LIQUID CHROMATOGRAPHY/INFRARED SPECTROSCOPY

Liquid Chromatography/Infrared Spectroscopy

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1 Introduction

IR spectroscopy has a high potential for the elucidation of molecular structures. The IR spectrum of a polyatomic molecule is based on molecular vibrations, each specifically dependent on atomic masses, bond strengths and intra- and intermolecular interactions. As a consequence, the entire IR spectrum of an organic compound provides a unique fingerprint, which can be readily distinguished from the IR-absorption patterns of other compounds including isomers. In other words, when reference spectra are available, most compounds can be unambiguously identified on the basis of their IR spectra. Moreover, characteristic absorption bands can be used for compound-specific detection.

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, much effort has been – and still is – devoted to the coupling of LC with spectrometric techniques such as the IR spectra are continuously measured. The merits of flow-cell IR detection include ease of operation, real-time detection and low maintenance, but its main disadvantage lies in the significant IR-absorption of the solvents commonly used in LC. These absorptions seriously limit both the detection sensitivity and the obtainable spectral information. The second approach involves elimination of the LC solvent prior to IR detection. In this approach, an interface is used to evaporate the eluent and deposit the separated compounds onto a substrate suitable for IR detection. The primary advantages of solvent-elimination LC/IR are the possibility to obtain full spectra of the analytes and the considerably enhanced sensitivity when compared to flow-cell detection. Unfortunately, common LC solvents, and particularly aqueous eluents, are not easily removed and therefore the evaporation interfaces are often rather complex. This article reviews the developments, practical aspects, applications and current status of LC/IR, covering both coupling approaches. It follows that despite the unfavorable detection limits, flow-cell LC/IR can be useful for the specific and quantitative detection of major components of mixtures. However, solvent-elimination-based IR-detection should be used when small amounts of sample constituents have to be characterized with a high level of confidence. In general, the practical use of IR detection in LC is still limited, but the advent of various (commercial) flow-cell and interface designs shows that LC/IR is more and more being recognized as a feasible and rewarding technique.

1 INTRODUCTION

Analytical techniques that combine liquid chromatography (LC) and infrared (IR) spectroscopy have been developed primarily to permit specific detection and/or identification of sample constituents. LC is an important and extensively used method for the separation of mixtures into their individual components. IR spectroscopy is very useful for the characterization of functional groups and has strong compound-identification capabilities which are especially suited for the differentiation of structural isomers. Over the past years the coupling of LC and IR spectroscopy (LC/IR) has been accomplished by two different approaches. The first and simplest approach is to use a flow cell through which the effluent from the LC column is passed while
as mass spectrometry (MS), IR and nuclear magnetic resonance (NMR) spectroscopy. Today, with modern Fourier transform infrared (FTIR) instrumentation routinely available, 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 IR spectroscopy (LC/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 ultraviolet/visible (UV/VIS) absorption spectroscopy or MS, the use of IR detection in LC is still rather limited. Nevertheless, progress in interfacing techniques during the last decade has brought LC/IR to a stage of analytical utility which suggests that LC/IR may well become a commonly available and applied technique.

2 COUPLING MODES AND OPERATING PRINCIPLES

In the first LC/IR systems\(^1,2\) flow cells were used in a fashion analogous to LC with on-line UV/VIS absorption detection. In order to circumvent interfacing difficulties related to the IR absorptions of the mobile phase, in 1979 Kuehl and Griffiths\(^3\) developed the first solvent-elimination based LC/IR set-up in which the eluent is evaporated prior to IR detection. Since then two approaches can be discerned in LC/IR, namely, the flow-cell approach and the solvent-elimination approach. In the contemporary practice of LC/IR both approaches are applied, although the detection limits and spectral information obtained with either approach may differ considerably. The principles, applications, merits and limitations of flow-cell and solvent-elimination LC/IR have been reviewed in a number of books and papers.\(^4\)–\(^8\)

2.1 Flow-cell Approach

The simplest way to couple LC and IR is to let the column effluent pass directly through a flow cell suited for IR measurements. The IR absorption of the LC effluent is continuously monitored and spectral data are collected on-the-fly and stored throughout the chromatographic run. During or after the run, the spectra and/or IR chromatograms are computed and absorption due to the mobile phase is subtracted. In a flow-cell design, band broadening caused by detection is easily minimized.

