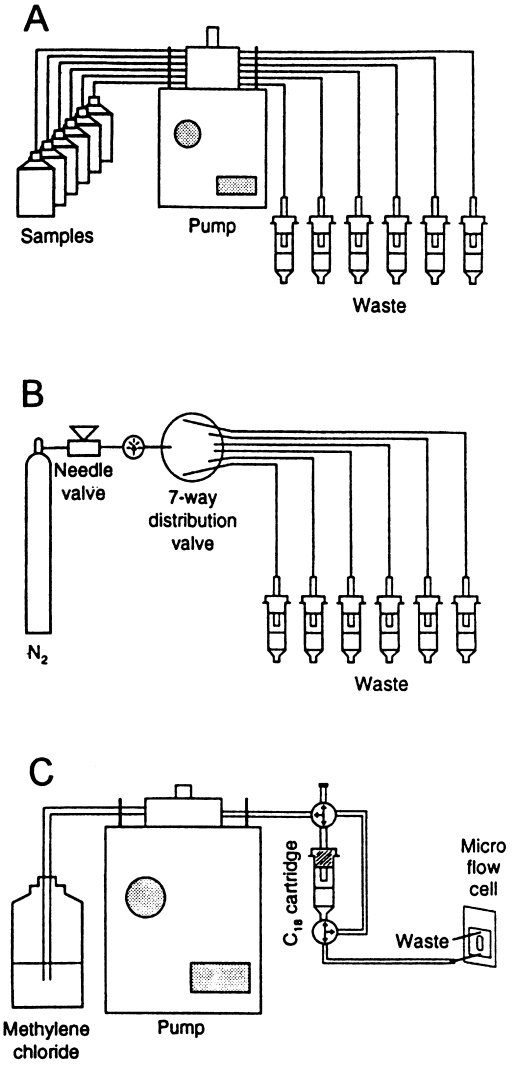

Fig. 3. SPE–FIA–FT-IR set-up for the determination of carbaryl; (A) preconcentration on C_{18} cartridges, (B) drying of the loaded cartridges, (C) on-line elution and FT-IR detection [29].

3. Solvent-elimination LC–FT-IR

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 is 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. In this way, interference-free FT-IR spectra of the deposited compounds can be recorded independently from the LC conditions and the sensitivity of the FT-IR spectrometer can be fully exploited. Next to the possibility to acquire complete spectra, there are some additional advantages of the solvent-elimination approach. By careful control of the interface performance and the speed of the substrate, concentrated deposits may be obtained which will enhance analyte detectability. Spectral analysis of the stored chromatogram can be performed without any time constraints so that signal

averaging can be used. Moreover, spectrometric detection can, in principle, be carried out repeatedly at any convenient time or place. Evidently, solvent-elimination LC–FT-IR is more complicated than on-line FT-IR detection. It requires an interface which should adequately effect the evaporation of the eluent and, at the same time, maintain the chromatographic resolution during the deposition process. In this respect, the LC flow-rate, the composition of the eluent and the nature of analytes and substrate (see Sections 3.1 and 3.2) are important factors.

With most described solvent-elimination set-ups, after immobilization of one or more chromatograms, the substrate is transferred to the FT-IR spectrometer where spectra of the deposited compounds are recorded. The deposited traces may be moved through the interrogating IR beam so that continuous FT-IR chromatograms can be constructed. Dependent on the type of substrate and/or size of the deposited spots, often special optics such as a (diffuse) reflectance unit, a beam condensor or an FT-IR microscope are used to scan the deposited substances.

3.1. Deposition substrates and detection modes

In solvent-elimination LC–FT-IR basically three types of substrates and corresponding IR modes can be discerned, viz., powder substrates for diffuse reflectance (DRIFT) detection, metallic mirrors for reflection–absorption (R–A) spectrometry and IR-transparent windows for transmission measurements. DRIFT detection of analytes on potassium chloride (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 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. [40,41] 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. Repeatability is not easily achieved since in DRIFT factors such as sample homogeneity, sample load and compactness of the powder layer, significantly influence the DRIFT analysis. Reorientation of the DRIFT matrix as a result of sample deposition may lead to a poor background compensation. Careful filling of cups or trays with the powder substrate is very time-consuming and has to be repeated for every 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. Some authors have used diamond powder as a water-resistant DRIFT substrate, but it is expensive (and thus not disposable) and not easy to clean.

Front-surface aluminium mirrors which are suitable for FT-IR detection by R–A, are compatible with aqueous eluents and are easy to handle. Compound deposition on this type of substrate requires efficient solvent-elimination interfaces because residual solvent that hits the substrate will easily spread over the hard and smooth reflective surface. 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 will therefore be largely governed by a double-pass transmittance mechanism, so that data analogous to transmission data are obtained. Useful results of solvent-elimination LC–FT-IR using mirrors have been reported (see Section 3.4); however, several authors [42–44] have reported evidence of band asymmetry and spectral distortions. 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 aluminium mirrors [44]. In order to

reduce spectral distortions, the use of an IR-transparent germanium disc with a reflective backing has been proposed as deposition substrate for R–A measurements [45]. This type of disc is used in the commercially available LC-Transform LC–FT-IR interfaces [5].

The most favorable results in solvent-elimination LC–FT-IR have been obtained with IR-transparent deposition substrates that allow straightforward transmission measurements. So far mainly KBr and ZnSe windows have been applied in experimental LC–FT-IR set-ups. Since the LC eluent is rarely eliminated completely before it reaches the substrate, 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. 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 [44]. ZnSe windows also cause fewer spectral artefacts than mirror substrates for R–A measurements [43,44].

Many studies have demonstrated that spectra obtained using ZnSe, closely resemble conventional KBr disc transmission spectra. Consequently, existing spectral libraries can be used for identification purposes which is very important for the acceptance of FT-IR as a valuable detection technique in LC. So far, the choice of search algorithms in LC–FT-IR for the automated retrieval of reference spectra that match the recorded analyte spectra, has been quite arbitrary. However, in a study on the LC–FT-IR analysis of herbicides in river water, Somsen et al. [46] showed that the performance of different search procedures is not necessarily the same. Based on a data set of 45 spectra covering two herbicide classes it was concluded that a search routine which used the matching of spectral peak frequencies only, was most suitable to identify the analytes at the trace level.

The repeated use of one substrate is common practice in LC–FT-IR with ZnSe windows and front-surface mirrors. In other words, the possibility to clean substrates between analyses is of importance. ZnSe is inert and deposited compounds can be removed simply and quickly with e.g., water, alcohol or acetone. 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. 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.

In solvent-elimination LC–FT-IR the identification limits usually improve when the width of the analyte spots is decreased. 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 condensor is too large and the use of an FT-IR microscope is indicated. Frequently, this enhancement is rationalized by considering the relatively increased spot thickness only (Beer's law), but this approach is too simple. From more complete signal-to-noise considerations it follows that the good sensitivity of FT-IR microscopic detection essentially results from the low noise level of the IR detectors in FT-IR microscopes [47]. In other words, to achieve the most sensitive IR detection in LC, the width of the analyte deposits should have the same dimensions as the microscope detector area (typically, 0.01–0.04 mm^2). Of course, as with any IR experiment, the signal-to-noise ratio also 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, although at the cost of an increased time of analysis.

3.2. Analyte characteristics

For successful deposition, the compounds ana-

lyzed by solvent-elimination LC–FT-IR should, of course, be considerably less volatile than the eluent. Since LC is used for non-volatiles in particular, generally this condition is met. 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. Spectra recorded from amorphous deposits often reveal broadened bands and, therefore, may differ from spectra present in reference libraries which generally are obtained from crystalline compounds. Also, some analytes will deposit as a smooth film, whereas others may form irregular clusters. When the deposits are crystalline, this phenomenon commonly will not induce spectral differences and the recorded analyte spectra will resemble 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. A morphological transition may take place some time after deposition, so that the spectral appearance depends on the time of recording the spectra. Finally, acidic compounds may be converted into their salts during the evaporation/deposition process yielding deviated spectra.

The aforementioned (spectral) phenomena are rather exceptional and 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.

3.3. 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. 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 at an early stage.

