3 Coupling of liquid chromatography and Fourier-transform infrared spectroscopy

3.1 Introduction

Fourier-transform infrared (FTIR) spectroscopy deals with the quantitative measurement of the interaction between IR radiation and materials. FTIR reveals molecular-vibrational transitions and provides characteristic information on molecular structure [1, 2]. The combination of liquid chromatography (LC) and FTIR can be highly useful when specific detection or identification of separated compounds is required. The high speed and multiplex nature of FTIR allows spectra to be recorded in real time at any point in the chromatogram. During or after the LC separation, software can be used to calculate a total IR-absorption-based chromatogram (via Gram-Schmidt vector orthogonalization) or to reconstruct functional-group chromatograms at one or more specific wavelengths. The application of FTIR spectroscopy in LC is, however, still rather limited, mainly because solvents commonly used in LC are strong IR absorbers, limiting both sensitivity and obtainable spectral information. Because of this fundamental incompatibility, the combination of LC and FTIR has been subject of research for more than twenty-five years now. In the development of LC–FTIR techniques two basically different coupling methodologies can be discerned, namely one that involves flow cells [3–26] and one that involves solvent-elimination interfaces [27–95]. In the flow-cell approach, the eluent is led directly through a cell where IR spectra are recorded continuously, offering fast and relatively easy detection of eluting analytes. The significant IR absorption of the eluent, however, may obscure large parts of the IR spectrum and dictates the use of short optical pathlengths. The solvent-elimination approach involves an evaporation interface for the removal of the interfering eluent and subsequent analyte deposition onto a suitable substrate, prior to FTIR detection of the analyte. In this case detection is no longer affected by the IR characteristics of the mobile phase and full spectra of relatively low amounts of compound can be obtained. The challenge of an effective solvent-elimination technique lies in the eluent evaporation and subsequent analyte deposition, while maintaining the integrity of the obtained LC separation. This chapter provides an overview of the principles, practical aspects and current status of LC–FTIR, covering both flow-cell and solvent-elimination interfaces.

3.2 Flow-cell interfaces

Flow cells offer a simple and straightforward means for the on-line coupling of LC and FTIR. The effluent of the LC is passed directly through a flow cell and IR spectra are acquired in real time. The merits of the approach include low cost, instrumental simplicity, ease of operation, low maintenance, and the possible use of non-volatile buffers. The

analyte can be studied without any orientation or crystallization effects [27, 28], oxidative degradation [25], or evaporation, which might occur during or after solvent elimination. Because flow-cell detection takes place in real-time, it is also potentially useful for on-line reaction monitoring. On the other hand, the dynamic nature of the IR measurements leaves less time to collect spectra, limiting the signal-to-noise ratio (SNR).

The major drawback of flow-cell LC–FTIR is the rather limited choice of eluents [17]. For example, water obscures big parts of the mid-IR region, prohibiting a practical combination of reversed-phase (RP) LC and FTIR using a flow cell. Only some organic solvents (*e.g.* chloroform) show sufficient transparency in (parts of) the IR spectrum to actually be useful. This essentially limits the application area of flow-cell LC–FTIR to normal-phase (NP) LC and non-aqueous size-exclusion chromatography (SEC). Gradient elution cannot be applied, as accurate background subtraction with changing eluent composition is virtually impossible [96, 97].

3.2.1 Cell-window materials

Cell windows or crystals are available from many materials and the choice depends on the application (Table 3.1) [17]. The materials must be chemically resistant to the eluent used in the chromatographic method, withstand high pressures, and offer sufficient transmittance to maintain a reasonable IR-energy throughput.

Calcium fluoride (CaF_2), zinc selenide (ZnSe) and, to a lesser extent, germanium (Ge), are frequently applied, but rather expensive flow-cell materials. Potassium bromide (KBr) and

Table 3.1: Optical and physical properties of window materials for use in IR flow cells and as deposition substrates in solvent-elimination interfaces.

material	transmission range (cm^{-1})	transmittance (thickness)	refractive index[a]	hardness (kg/mm^2)	sensitive to	solubility in water
calcium fluoride (CaF_2)	50,000–1111	90.0% (4.0 mm)	1.39	158	ammonium salts, acids	slightly soluble (0.013 g/l)
germanium (Ge)	5,500–475	50% (2 mm)	4.0	550	sulfuric acid, aqueous reagents	insoluble
potassium bromide (KBr)	40,000–400	90.5% (4.0 mm)	1.52	7	lower alcohols, water	highly soluble
sodium chloride (NaCl)	40,000–625	91.5% (4.0 mm)	1.49	15	lower alcohols, water	highly soluble
zinc selenide (ZnSe)	20,000–454	65% (1.0 mm)	2.4	137	acids, strong bases	insoluble

[a] at 1000 cm^{-1}

sodium chloride (NaCl) are cheap alternatives and offer complete transparency in the mid-infrared range. In addition, their low refractive indices minimize the risk of spectral fringes at certain optical pathlengths [17]. However, these materials cannot resist excessive pressures and their strongly hygroscopic properties limit their use to non-aqueous eluents. High-refractive-index materials (such as ZnSe) are required in ATR flow cells in order to maintain total reflection at the crystal boundaries.