Unfortunately, the absolute sensitivity of IR spectroscopy is relatively poor compared to spectrometric techniques like MS, UV/VIS and fluorescence spectroscopy. Moreover, solvents suited for LC generally have many absorption bands in the IR region, which leads to serious limitations of flow cell LC/IR interfacing. Firstly, absorption bands of analytes may be obscured by eluent absorptions. In other words, in flow-cell LC/IR, the spectral information that can be obtained is limited and depends on the window provided by the mobile phase used. Moreover, ill-considered subtraction of solvent absorption bands may lead to the erroneous conclusion that there is no absorption of the analyte in the corresponding spectral regions. Secondly, gradient elution can hardly be applied as the changing composition of the mobile phase frustrates proper subtraction of the background. To some extent, the second derivative of a spectrum can be used to compensate for this problem, but accurate spectral correction is virtually impossible. Thirdly, the pathlength of the flow cell has to be limited to ensure a certain spectral window and sufficient energy reaching the IR detector. For organic solvents commonly used in LC/IR, the effective pathlength rarely exceeds 1 mm which, bearing in mind Beer’s law, seriously reduces the analyte detectability. For aqueous eluents, the largest tolerable pathlength is even shorter. As can be seen from the spectrum of water in Figure 1, most of the mid-IR region between 4000 and 1000 cm\(^{-1}\) is opaque, even at a very short pathlength of 10 μm. It implies that a practical combination of reversed-phase liquid chromatography (RPLC) and IR via a transmission flow cell is restricted to specific applications. Finally, the use of signal averaging to improve the signal-to-noise ratio (S/N) of the spectra is limited owing to the short time that is available for analysis under dynamic conditions. Occasionally, the principle of stop-flow can be used to enhance the S/N without too much loss in chromatographic performance.

The choice for a certain mobile phase and flow-cell properties is primarily determined by chromatographic considerations and the desired spectral information. In principle, the spectral window of the solvent can be very small for quantitative analysis as the measurement
of a single wavenumber, e.g. the band maximum, is sufficient. Contrary, qualitative information desires IR transparency over a much wider spectral region in order to determine the presence or absence of functional groups, or to identify a compound by its IR fingerprint region (1300–600 cm\(^{-1}\)). Obviously, the spectral window of the mobile phase is inversely proportional to the cell thickness and thus, an excess of sensitivity can be traded for more spectral information and vice versa. The experimental parameters in LC/IR are, therefore, a compromise between chromatographic and spectroscopic considerations giving maximum cell thickness and solvent compromise between chromatographic and spectroscopic performance. Examples of such techniques are gel permeation chromatography (GPC), also referred to as size-exclusion chromatography (SEC), and flow-injection analysis (FIA). An important feature of the flow-cell LC/IR technique is the ability to monitor the elution of compounds with specific structural characteristics, i.e. functional groups. Besides, apart from regions of solvent opacity, spectra can be obtained instantaneously from any point in a chromatographic peak.

2.2 Solvent-elimination Approach

The compatibility and time-domain difficulties connected to flow-cell IR detection can be circumvented by coupling LC and IR spectrometry via a substrate suitable for IR detection. In this indirect approach, the eluent is eliminated and the chromatographically separated compounds are immobilized on the substrate prior to the collection of IR spectral data. The immobilization of the chromatogram is accomplished by using an interface which evaporates the eluent and continuously deposits the column effluent onto the moving substrate. In this way, interference-free IR spectra of the deposited compounds can be recorded independently from the LC conditions and the sensitivity of the FTIR spectrometer can be fully exploited. A schematic of a solvent-elimination LC/IR set-up is depicted in Figure 2.

Besides the possibility to acquire full spectra, there are some additional advantages of the solvent-elimination approach with respect to flow-cell IR detection. 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 can be performed without any time constraints since the chromatogram is stored on the substrate. This implies that signal averaging can be used and analyte spots can be analyzed repeatedly. For instance, after rapid screening of the deposited trace applying low spectral resolution and only one scan per spectrum, high-resolution spectra with a high S/N can be recorded for a few interesting parts of the chromatogram. Moreover, spectrometric analysis of the stored chromatogram in principle can be carried out at any convenient time or place, which may be helpful when suitable IR facilities are limited and spectrometers have to be shared.