After some less successful attempts [48], Kuehl and Griffiths [9,49] designed an adequately working interface for the coupling of conventional-size normal-phase (NP) LC and FT-IR. The organic eluent was led through a heated concentrator tube and dropped into a series of cups filled with KCl powder suited for DRIFT analysis. Nitrogen was passed through the cups and a carousel rotated them into the FT-IR spectrometer where identifiable spectra could be recorded for sub-μg amounts of analyte. In order to allow for RPLC separations, the aqueous effluent was first on-line extracted with dichloromethane which, after continuous phase separation, was directed through the concentrator to the KCl cups [50]. For extracted compounds good-quality spectra were obtained. The carousel–DRIFT method was also adopted for narrow-bore NPLC (1 mm I.D. columns) by reducing the size of the KCl cups and by omitting the concentrator tube [51]. Using a similar set-up, Kalasinsky and co-workers [52,53] coupled both narrow-bore NP- and RPLC with DRIFT. The KCl powder 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 post-column reaction with 2,2′-dimethoxypropane. The identification limits of these systems were typically 1–3 μg.

The early DRIFT-based systems for the first time demonstrated that solvent-elimination LC–IR can provide (much) better sensitivity and spectral quality than flow-cell based LC–IR. However, the systems were mechanically complex and tedious to work with, and DRIFT detection appeared to be strongly affected by small disturbances of the KCl powder surface and by the presence of (residual) water.

Jinno and co-workers [54,55] proposed the use of micro-LC columns (0.3 mm I.D.) in solvent-elimination LC–IR in order to alleviate the problem of the evaporation of large eluent volumes. The effluent (5 μl/min) from either a SEC or an NPLC system was

led directly to a moving KBr plate which was covered by a stream of heated nitrogen. Subsequently, the plate with the deposited track was scanned by IR transmission spectroscopy using a 3× beam condensor. The potential of the approach (termed ''buffer-memory'' technique) was illustrated by the analysis of a mixture of dithiocarbamate metal complexes by three spectroscopic techniques [55]. In a modified set-up (Fig. 4) the linearly moving KBr plate was replaced by a rotating KBr disk. After the chromatographic run was finished, it was transferred to a special rotation module in the IR spectrometer [56]. In order to permit the use of RPLC, a stainless steel wire net was used instead of an KBr window [57]. IR transmission measurements were possible because after deposition and drying the analytes were partly suspended in the metal meshes.

The ''buffer-memory'' technique demonstrated the usefulness of the storage of a continuous chromatogram on a flat substrate. Besides, it was considerably simpler than the DRIFT methods. However, at least several micrograms of analyte were needed for a positive IR identification. These amounts often exceeded the sample capacity of the micro-columns and required unrealistically high concentrations to be injected.

3.4. Spray-type interfaces

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

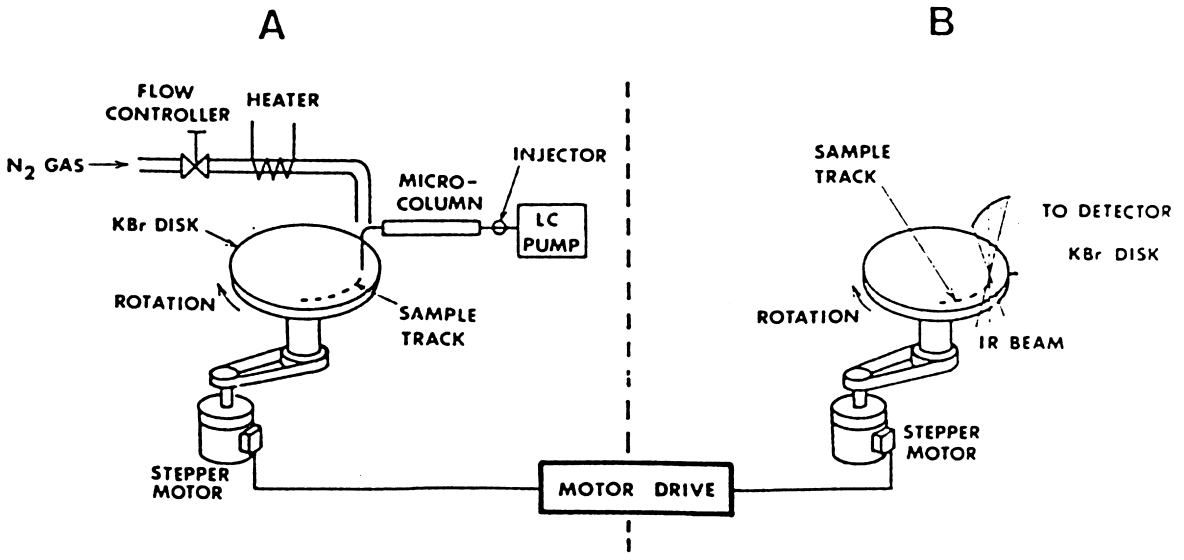

Fig. 4. Schematic of micro-LC–FT-IR system in which (A) the LC effluent is deposited on a rotating KBr disc and (B) IR spectral data are recorded from the deposited compounds [56].

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.

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. The following classification of these LC–FT-IR interfaces is primarily based on the applied method of solvent elimination.

3.4.1. Thermospray interface

In the thermospray (TSP) interface the LC eluent is led through a directly heated vaporizer tube. In the tube, part of the liquid evaporates to an expanding vapor and, as a result, a mist of desolvating droplets emerges from the end of the tube. When using 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 [58] 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 (0.8 ml/min) as eluent, and the analytes were deposited on diamond powder via a TSP. The TSP did not completely evaporate the aqueous eluent, so that KCl powder could not be used. Heating of the diamond powder allowed evaporation of the residual eluent, but phenol itself could not be detected, probably because it evaporated. Satisfactory spectra of the three remaining phenols were obtained when μg amounts were injected. Improved analyte detectability was achieved by coupling the TSP to a narrow-bore LC column and using a flow-rate of 20–50 μl/min [58].

Jansen [59] 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 steel tape which moved through an adapted IR reflectance accessory mounted in the sample compartment of the FT-IR spectrometer (Fig. 5). Most of the eluent was eliminated directly by the TSP and residual solvent, if any, was evaporated off the tape by an infrared lamp. The immobilized chromatogram was monitored continuously and solvent-interference-free spectra were recorded. The practicality of the system was demonstrated by analyzing several simple polymer samples (20–80 μg injected) by SEC–FT-IR with dichloromethane or tetrahydrofuran 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 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 and co-workers [60–62], mainly for RPLC–FT-IR. After a preliminary study [60] in which amino acids were analyzed, the interface design was optimized and the analyte-deposition efficiency and analytical potential were studied [61]. The TSP temperature and the TSP height above the moving tape were varied in order to obtain deposited spots that matched the IR beam (ca. 2 mm) of the IR reflectance 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 saccharides and aliphatic carboxylic acids. However, with saccharides the spectra showed significant band broadening in the fingerprint region due to thermal effects. The TSP–FT-IR system was also used for polymer analysis: to identify antioxidants (Fig. 6) [61] and to characterize polyesters and polystyrenes [62,63].