Types of flow cells

Three types of flow cells can be discerned for on-line LC–FTIR coupling. These are based on transmission, attenuated-total-reflection (ATR) and specular-reflection measurements, respectively. The spectral range (*i.e.* detection-wavenumber range) of these interfaces is determined by the IR characteristics of the applied cell-window material and by the mobile phase used for the chromatographic separation.

The most frequently used type of flow cell is the transmission cell [3–12], which can either consist of an IR-transparent cavity or of two IR-transparent windows separated by a metal or Teflon spacer. The LC eluent enters and exits the cell through capillary tubing and is sampled by the IR beam passing perpendicularly. Depending on the application, the optical pathlength (and thus the internal volume) can be adjusted. The pathlength ranges from 0.001 to 2 mm. Transmission flow cells are available from several manufacturers and can include high-temperature options [7, 9]. Special "zero-dead-volume" (ZDV) flow-cells, with an internal volume of 0.33 µl, have been developed for use in microbore LC [10, 11]. The eluent is led through a sample cavity consisting of a 0.75-mm hole drilled in a block of potassium bromide or calcium fluoride (Figure 3.1). The IR beam crosses the eluent stream perpendicularly, yielding detection limits in the range of 40–50 µg when chloroform is used as mobile phase.

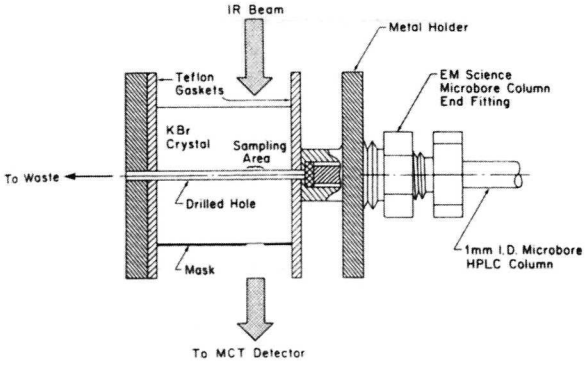

Figure 3.1: Principle of a transmission zero-dead-volume microbore LC–FTIR flow cell (cross-sectional view) [10].

The second category of flow cells is based on the ATR principle [13, 14]. One type of cell consists of a cylindrically shaped ATR crystal with cone-shaped ends (Figure 3.2). The crystal is incorporated in a flow cell with the cone ends outside the cell body. The effluent passes through the flow-cell cavity surrounding the crystal. Cassegrain optics are used to focus the IR beam on the crystal at one end and to direct the IR radiance emerging from the other end to the detector. To achieve adequate sensitivity, the number of reflections in the optical element is typically 10 or 11. The internal volume of the flow cell is between 1 and 25 µl. Spectra collected from ATR flow cells may exhibit typical band-shape distortion due to the refractive-index changes around absorption bands [98], complicating spectral interpretation. In addition, the wavelength-dependent penetration depth of IR radiation complicates quantitation. However, ATR techniques can be very useful when spectral information has to be obtained from aqueous solutions, as the optical pathlength (*i.e.* penetration depth) is in the low-micrometer range, thereby limiting absorption by the eluent.

The third type of flow cell is based on specular-reflection measurements and consists of a trough-shaped stainless-steel cell body, covered with an IR-transparent window (Figure 3.3) [15, 16]. An external mirror is used to direct the IR beam towards the flow-cell window under near-normal incidence angles, reducing the reflection losses at the air-window interface. After passing the cell-window, the IR beam is reflected via a mirror surface inside the cell cavity, crossing the effluent flow path twice, and directed towards the detector via a second external mirror. The actual optical pathlength is twice the thickness of the sample cavity and it can be adjusted from 50 µm to 2 mm, corresponding to cell-volumes of 1 to 40 µl. AABSpec (Waterford, Ireland) supplies this type of cell.

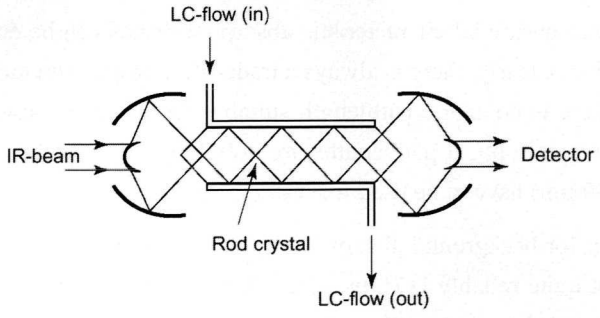

Figure 3.2: Principle of an ATR flow cell.

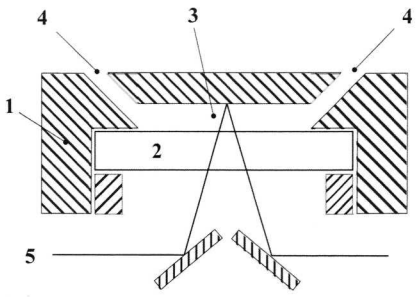

Figure 3.3: Principle of a reflection flow cell. 1, cell body; 2, IR-transparent window; 3, flow-cell cavity; 4, LC-flow path; 5, IR-beam path [99].

Eluent absorption

Ideally, the mobile phase used in flow-cell LC–FTIR should not exhibit serious background absorption, because this may obscure analyte absorption bands. Unfortunately, just about all organic solvents used in LC show intense IR spectra [17]. Furthermore, in most cases the choice of eluent is largely determined by the required chromatographic properties. As a consequence, the obtainable qualitative (molecular) information often is limited to the spectral window(s) provided by the eluent [17]. The magnitude of solvent absorption can be decreased by adjusting the optical pathlength of the cell, although this obviously will affect the analyte absorption too. The optimum pathlength also depends on the analytical query at hand. For example, when specific, accurate and sensitive detection of an analyte is required at a particular wavenumber where the solvent shows absorption, an optical pathlength resulting in an eluent absorption of approximately 0.4 AU (*i.e.* transmission of e^{-1}) has been recommended to obtain an optimum SNR [17, 18]. On the other hand, when the primary goal of the experiment is the characterization or identification of the analyte(s), the optical pathlength is chosen such that the eluent absorptions are minimized throughout the spectrum in order to ensure all characteristic absorption bands can be detected for reliable structure elucidation. Clearly, there is always a trade-off between structural information and sensitivity, and there is no single pathlength suitable for all eluents used in LC [17]. For organic solvents typical optical pathlengths are 100-2000 μm, while much shorter optical pathlengths (10–50 μm) have to be used for water.

In order to correct for background absorption by the eluent, background subtraction often can be carried out quite reliably [17], provided that isocratic LC is used. FTIR allows the acquisition of spectral data on an extremely precise wavenumber scale [25]. However, one must be aware of 'ghost bands' or spikes in the region where the eluent is completely opaque. These may be falsely interpreted as analyte-absorption bands.

To circumvent problems associated with excessive eluent absorptions that prohibit FTIR-transmission detection, some remedies exist. ATR flow cells [13, 14] can be used to inherently reduce the optical pathlength. Another option is post-column extraction of the analytes from the LC effluent into a more IR-transparent solvent [11]. Also, deuterated solvents can be used to switch eluent-absorption bands to lower wavenumbers and to potentially reveal analyte absorption bands [6].

A more recent option to cope with eluent absorption is the increase of the IR-source intensity, by using quantum-cascade lasers operating in the mid-IR region [19–22]. Though molecular structure information cannot be obtained when using a monochromatic source, quantitative measurements of specific functional groups can be achieved. The powerful emission of the IR laser allows larger optical pathlengths to be used in combination with aqueous eluents. This improves the SNR with a factor of 50 and extends the application of flow-cell LC–FTIR to biological samples.

Applications

Notwithstanding the limitations, there are a number of specific applications in which flow-cell LC–FTIR can be quite useful to obtain specific quantitative and structural information in a convenient manner. The application area of flow-cell FTIR is limited to samples with relatively high analyte concentrations, as is the case in, for instance, the analysis of sugars in non-alcoholic beverages [100]. SEC, as used for the separation of synthetic polymers, is also well suited to be coupled with FTIR by using flow cells. The separation process in SEC is essentially independent of the choice of the eluent, provided that the sample is fully soluble and that no interactions take place between the analyte and the stationary phase [102, 103]. Consequently, eluents that are favorable for IR spectroscopy can be selected. Next to a distribution in molecular weight, synthetic polymers can exhibit additional distributions (*e.g.* chemical-composition and end-group distributions) that can in principle be detected by IR spectroscopy. Conversely, the characterization of synthetic polymers by LC-MS is of limited value, because ionization efficiency and MS response may differ among analytes within one distribution. Moreover, certain types of polymers (*e.g.* polyolefins) are simply not amenable to MS. Therefore, SEC-flow-cell–FTIR is a valuable tool for the rapid, selective and quantitative determination of the chemical composition of polymers as a function of their hydrodynamic volume.

3.3 Solvent-elimination interfaces

The strong IR absorption of most eluents increases the attainable detection limits in flow-cell FTIR and has directed LC–FTIR research towards a solvent-elimination approach, in

which the eluent is removed prior to detection. To accomplish this, the eluent is generally directed to a nebulizer, often aided with (heated) nebulizer gas. Almost simultaneously, the separated analytes are deposited (immobilized) on a substrate, which can be moved step-wise or continuously to collect the analytes individually and to retain the chromatographic integrity. After deposition, IR spectra from the immobilized chromatogram are acquired. Dependent on the type of substrate used (see below) and on the size of the deposited spots, special optics, such as a (diffuse) reflection unit, a beam condenser, or an IR microscope may need to be used.