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

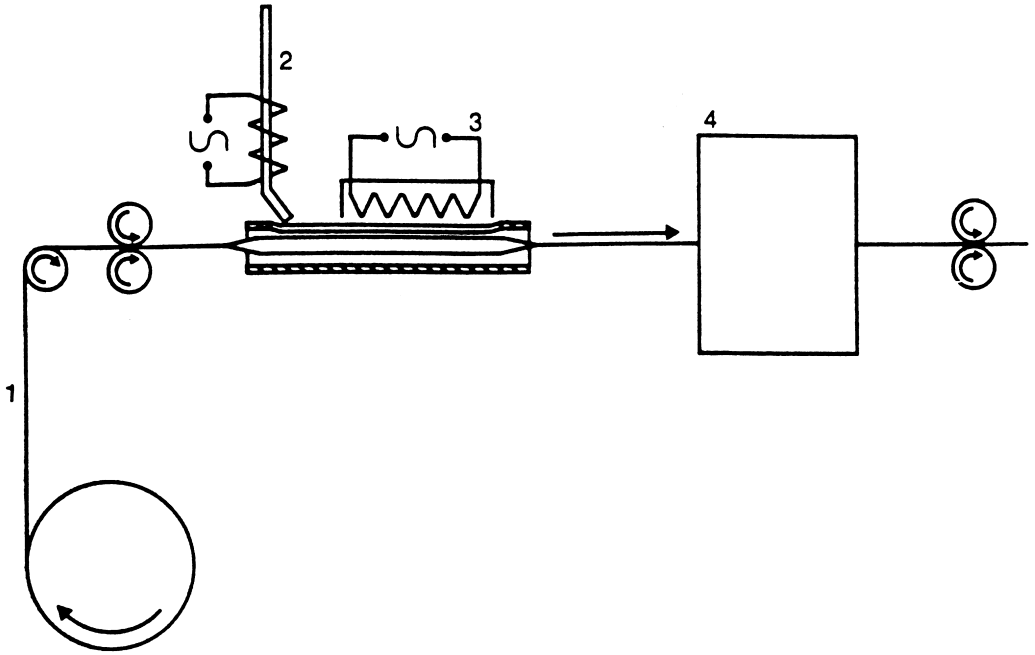

Fig. 5. Schematic of thermospray–moving belt interface for LC–FT-IR; 1=moving stainless steel tape; 2=thermospray interface; 3=infrared lamp; 4=diffuse reflectance cell mounted in the sample compartment of the FT-IR spectrometer [59].

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 which results in a moderate FT-IR sensitivity.

3.4.2. Particle beam interface

The particle beam (PB) interface, a solvent-elimination interface originally developed for LC–MS, was modified for LC–FT-IR by de Haseth and co-

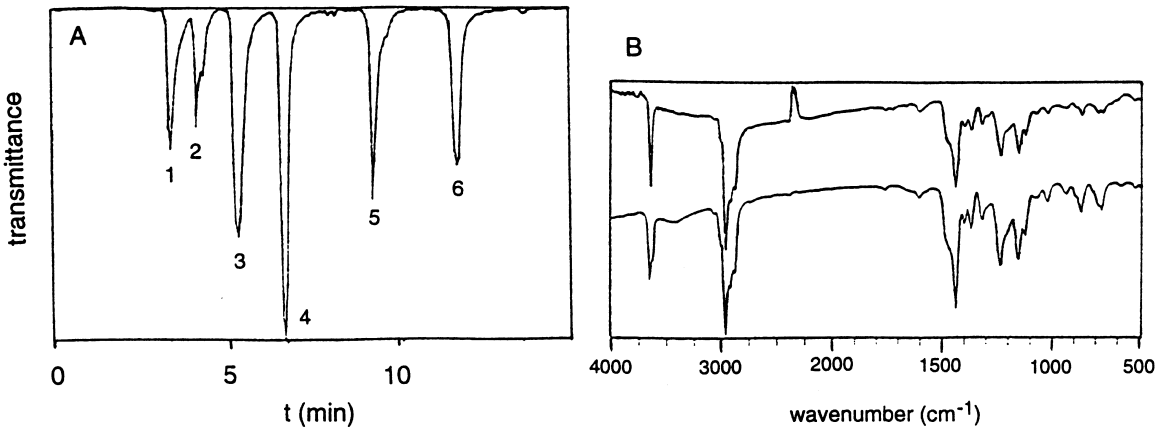

Fig. 6. (A) LC–TSP–FT-IR functional group (3100–2800 cm^{-1}) chromatogram of phenolic antioxidants. Peaks: 1=Irganox 3114 and 1035; 2=Irganox 1010; 3=Irganox 1330; 4=Irganox 565; 5=Irganox 1076; 6=Irgafos 168. (B) LC–TSP–FT-IR spectrum (top) and standard FT-IR spectrum (bottom) of Irganox 1330 [61].

workers [64–71] and Wood [72]. 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, vapor and condensed 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 vapor (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; that is, no complete chromatograms, but fractions were analyzed.

A preliminary study [65] 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 and via the PB interface the analytes (50 μg each) were deposited individually on a KBr disc. Subsequently, transmission spectra were recorded for both compounds using a 5× beam condensor accessory (Fig. 8). Wood [72] studied LC–PB–FT-IR using dioctyldiphenylamine as model compound. With hexane as eluent a spectrum of 2 μg of the analyte could be obtained after its deposition on KBr.

In solvent-elimination LC–FT-IR the presence of buffer salts in the eluent seriously disturbs the

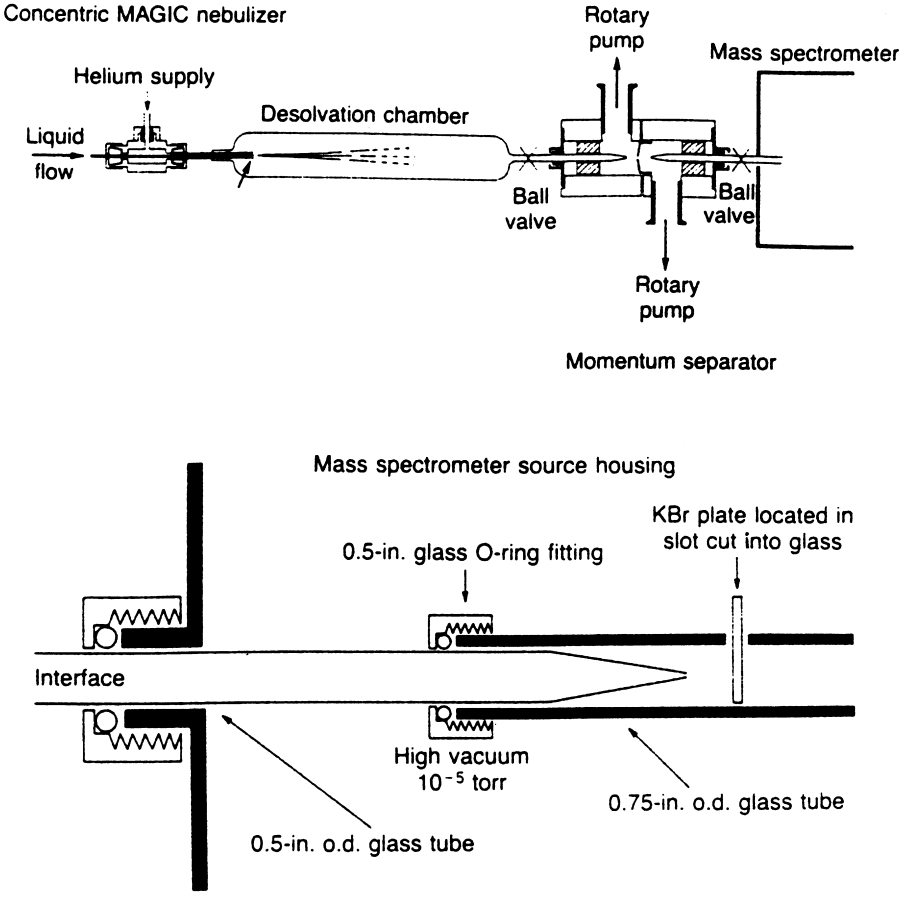

Fig. 7. Schematic of particle beam interface for LC–FT-IR as used by Wood [72].

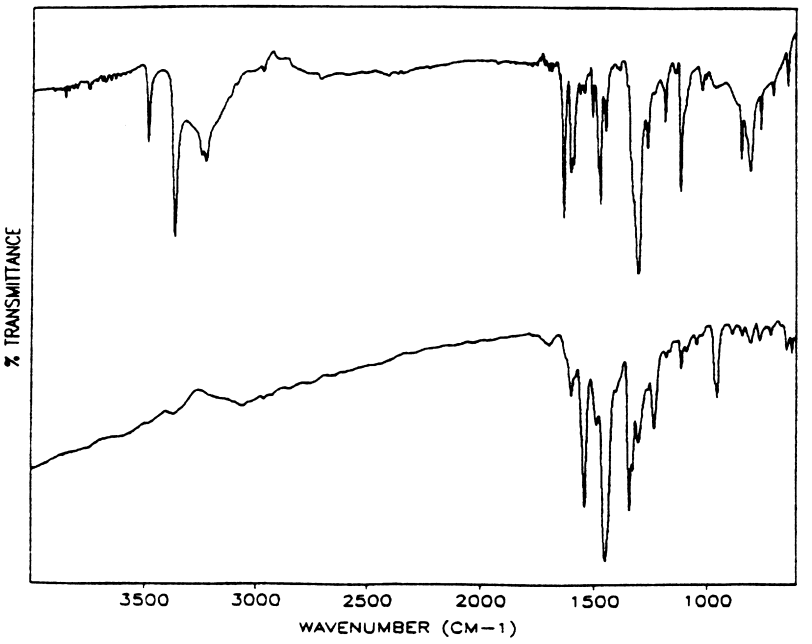

Fig. 8. LC–PB–FT-IR spectra of (A) *p*-nitroaniline and (B) erythrosin [65].

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. [66] studied the deposition and IR detection of caffeine from several buffered solvent systems. The interface indeed appeared to be able to process a 0.3 ml/min flow of buffered eluent; however, the buffer salts were never completely eliminated. Best results were obtained with eluents buffered with ammonium acetate, although buffer bands were clearly present in the FT-IR spectra recorded from μg amounts of analyte. 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.

The PB–FT-IR interface has been used as a tool for the determination of protein structures [67–69]. 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. On the basis of major IR amide bands, structural changes of the protein caused by the LC stationary phase and the organic modifier were determined [69]. The sample loads in these experiments generally were quite high (5–500 μg). However, when using an FT-IR microscope a spectrum was obtained for 100 ng α-chymotrypsin, although some interfering bands from an eluent impurity were also present. Recently, Turula and co-workers [70,71] demonstrated the combined use of LC–PB–FT-IR and LC–electrospray–MS for the structural characterization of peptides and tryptic digests of β-lactoglobulin.

The PB interface can effectively remove both organic and aqueous solvents; however, relevant applications in LC would 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 [64], 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.

3.4.3. Electrospray interface

The feasibility of electrospray (ESP) nebulization as a means of coupling micro-LC and FT-IR was studied by Raynor et al. [73]. A high electrical field is used to produce a spray of charged droplets at the end of a capillary filled with flowing liquid. As a result of solvent evaporation and charge density, the initial droplets break up into smaller droplets which further facilitates solvent evaporation. Use of low flow-rates (typically, 1–20 μl/min) is indicated in order to obtain stable ESP operation.

Raynor et al. used an ESP interface to deposit the effluent from a micro-RPLC column (4 μl/min) on a 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 (20 ng each) was separated with methanol–water and could be analyzed successfully using an FT-IR microscope (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 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.

3.4.4. 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.

Gagel and Biemann [74] 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

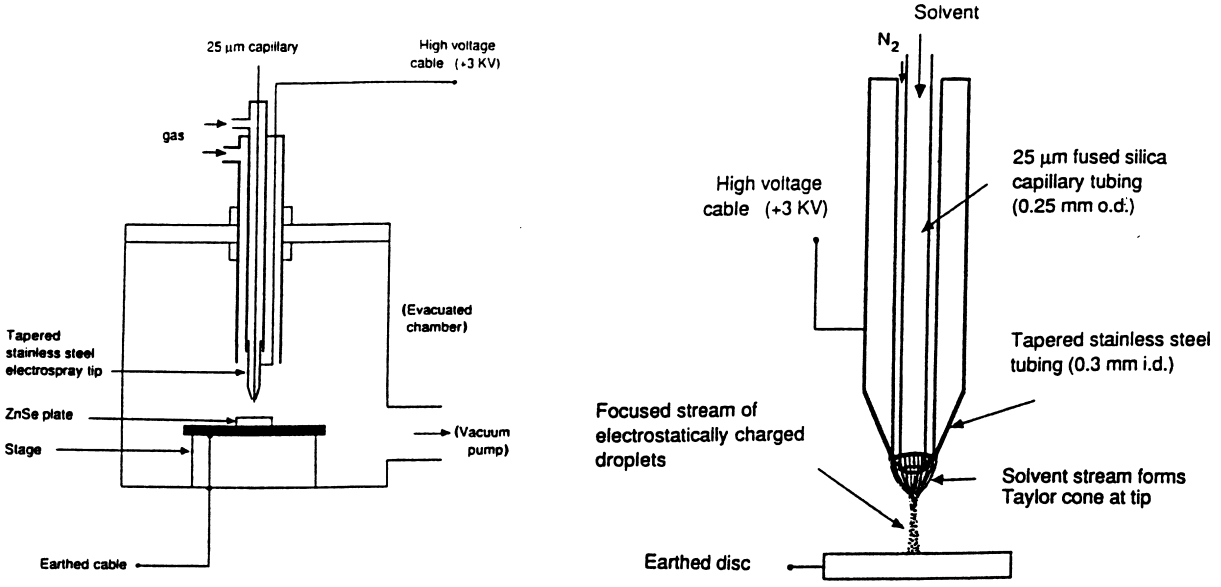

Fig. 9. Schematic of electrospray interface for micro-LC–FT-IR (left) with the electrospray tip in detail (right) [73].

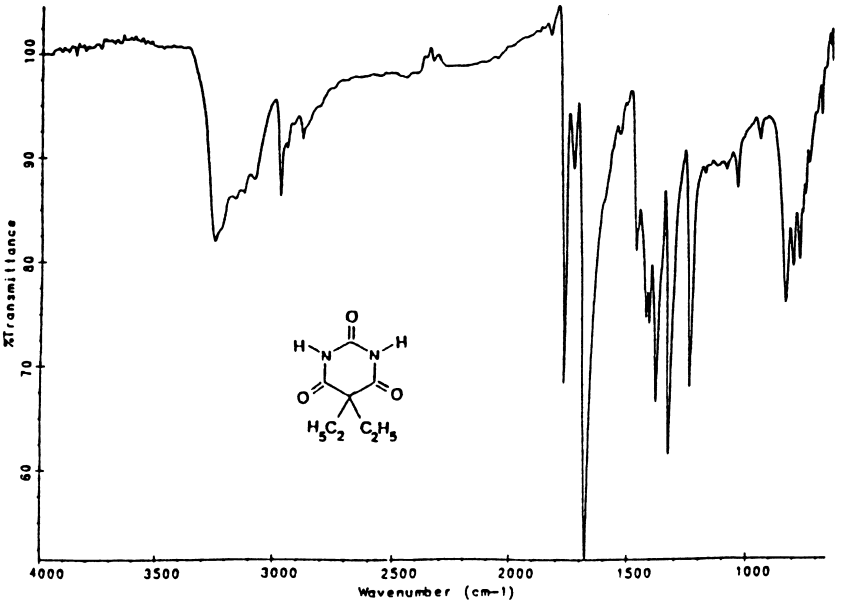

Fig. 10. LC–ESP–FT-IR spectrum of barbital [73].

needle from which a fine spray emerged resulting in a 1–2 mm wide deposition track (Fig. 11). The immobilized chromatogram was analyzed by rotating the disc through a 3× condensed IR beam while recording R–A spectra. The performance of the system was tested with a mixture of polycyclic aromatic compounds (200–800 ng each) which were separated on a 1 mm I.D. silica column using hexane–dichloromethane (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.

In order to accomplish elimination of aqueous solvents, the nebulizer design was improved [42]. The syringe needle was placed inside a nozzle through which heated nitrogen gas flowed to enhance solvent evaporation and focus the spray. 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 naph-

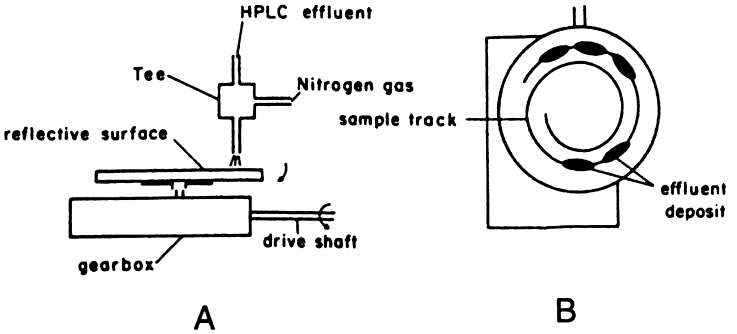

Fig. 11. Schematic of the narrow-bore LC–FT-IR system of Gagel and Biemann [74]; (A) side view during deposition; (B) top view of collection mirror.