Solvent-elimination LC–FTIR offers a number of distinct advantages when compared with flow-cell LC–FTIR approaches. Firstly, the absence of interfering eluent absorption bands permits spectral interpretation over the entire wavenumber range, allowing full exploitation of the identification possibilities of IR spectroscopy. Secondly, the immobilized chromatogram is still available after the chromatographic run has been completed. The signal-to-noise ratio (SNR) can be greatly enhanced by employing increased scanning

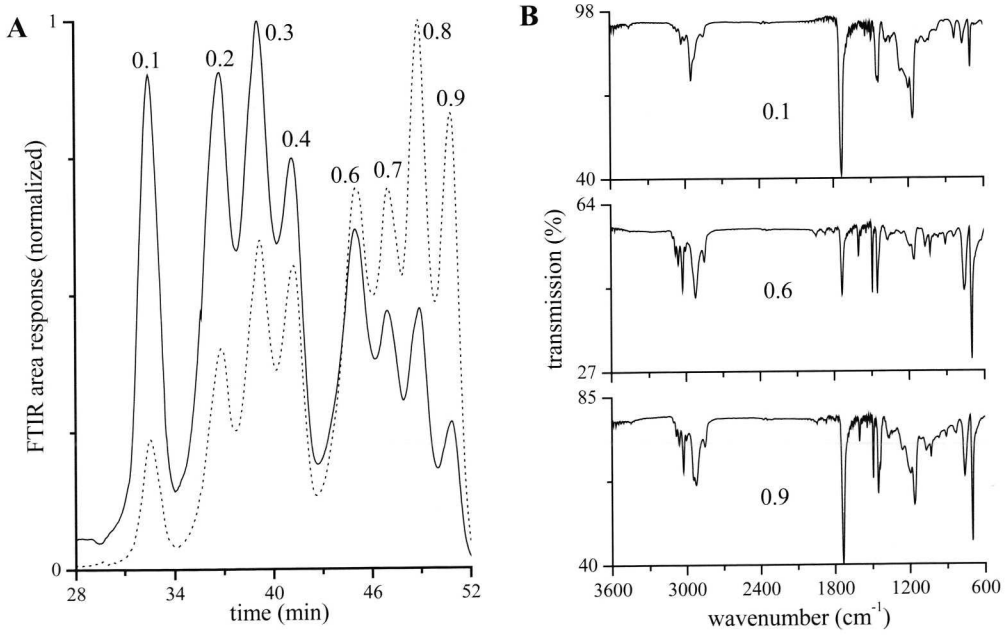

Figure 3.4: Application of solvent-elimination gradient-elution LC–FTIR for the analysis of styrene-methylacrylate (SMA) copolymers with increasing styrene fraction as indicated in Figure A. (A) Functional-group chromatograms for methylacrylate (C=O: 1744–1724 cm^{-1}, solid line) and styrene (ring C=C: 688–708 cm^{-1}, dotted line). (B) FTIR spectra for SMA copolymers with varying styrene content at their corresponding elution times. Conditions: column, Waters Novapak C18, 150 × 3.9 mm I.D.; gradient, 50:50% (*v/v*) H_2O/MeCN to 100% (*v/v*) MeCN to 100% (*v/v*) THF (2% (*v/v*)/min); flow, 0.5 ml/min. [104]

times. The extra time available also allows recording of spectra with a greater optical resolution. The sensitivity can be increased even further by producing concentrated analyte deposits and by using appropriate IR optics. These aspects make solvent elimination the LC–FTIR methodology of choice when structural information is wanted for relatively small amounts of analytes. Finally, solvent-elimination interfaces are compatible with gradient LC by varying the nebulizer temperature during the chromatographic run to obtain a constant deposit quality (Figure 3.4).

Deposition substrates and spectral quality

Deposition of analytes in solvent-elimination LC–FTIR is performed on powdered substrates, mirrors or IR-transparent windows. Correspondingly, diffuse reflection Fourier-transform infrared (DRIFT) detection, transflection spectroscopy, or transmission measurements are applied to investigate the analyte deposits.

In early solvent-elimination interfaces, powdered potassium chloride (KCl) was used as substrate and the eluent was only partly evaporated when it impinged the KCl [29–32]. DRIFT, one of the most sensitive IR techniques, was subsequently used for detection and sub-μg detection limits could be achieved. However, when the eluent is not completely evaporated during analyte deposition, analyte solution may penetrate into the lower powder layers, which cannot be penetrated by the interrogating IR beam. Moreover, DRIFT is a very intricate technique. The homogeneity of the powder, the nature and load of sample, and the reorientation of the powder during deposition may all strongly affect the quality and reproducibility of the IR spectra acquired [33, 34]. Furthermore, common DRIFT substrates, such as KCl, are not compatible with aqueous eluents as used in RPLC. As an alternative, diamond powder can be used, but this is very expensive and difficult to recycle [103]. Also, a stainless-steel wire net has been proposed, in which the analytes are retained in the gaps of the mesh after deposition. In this case absorption band intensities strongly depend on the eluent composition and quantitative analysis has proven difficult [35]. This was attributed to the surface tension of the eluents used, leading to a variation in spot size. In some cases, the spots were larger than the IR beam diameter.

Water-resistant, front-surface aluminum mirrors can be used as deposition substrates, followed by spectral acquisition in transflection [36–38]. The smooth and hard surfaces of such mirrors complicate efficient analyte deposition when the eluent is not completely evaporated. The analyte solution may easily spread across the surface. The spectral data recorded from these substrates should closely resemble the spectra obtained from transmission measurements, because the band intensities are controlled by a double-pass transmittance mechanism. However, spectral differences between transflection and KBr-

disk spectra can still be observed, including absorption-band shifts and asymmetries [36–41]. It was suggested that specular reflection from the front surface, diffuse reflection from the bulk, and the optical configuration may contribute to these phenomena [41]. Furthermore, the effect of light scattering (Christiansen effect) may become apparent when the spot thickness exceeds a certain level and anomalous relative band intensities may be observed in transflection spectra of certain analytes deposited on flat substrates when compared to transmission spectra acquired from KBr disks [42]. In order to minimize these effects, a rear-surface aluminum-coated IR-transparent germanium disc can be used as deposition substrate [43]. However, the adverse spectral effects are never completely eliminated. A post-deposition annealing procedure with dichloromethane has been proposed to minimize the effects of light scattering and to produce homogeneous deposits [44, 45].