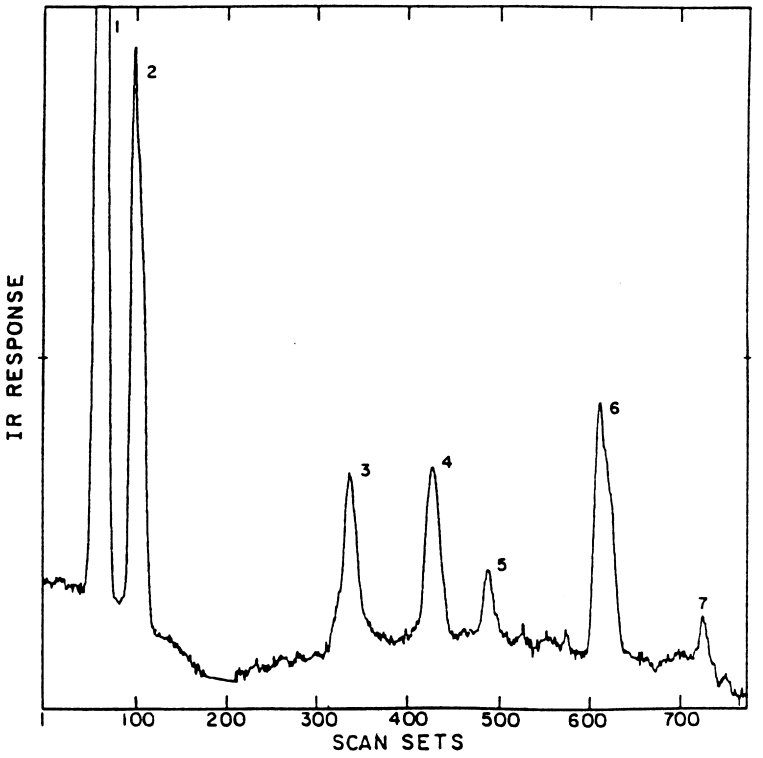

Fig. 12. LC–FT-IR chromatogram (Gram–Schmidt) recorded after deposition of a gradient elution separation of a mixture of (1) caffeine, (2) 2,7-dihydroxynaphthalene, (3) phenanthrenequinone, (4) carbazole, (5) anthrone, (6) 9-nitroanthracene and (7) anthracene [42].

thalenediols (500 ng each) was demonstrated and an identification limit of 31 ng (injected) was obtained for phenanthrenequinone. Again, 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 original aluminium disc by a 2-mm thick IR-transparent germanium disc with a rear surface of IR-reflective aluminium [45]. The authors claimed that the germanium layer prevents interference of the incident and reflected IR beams 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 [75–78]. The essentials of these polymer-composition characterization studies are summarized in Table 1. Jordan et al. [79] used the LC Transform for the identification of triclosan, an antibacterial agent, in toothpaste.

Lange et al. [80] constructed a simple but effective concentric flow nebulizer (CFN) for the coupling of narrow-bore LC and FT-IR. The interface consists of two concentric fused-silica capillaries. The LC column effluent is led through the inner capillary and helium gas through the outer capillary (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 [40] 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

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	[75]
Polystyrene–PMMA	1–6	0.2	THF	1	0.07	[76]
Styrene–butadiene rubber	–	–	THF	–	–	[77]
PC–ABS; HIPS	1	0.15	THF	–	0.1	[78]

[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.

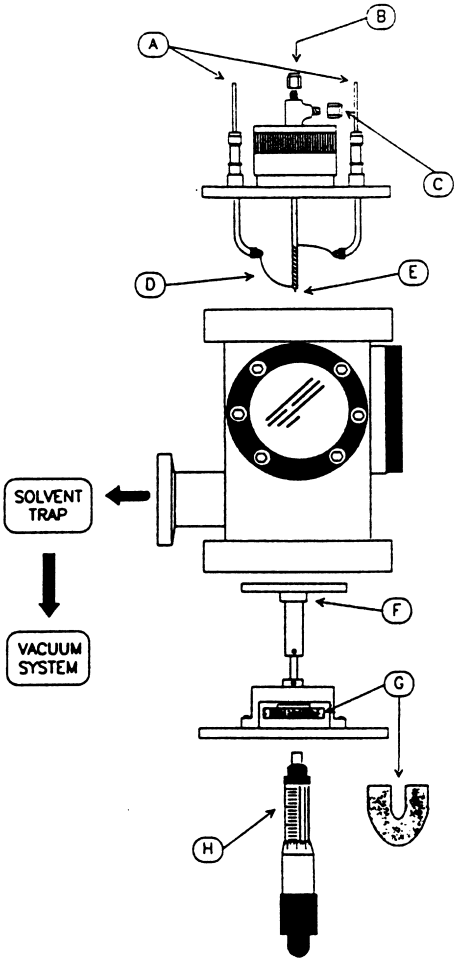

Fig. 13. Concentric flow nebulizer for narrow-bore LC–FT-IR (top) and interface chamber (bottom); A=electric connections; B=LC effluent inlet; C=helium gas inlet; D=heating wire; E=concentric fused-silica tubes; F=stage; G=magnet for stage rotation; H=vertical positioner [80].

was placed in a vacuum chamber (Fig. 13, bottom). Finally, 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 [81] 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, Düsseldorf, Germany) GC–FT-IR interface which allows spectral acquisition in real time. So far, only some preliminary results with this

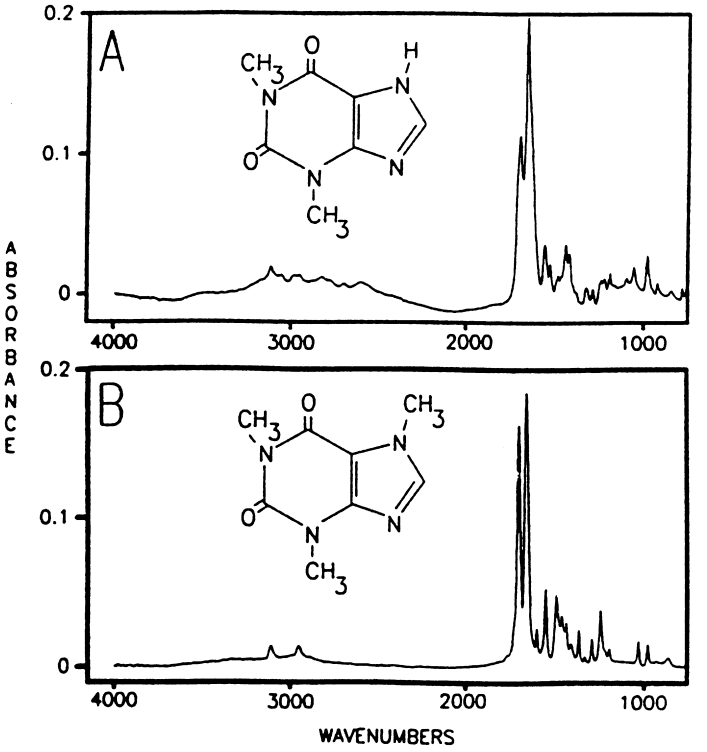

Fig. 14. LC–CFN–FT-IR spectra of (A) theophylline and (B) caffeine [80].

on-line LC–FT-IR system have been reported. These show reasonable IR peak shapes for theophylline (FIA of 140 ng) and real-time acquired spectra of 6–300 ng of the same compound [82].

The handling of buffered solvent systems by the CFN has also been studied [83]. 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 and co-workers [43,84–86] modified a spray-jet interface which was originally developed for the on-line hyphenation of LC and thin-layer chromatography, 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 [43] with quinones and polyaromatic hydrocarbons 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 [84] and for the characterization of a synthetic mixture of chlorinated pyrenes [85]. 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 [86]. For example, analysis of a poly-

(vinylchloride) 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 analyzing a polystyrene standard mixture [86]. 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.