The most favorable spectral results in solvent-elimination LC–IR are obtained when analytes are deposited on flat IR-transparent substrates (ZnSe, CaF_2, KBr) and measured in the transmission mode [33, 38, 42]. ZnSe is the preferred deposition substrate, because this material is inert and insoluble in water (compatible with RPLC) and because it offers a wide transmission range (Table 3.1). Deposits on ZnSe show better SNR values than transflection spectra of the same amounts of material deposited on aluminum. The spectra acquired from analytes deposited on ZnSe are of good quality, free from spectral distortions, and closely resemble KBr-disk transmission spectra, allowing reliable spectrum interpretation and automated library searches [33]. CaF_2 can be used as a cheap alternative when no spectral information has to be obtained in the low-wavenumber region (< 1111 cm^{-1}).

The quality and appearance of spectra is influenced by the morphology and layer thickness of the deposited analytes [47–50, 74]. The morphology will depend primarily on parameters such as eluent composition, evaporation rate, temperature, and nature of the substrate and the analytes. Upon solvent evaporation some compounds will form nice crystals, while others will deposit as amorphous layers. At a slow evaporation rate the analyte is more likely to form an oriented crystal on any smooth substrate. This can occur throughout the spot or in the center of a deposit, where not all the eluent has been evaporated during deposition. Over time, the morphology can change to the energetically most favorable state. Analyte morphology must be taken into consideration, because different forms of a given analyte may give rise to differences in the IR spectra. Library entries usually reflect a particular morphology. Some analytes may deposit as smooth films, whereas other analytes may form discontinuous spots showing numerous small (irregular) domains [51–53]. Empty substrate areas may be sampled by the narrow beam of an IR microscope, resulting

in great variations in spectral intensity or noise in a reconstructed chromatogram. Scanning over a larger substrate area using a somewhat broader beam can average out the spatial inhomogeneities. However, the sensitivity and the chromatographic resolution may be compromised. Effective deposition of low-viscosity, liquid-like compounds as distinctive spots may be a problem when hard and smooth substrates are used. Spreading and remixing of such analytes can be avoided by depositing or trapping them in the pores of either low-density polyethylene or PTFE membranes [105].

Types of solvent-elimination interfaces

In early LC-DRIFT interfaces the LC eluent was dripped via a heated tube into discrete KCl-filled cups and residual solvent was removed under a gentle stream of nitrogen before the acquisition of spectra [29, 30, 32]. In order to extend the applicability of the system to aqueous eluents, an on-line extraction with dichloromethane was performed and a phase-separator was installed before the heated tube [31]. These early LC-DRIFT systems demonstrated for the first time that solvent-elimination LC–FTIR was more sensitive and produced spectra of better quality than flow-cell-based LC–FTIR. However, the drawbacks of DRIFT discussed previously directed the focus to the use of non-porous, flat deposition substrates.

Flat KBr plates for transmission measurements were used in a method that is referred to as the "buffer-memory" technique [54–56]. Here a complete chromatogram is immobilized and stored on a substrate, allowing off-line scanning. For the rapid evaporation of eluent, the use of micro-bore LC and low flow rates (typically 5 µl/min) were proposed. In this interface, the eluent was directed to a constantly moving substrate via a stainless-steel capillary. Evaporation of the eluent was accomplished by a coaxial stream of heated nitrogen, producing a 2-mm wide trace of analytes. FTIR transmission microscopy was used for spectra acquisition. Following IR detection, it was possible to use other techniques to study the analytes. X-ray fluorescence (XRF) spectra were recorded directly from the KBr substrate to determine metals. Afterwards, the analyte deposits were scraped off the substrate and inserted in a mass spectrometer to generate direct-introduction electron-impact MS spectra [55]. With the buffer-memory technique it has been shown that immobilization and storage of the chromatogram is an attractive alternative for DRIFT-LC–IR.

In order to permit the use of higher (aqueous) flow rates (*i.e.* > 5 µl/min) in LC–FTIR, interfaces with an enhanced evaporation capacity are essential. Effective solvent elimination is also an important issue when LC is combined with MS. Therefore, several LC-MS interface types have been utilized for LC–FTIR. An example is the thermospray

(TSP) interface, which incorporates a heated capillary [57–59]. It produces a supersonic vapor jet when the eluent exits the capillary, thereby breaking up the eluent into a mist of fine droplets and enhancing evaporation of the eluent. Such a system has been used to evaporate aqueous eluents at 0.5 ml/min and to simultaneously deposit separated analytes in 2-3 mm spots on a metal IR-reflective ribbon that is continuously moved through an FTIR spectrometer equipped with a reflection accessory for spectra acquisition. The TSP interface was very well suitable for evaporating NPLC eluents and RPLC eluents containing up to 100% water at flow rates up to 1 ml/min. Detection limits as low as 1 μg could be achieved. Typical operating temperatures of the TSP interface ranged from 100 to 300°C and no degradation of the analytes was observed. However, it was not possible to deposit low-molecular-weight components, such as monomers.

The particle-beam interface originally developed for LC-MS was successfully used for the deposition of LC-separated compounds on KBr substrates [65–70]. The interface consists of three components. From the LC eluent a monodisperse aerosol is generated via nebulization and with the aid of a stream of helium. This aerosol is directed to a desolvation chamber, where the eluent is evaporated and condensed analyte molecules (*i.e.* particles) are formed. The mixture of gas, vapor and particles is then transferred to a momentum separator, where the gas and vapor are removed from the particles in a vacuum. The remaining particles pass a skimmer and are deposited as spots on the substrate. Solvent-elimination and analyte-deposition take place at atmospheric pressure and ambient temperature. The latter enhances the deposition and the detectability of thermally labile analytes. Aqueous eluents could be effectively evaporated at flow rates up to 0.3 ml/min and typical analyte spot widths are 100 μm. Analytes were successfully deposited and analyzed in the (high) microgram range. However, a device for the continuous collection of a complete chromatogram was not described and the interface was only used for the analysis of collected fractions.

Micro-LC–FTIR using an electrospray (ESP) interface is also possible. Up to 20 μl/min of solvent could be eliminated while depositing analytes on a ZnSe plate, attaining detection limits of 20 ng measured in transmission on ZnSe [71]. The system could be used with NPLC and RPLC eluents. However, the evaporation of pure water resulted in an unstable ESP and was not successful. Although the potential usefulness of LC-MS interfaces for solvent-elimination LC–FTIR has been demonstrated, the developed systems have never really matured and essentially were used by their designers only.

The most successful solvent-elimination LC–FTIR is achieved by employing pneumatic nebulization (Figure 3.5) [28, 49, 38, 72–85, 94]. These nebulizers use a high-speed gas flow to break up the eluent into small, fast-moving droplets, thereby greatly enhancing the

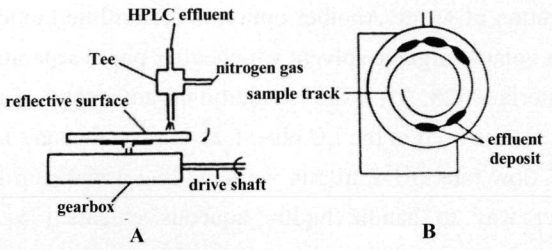

Figure 3.5: Schematic representation of a solvent-elimination interface. A, Side view during solvent-elimination and analyte deposition; B, Top view of analyte deposits on substrate [36].

evaporating capacity. At room temperature common organic eluents can be readily eliminated. The nebulizer gas is heated when (almost) complete removal of aqueous eluents is required. Following eluent evaporation, the analytes are deposited on a step-wise or continuously moving IR-transparent substrate. Depending on the focusing capacity of the nebulizer, deposition-trace widths of 200-500 μm are achieved, resulting in IR detection limits in the sub-μg range. Several LC–FTIR interfaces based on pneumatic nebulization are commercially available.

The concentric-flow nebulizer (CFN) consists of two concentric fused-silica capillaries [49, 72]. The effluent from a narrow-bore LC (50 μl/min) is passed through the inner capillary and heated nebulizer gas is passed through the outer one. The hot nebulizer gas facilitates vaporization of the eluent and produces a focussed spray resulting in 200-μm broad deposits. Using a ZnSe window as substrate and IR microscopy for detection, analyte quantities in the low-nanogram range could be detected.

In a very similar manner, a spray-jet interface has been used for the evaporation of eluents containing up to 20% water at flow rates of 20–30 μl/min [38]. In this interface, a narrow-bore LC is connected to a stainless-steel needle that is directed through a nozzle. Pneumatic nebulization is accomplished by heated nitrogen gas. IR microscopy was used for detection of the analytes deposited on a ZnSe substrate and identification limits in the 10 to 20-ng range were achieved. However, the system was less successful in RPLC with high eluent flow rates (*viz.* > 30 μl/min), highly aqueous eluents, and buffers.

In summary, with pneumatic nebulization for LC–FTIR, optimum mass sensitivity is achieved when microbore-LC (typical flow rates 20-50 μl/min) is used in combination with a ZnSe deposition substrate and IR microscopy for detection. With such systems it is possible to acquire full spectra from 1-10 ng of analyte. In order to achieve complete evaporation of 100% aqueous eluents, enhanced solvent-elimination power is required. One solution to this problem is the placement of the nebulizer inside a vacuum chamber to

facilitate the evaporation of water. Another option is the on-line liquid-liquid extraction of the LC eluent with a volatile organic solvent which, after phase separation, is being directed to the pneumatic interface [28, 31, 47]. An additional advantage of this approach is that non-volatile buffers can be used in the LC eluent, as long as they are not extracted. Further reduction of the LC flow rate to 1-2 µl/min while adding a make up flow of 20 µl/min of methanol is another way to handle highly aqueous eluents [76]. In the latter case evaporation conditions are essentially independent of the water content and even gradient elution can be used.