When RPLC is used, 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 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

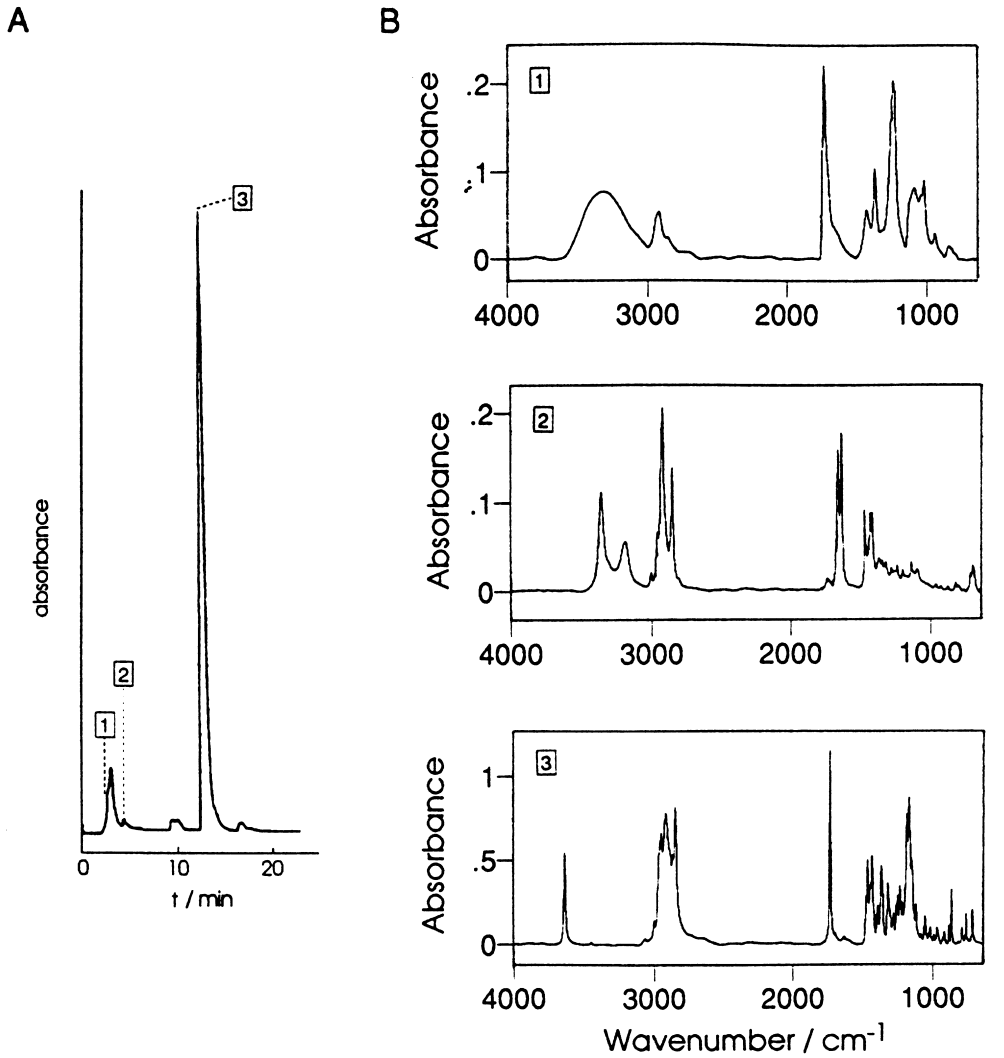

Fig. 15. (A) LC–UV chromatogram (275 nm) of a poly(vinylchloride) sample extract. (B) FT-IR spectra of peaks 1–3. On the basis of library search and spectral interpretation the spectra were assigned to (1) a monoesterified *N*,*N*-bis(hydroxyethyl)alkylamine, (2) oleamide and (3) Irganox 1076 [86].

interface [87]. 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 3.4.2 and 3.4.4). Since the salts are not extracted, phosphate-buffered eluents (0.01 *M*) can be used without causing interferences [46,87]. 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) [46].

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 [88,89]. 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 became essentially independent of the water content of the eluent and the system allowed 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 ml, the identification limits were improved to 10–160 μg/l.

3.4.5. 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. Castles et al. [90] used ultrasonic nebulization for the deposition of compounds separated by narrow-bore RPLC onto diamond powder suitable for DRIFT detection. Spectra of satisfactory quality were obtained for 3 μg of analyte. 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 [91] developed an interface for high-temperature SEC–FT-IR which involved an ultrasonic nebulizer [92]. The nebulizer was placed in a vacuum chamber and sprayed the SEC effluent on a set of heated KBr discs which were subsequently analyzed by FT-IR transmission spectrometry. With trichlorobenzene as SEC eluent, the system was applied to the determination of compositional changes of ethylene–propylene rubbers and a block polymer reaction product. An interface comprising an ultrasonic nebulizer in a vacuum chamber is used by Lab Connections in their LC Transform 300 Series [5]. This 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 3.4.4). The system has been used for the quantitative analysis of copolymers by SEC–FT-IR [93] and also for steroid analysis by RPLC–FT-IR [94].

4. Conclusions and future developments

In the last 15 years, LC–FT-IR has emerged as a potentially powerful tool for the specific detection of major components (flow-cell approach) or for the identification of (minor) constituents of complex mixtures (solvent-elimination approach). Nowadays, both coupling approaches are applied, but their purposes often are quite different.

4.1. Flow-cell IR-detection

The flow-cell procedure has developed into a special-purpose method with a somewhat restricted