Next to pneumatic nebulization, ultrasonic nebulization can be applied for solvent-elimination LC–FTIR [77–83]. The eluent spray is now formed by disrupting the liquid surface at ultrasonic frequencies. Carrier gas can be used to enhance eluent evaporation and to focus the spray towards the deposition substrate. A further increase in the evaporation capacity is accomplished by placing the ultrasonic nebulizer and substrate in a vacuum chamber. Such a system is suitable for the successful evaporation of high-boiling eluents as used in high-temperature SEC–FTIR (HT-SEC–FTIR) at relatively high flow rates (100-200 µl/min) [80]. Various manufacturers have commercialized ultrasonic nebulizers for LC–FTIR.

As mentioned earlier, the highest sensitivity in LC–FTIR is achieved when analytes are deposited on the IR substrate as small spots, because then the advantages of IR microscopy can be fully exploited [33, 84]. Effectively evaporating a stream of eluent and depositing analytes in a narrow trace is not an easy task, but developments in this direction are on-going. This is illustrated, for example, by the use of a state-of-the-art piezo-actuated flow-through microdispenser in the analysis of glucose and fructose by LC–FTIR [85]. The interface is based on the principle used for inkjet printing and its design has been adapted to operate in the flow-through mode for use in LC–IR (Figure 3.6). The droplets produced by

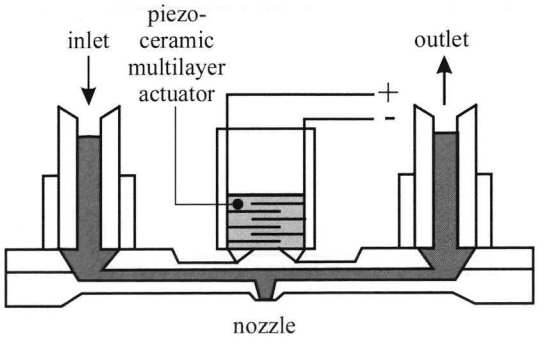

Figure 3.6: Schematic representation of a piezo-actuated flow-through microdispenser interface (cross-section) [85].

the interface are about 50 pl in volume and they are readily evaporated at room temperature and atmospheric pressure without additional heating or nebulizer gas, offering mild deposition conditions. As a result the deposits are concentrated in 40-80 µm narrow spots on a deposition substrate of calcium fluoride, which is optimal for detection by IR microscopy.

Applications

The LC–FTIR detection limits obtained with pneumatic and ultrasonic nebulizers are adequate for a number of practical applications. In real trace analysis, a sample-enrichment procedure, such as solid-phase extraction, will be necessary to allow analyte detection by LC–FTIR. The usefulness of solvent-elimination LC–FTIR has been successfully demonstrated by solving a variety of analytical queries, where structural information and/or identification of (unknown) compounds were required. The wide range of compounds analyzed comprises environmental pollutants (polycyclic aromatic hydrocarbons,

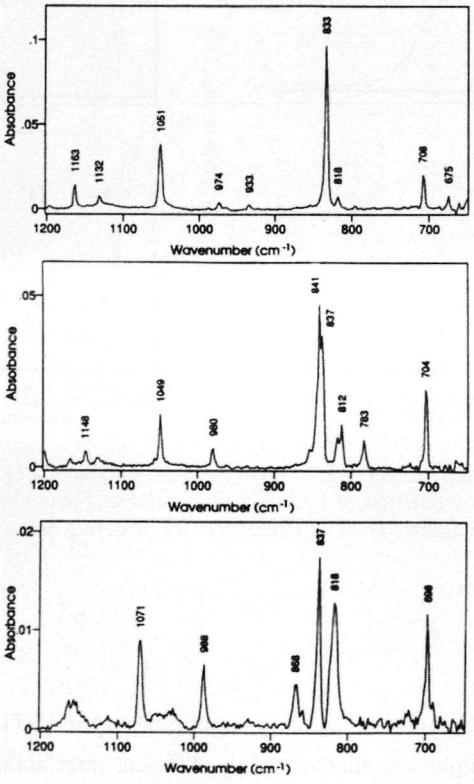

Figure 3.7: FTIR spectra of isomeric chloropyrenes recorded after solvent-elimination LC–FTIR of a chlorinated pyrene sample. Based on the spectral data the isomers could be identified (from top to bottom) as 1,6-dichloropyrene, 1,8-dichloropyrene and 1,3-dichloropyrene, respectively [106].

pesticides, and herbicides), pharmaceuticals (*e.g.* steroids and analgesics) and their impurities, drug metabolites, polymer additives, dyes, non-ionic surfactants and fullerenes [48, 50, 73, 74, 82, 83, 60–64]. FTIR detection can be especially useful when isomeric compounds have to be distinguished (Figure 3.7). Even the secondary structure of proteins, such as ß-lactoglobulin and lysozyme, has been studied by solvent-elimination LC–FTIR [69]. LC–FTIR can be particularly beneficial in the analysis of synthetic polymers, revealing the chemical composition of (co-)polymers (Figure 3.8). A special application area is the use of ultrasonic nebulizers for HT-SEC–FTIR, where composition studies have been carried out for polyolefins with the high-boiling trichlorobenzene as eluent [80].