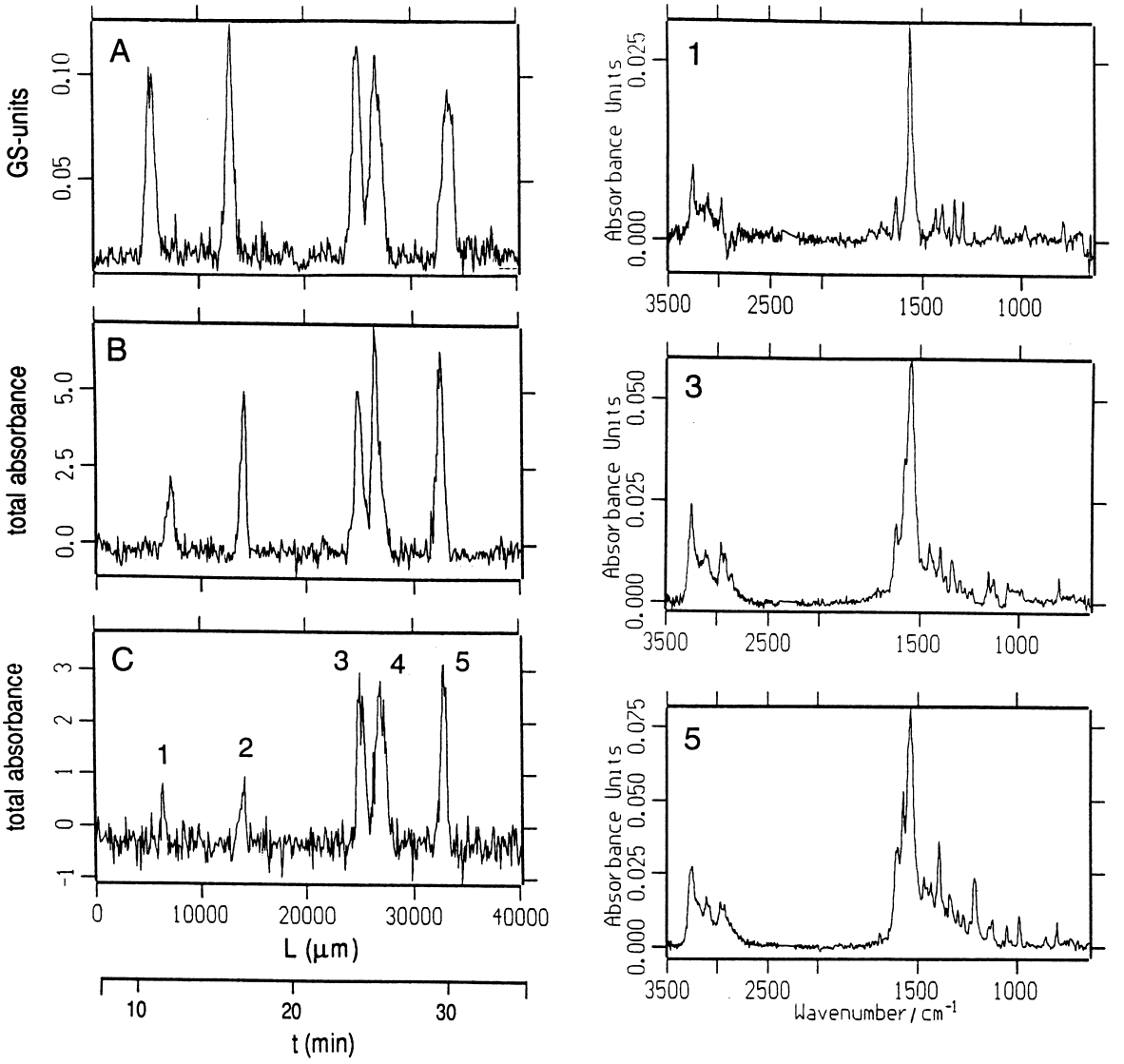

Fig. 16. (Left) SPE–LC–LLE–FT-IR chromatograms of River Meuse water samples spiked with five triazines: (A) 20 ml (30 μg/l), (B) 50 ml (6 μg/l) and (C) 100 ml (2 μg/l). (Right) FT-IR spectra of peaks 1, 3 and 5. Chromatogram representation, (A) Gram–Schmidt or (B and C) spectral window (1650–1500 cm^{-1}). Peaks: 1=simazine; 2=atrazine; 3=sebutylazine; 4=propazine and 5=terbutylazine [46].

applicability. Since IR absorptions of any solvent invariably take up parts of the mid-IR spectral region, the identification power of FT-IR spectrometry cannot be fully exploited in flow-cell FT-IR detection. Moreover, with respect to common LC detection techniques like e.g., UV absorption detection, the sensitivity of flow-cell FT-IR detection is rather poor. Nevertheless, when an eluent with proper spectral windows is selected, it is possible to monitor absorptions that are specific for the analyte or for a certain functional group. Therefore, flow-cell LC–FT-IR is used occasionally as a universal and relatively simple method to obtain quantitative (and sometimes structural) information on major constituents of samples. Various types of flow cells are commercially available, and the experimental set-up

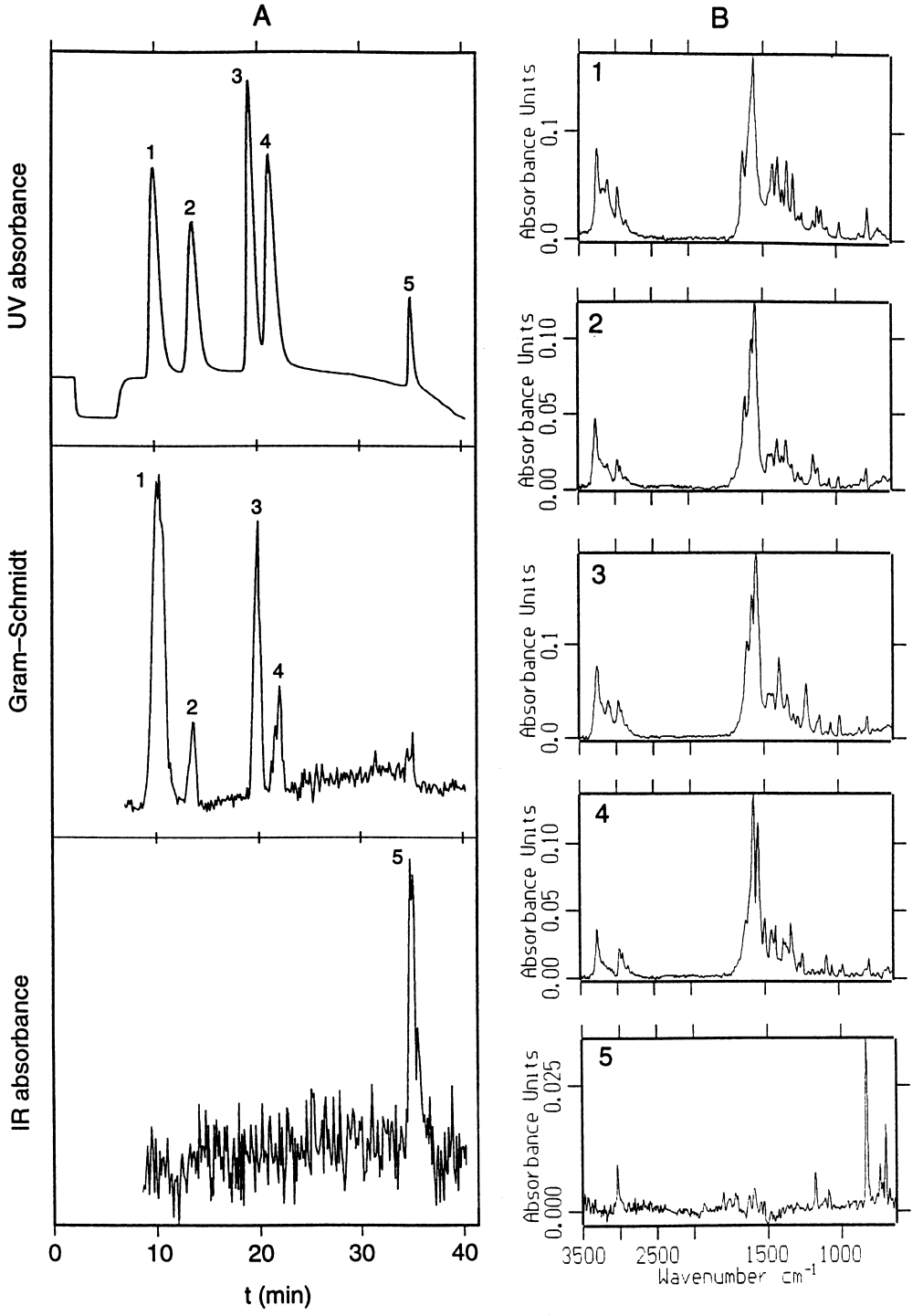

Fig. 17. (A) Gradient-LC–UV (top) and gradient-LC–FT-IR (centre and bottom) after on-line trace enrichment of a 40-μl mixture of test compounds (1 mg/l each). (B) FT-IR spectra recorded of peaks 1–5. FT-IR chromatogram representation, (centre) Gram–Schmidt or (bottom) spectral window (850–820 cm^{-1}). Peaks: 1=simazine; 2=atrazine; 3=terbutylazine; 4=trietazine and 5=pyrene [89].

of on-line LC–FT-IR is well-suited for routine applications.

As the limitations of flow-cell LC–FT-IR are inherent in the technique, no dramatic improvements can be anticipated in the near future. Some gain in performance may be achieved by optimization of the flow-cell design and the use of advanced FT-IR spectrometers, but these improvements will be modest and not essentially change the applicability of on-line LC–FT-IR. Probably, the use of flow-cell FT-IR detection will shift more and more to FIA and sensor-type applications, – that is, to situations where the multiplex character of FT-IR spectrometry can be used to specifically (and simultaneously) detect one or a small number of analytes. In such cases employing advanced chemometrical techniques will be essential because the recorded signal or spectrum is a complex result of the absorbances of various substances [35]. Recently, it was demonstrated that, when the proper precautions are taken, it is possible to detect compounds such as carbohydrates and alcohols in aqueous matrices or even whole blood [20,21,95]. These observations may lead to a further (re)appreciation of flow-cell FT-IR detection. In this respect, the on-going development of flexible, IR-transparent optical fibers is important because, in time, it will open up the possibility to perform ATR-like FT-IR detection in small volumes of biofluids. Such a set-up could be used, for example, for the determination of glucose in microdialysates [96,97].

4.2. Solvent-elimination LC–FT-IR

As has been outlined above, on-line LC–FT-IR cannot get around the detection limitations imposed by the LC eluent and, therefore, coupling procedures based on analyte deposition prior to FT-IR detection offer a number of advantages. These include the possibilities (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. Not surprisingly, the commercial LC–FT-IR systems which are presently available [5,6], are solvent-elimination devices.

Interestingly, also in GC–FT-IR and supercritical fluid chromatography–FT-IR (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 [98–101]. Indeed, mutually similar interfaces have been developed for the coupling of GC [2], SFC [102] and LC [43,80] 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.

4.2.1. State-of-the-art

Today, the vast majority of LC separations is carried out by means of RPLC and the more recent research in the field of solvent-elimination 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 best, moder-

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	[57]
Thermospray	800	Diamond powder	DRIFT	2000	–	[58]
	50	Diamond powder	DRIFT	10	–	[58]
	500	SS tape	R–A	10 000	–	[59]
	1000	SS tape	R–A	1000	25	[61]
Particle beam	300	KBr window	trans	1000	200	[65,66]
Electrospray	4	ZnSe window	trans-micr	1	10	[73]
Pneumatic nebulizer	30	Al mirror	R–A	30	30	[42]
	2	ZnSe window	trans-micr	0.5	8	[80]
	50	ZnSe window	trans-micr	1	17	[81]
	20	ZnSe window	trans-micr	5	3	[43,85]
Ultrasonic nebulizer	40	Diamond powder	DRIFT	1000	1000	[90]
	500	Ge disc	R–A	100	20	[94]

[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.

ate (100 ng) and often unfavorable (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 essentially represent the state-of-the-art, will be summarized below.

The pneumatic interfaces combine rapid solvent elimination with a relatively narrow spray. The latter aspect allows analytes to be deposited on e.g., ZnSe in narrow spots, so that FT-IR transmission microscopy can be applied to achieve mass sensitivities in

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	[52]
DMP/concentrator	500	KCl powder	DRIFT	5000	1000	[53]
LLE/concentrator	800	KCl powder	DRIFT	100	10	[50]
LLE/pneumatic nebulizer	200	ZnSe window	trans-micr	30	0.2	[87]
	200	ZnSe window	trans-micr	50	0.001[c]	[46]
Make-up/pneumatic nebulizer	2	ZnSe window	trans-micr	20	0.02[c]	[89]

[a] 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 solid-phase extraction.

the low- or even sub-ng range. It is noteworthy that with a microscope often only part of the injected amount of analyte is actually analyzed. In other words, 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.

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- 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, even pure water can be eliminated efficiently. However, for flow-rates of 20–50 μl/min enhancement of the solvent evaporation efficiency is required, for example by mixing the effluent with nitrogen gas [42] or by placing both the nebulizer and the deposition substrate inside a vacuum chamber [81]. With the latter set-up, 50 μl/min flows of pure water can be eliminated, although the 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 also be circumvented by on-line LLE of the aqueous effluent with an organic solvent which subsequently is led to the evaporation interface [46,87]. With such a 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. Solvent-elimination RPLC–FT-IR with gradient elution poses the problem of the efficient evaporation of an eluent with a changing water content. One solution involves the increase of the temperature of the nebulization gas during the gradient run [42]. Another option is the addition of excess methanol to a micro-LC effluent in order to mask the changes in its water content and, thus, eliminate the need to change the interface conditions during the gradient run [88,89].

The use of buffered eluents is generally avoided in solvent-elimination LC–FT-IR, since buffer salts may seriously affect the deposition and detection of the analytes. 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 [46,87].

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 which is certainly adequate for a number of analytical applications [84–86]. By using the LLE–pneumatic nebulizer combination, analyte detectability can be improved to sub-mg/l levels 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. Fortunately, this problem can largely be overcome by combining LC, preferably on-line, with SPE to allow analysis of sample volumes of 1–100 ml. Using SPE–LC–LLE–FT-IR, herbicides present in river water could be identified at the low-μg/l level [46]. 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 be obtained in micro-LC–FT-IR using micro-precolumns for the injection of relatively large sample volumes (0.1–1 ml) [89].

The effective solvent elimination by pneumatic nebulizers allows the use of deposition substrates with a hard and smooth surface such as ZnSe windows. With these substrates interference- and distortion-free transmission spectra can be obtained which can be readily compared with KBr disc IR spectra. This implies that currently available libraries of condensed-phase reference spectra can be used for spectral recognition and identification purposes. Compounds of various nature such as quinones, steroids, drugs, polymer additives and herbicides have been analyzed successfully by pneumatic-

nebulization-based LC–FT-IR. The interfaces can handle most types of analytes, although too volatile compounds will be evaporated by the nebulizer gas and, therefore, will not be deposited on the substrate. For example, low-molecular-mass polyaromatic hydrocarbons such as naphthalene and anthracene could not be analysed by solvent-elimination LC–FT-IR [43]. Thermal degradation of analytes is usually 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. This is nicely illustrated by the LC–FT-IR analysis of thermolabile phenylurea herbicides using a nebulization temperature of 150°C: spectra of the parent compounds were obtained and no degradation products were observed [46,87].

4.2.2. Perspectives

The usefulness of solvent-elimination LC–FT-IR to provide structural information and/or identification of sample constituents has been demonstrated convincingly during the last decade. Unfortunately, the development of coupling techniques proceeded, and still proceeds, quite slow and until now most LC–FT-IR interfaces have been used only by their designers. Nevertheless, the difficulty of solvent-elimination LC–FT-IR, i.e., simultaneous eluent evaporation and analyte deposition, seems to be a technical rather than a fundamental problem. In other words, the development of an overall effective and routinely applicable interface probably seems to be a matter of time, effort and technological innovations.

Of course, solvent-elimination LC–FT-IR has to compete with other analyte-characterization identification techniques. Presently, on-line LC–MS undoubtedly is the most important and versatile identification technique. 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 order to enhance the use of LC–FT-IR, several items of interest should be considered. The practicality of the technique for real-life samples should be demonstrated more extensively. The applications described so far [46,84–86] indicate that LC–FT-IR can indeed be used for the characterization and unambiguous identification of target and unknown compounds. LC–FT-IR is particularly useful for the distinction of isomeric compounds [46,85,89] which cannot be distinguished by LC–MS. For the general application of solvent-elimination LC–FT-IR, the availability and use of commercial interfaces is essential. The LC-Transform (Lab Connections) interfaces [5] have been available now for several years but, although a considerable number of application notes is available, unfortunately only few applications have been reported in literature. Because this solvent-elimination system uses a mirror substrate disc and standard FT-IR equipment, both the FT-IR sensitivity and spectral quality are limited. Very recently, Lab Connections marketed an accessory which allows analysis of the optical disc by FT-IR microscopy. However, the deposits produced by their interfaces generally are too wide to fully benefit from the sensitivity of FT-IR microscopes. In a more viable approach an IR-transparent substrate should be used together with a narrow-spraying interface and microscopic FT-IR detection. Such a configuration is used by the Infrared Chromatograph (Bourne Scientific) interface [6]. 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 Infrared Chromatograph seems promising but, as it has been introduced only recently, it is still too early to assess its merits.

Another item of attention is the development and use of appropriate on-line sample-treatment procedures to improve analyte detectability. The first studies indicating the advantage of on-line SPE in

solvent-elimination LC–FT-IR have been reported [46,89]. Proper 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.

Finally, it is noted that the solvent-elimination approach in LC is not restricted to FT-IR detection but can, in principle, be applied to any spectrometric technique which requires the compounds of interest to be present as deposits [103]. An example of such an analyte-deposition-based detection technique is matrix-assisted laser desorption/ionization (MALDI) MS. From a technical viewpoint the collector systems developed for the coupling of LC or capillary electrophoresis with MALDI-MS show a strong similarity with solvent-elimination LC–FT-IR systems. Lab Connections has recognized this and recently introduced the LC-Transform Series 500 interface for the semi on-line coupling of SEC and MALDI-MS. Obviously, an exchange of designs, methods and ideas between the various domains of hyphenation-in-LC which involve analyte deposition, will be beneficial, if not essential, to further improve interface performance.

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