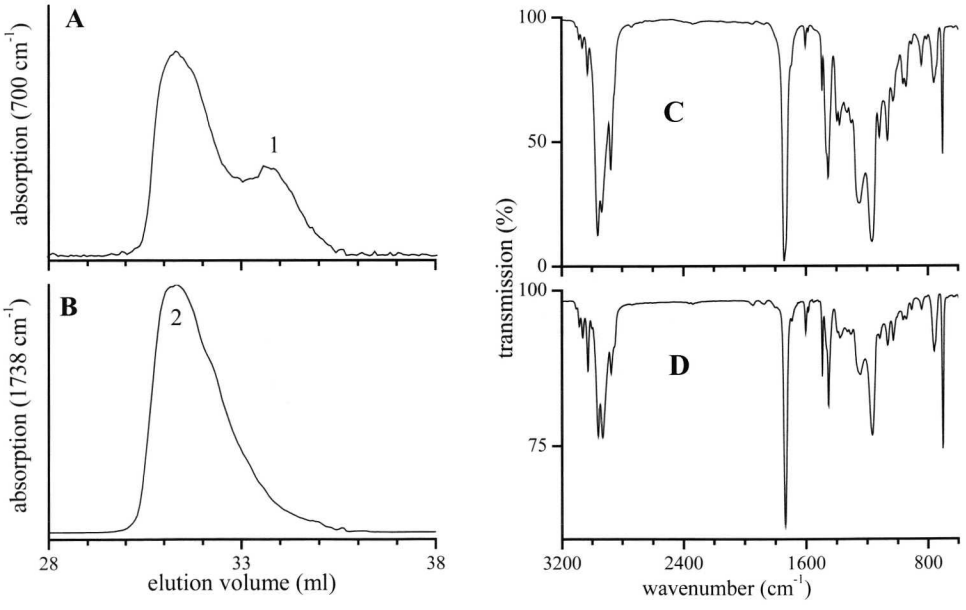

Figure 3.8: Solvent-elimination SEC–IR of a poly(styrene-butylacrylate) sample, revealing changes in chemical composition as function of hydrodynamic volume. Functional-group chromatogram for (A) styrene and (B) butylacrylate. The FTIR spectra at the peak maximum (1 and 2) are shown in (C) and (D), respectively [107].

3.4 Conclusion

Over the last decades, the progress made in combining LC and FTIR has led to two distinct coupling techniques employing fundamentally different interfacing approaches. Flow-cell LC–FTIR is relatively simple and straightforward. It has developed into a niche technique that can be used in a routine fashion for monitoring major mixture constituents with specific functional groups. Solvent-elimination LC–FTIR is somewhat more complicated. It

requires (sometimes complex) evaporation interfaces, but it allows characterization of minor sample components with a high level of confidence in NPLC, as well as in RPLC. Obviously, the choice for the type of LC–FTIR interface depends on the particular application. Aspects such as the type of spectral information needed, the required sensitivity and the ease of use are main criteria.

Solvent elimination is technically challenging due to eluent evaporation and subsequent analyte deposition in narrow traces. However, the advantage of increased signal-averaging in post-run spectra collection (signal-to-noise enhancement) makes solvent elimination the most favorable LC–IR technique. Preferably, IR-microscopy is used for the acquisition of transmission spectra from deposits on flat substrates. Miniaturized liquid-handling technologies constitute a promising development for obtaining small spot sizes under mild deposition conditions (*viz.* no heating of the eluent). However, in solvent-elimination LC–IR the deposit quality depends on the nature of the substrate (*e.g.* roughness), the nature of the sample (*e.g.* viscosity, tendency to crystallize), and the evaporation capacity of the interface.

When it comes to the identification of mixture components, LC-MS currently is the leading technique, while LC-NMR is gaining importance. However, there always will be particular applications (*e.g.* discrimination between isomers in polymer analysis) where IR data on separated compounds can be highly valuable. Furthermore, for solving complex analytical problems the possible integration of the information on molecular structure provided by FTIR, MS and/or NMR would be highly advantageous. Illustrative for this statement is the recent development of hyphenated systems employing multiple interfacing of the same LC system to several spectrometric detectors (UV absorption, MS, NMR and FTIR) [88, 89]. The complementary nature of the data provided by each spectrometric technique leads to an enormous information provided by the total system.

At present, the practical use of FTIR detection in LC is still quite limited. Nevertheless, developments over the last years have led to the situation that almost every type of LC has been or can be effectively coupled to FTIR. In addition, expansion of the application field can be expected. For instance, a separation technique such as critical chromatography (CC) shows good perspectives to be coupled via a flow cell to FTIR. CC operates on the boundary of liquid-adsorption chromatography and size-exclusion chromatography and separates polymers according to their functionality. Because IR-compatible chlorinated solvents and alkanes are frequently used as eluents in CC, a wide detection window free of eluent interferences is offered. All hyphenated techniques, including LC–FTIR, have their limitations. However, LC–FTIR is a unique and powerful analytical technique with a significant potential.

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