Figure 2 Schematic representation of a set-up for solvent-elimination LC/IR: LC, liquid chromatograph; IF, interface; Sub, deposition substrate; T, transfer; S, spectrometer.
Evidently, the solvent-elimination approach to LC/IR is (technically) more complicated than on-line 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, the nature of the analytes and the substrate material are important factors. For example, small volumetric flows of a volatile solvent may be readily evaporated, while rapid elimination of aqueous eluents will require an interface with enhanced solvent-elimination power. Elimination of the eluent may also be hampered by the presence of non-volatile additives such as buffer salts. Next to eluent evaporation, ideally the interface also should provide compound deposition into narrow spots in order to minimize band broadening and achieve optimum IR sensitivity. However, complete eluent evaporation and compact analyte deposition may well be irreconcilable goals. Therefore, in solvent-elimination LC/IR reduced flow rates, non-buffered eluents and/or eluents with a low (or even zero) water percentage are frequently used.

The compounds analyzed by solvent-elimination LC/IR, of course, should be considerably less volatile than the eluent to accomplish their deposition. Since LC is used for non-volatiles in particular, this condition is generally met. The quality of the used substrate should not be affected by either the eluent or the deposited compounds. The substrate also must be compatible with the selected IR mode without introducing additional interferences. Furthermore, the physico-chemical properties of the substrate may influence the efficiency of analyte immobilization. For instance, residual eluent easily spreads over a substrate with a hard and smooth surface, while it may be effectively sorbed by a powder.

During the last decades, the combination of LC and IR via solvent elimination has been pursued by several research groups, which designed quite a number of different interface concepts with varying success. The common goal of all these LC/IR systems is to sensitively acquire IR spectra of mixture constituents that are free from spectral interferences and can be used for identification purposes. Through the years, the IR detection limits obtained with solvent-elimination LC/IR have improved gradually from the microgram to the low- or sub-nanogram range. Despite the progress, today there is no single "perfect" solvent-elimination interface available: every described system has its specific limitations with respect to, for example, flow rate, composition of the eluent and/or achievable sensitivity. The research activities in the field, which until now have been dominated by the problem of simultaneous eluent-evaporation and compound-deposition, are still on-going. Nevertheless, during the last years, several new commercial interfaces based on the solvent-elimination approach have been introduced.\textsuperscript{10,11}

3 FLOW-CELL TECHNIQUES

3.1 Cell Types and Infrared Detection Modes

A variety of flow cells, differing in optical material, pathlength and cell volume, is available for LC/IR detection purposes. The cells, including corresponding beam condensing optics, generally fit in the standard optical bench of the IR spectrometer. The distance between the flow cell, i.e. the IR spectrometer, and the LC system is kept as short as possible in order to minimize deterioration of the chromatography. As noted in section 2.1, the selection of a certain cell type and, particularly, the optical pathlength, depends on the required information.

The choice for a specific window material is mainly determined by the properties of the LC eluent and the spectral region that has to be monitored. A fully IR-transparent material such as potassium bromide (KBr), for instance, cannot be used with RPLC. Instead, more expensive water-insoluble materials such as calcium fluoride and zinc selenide (ZnSe) have to be chosen. The optical and physical properties of some commonly used IR-window materials are presented in Table 1. In all cases, in order to obtain an identifiable IR spectrum a minimum amount of analyte has to be present in the detection cell during the time of measurement. According to Beer’s law, the minimum identifiable concentration

<table>
<thead>
<tr>
<th>Material</th>
<th>Spectral window (cm(^{-1}))</th>
<th>Solubility in water</th>
<th>Sensitive to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silver chloride</td>
<td>4000–350</td>
<td>slightly soluble</td>
<td>complexing agents</td>
</tr>
<tr>
<td>Calcium fluoride</td>
<td>4000–1100</td>
<td>insoluble</td>
<td>ammonium salts; acids</td>
</tr>
<tr>
<td>Quartz</td>
<td>4000–2400</td>
<td>insoluble</td>
<td>hydrofluoric acid; hot sulfuric acid</td>
</tr>
<tr>
<td>Potassium bromide</td>
<td>4000–400</td>
<td>very soluble</td>
<td>water; methanol</td>
</tr>
<tr>
<td>Zinc selenide</td>
<td>4000–450</td>
<td>insoluble</td>
<td>acids; strong alkalis</td>
</tr>
<tr>
<td>KRS-5</td>
<td>4000–250</td>
<td>slightly soluble</td>
<td>complexing agents</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>630–30</td>
<td>insoluble</td>
<td>organic solvents</td>
</tr>
</tbody>
</table>
decreases when the pathlength of the cell is increased. However, extending the pathlength also results in an increase of the eluent absorbance, thus limiting the spectral window. Commonly, the volume of the flow cell has to be minimized to such an extent that only a small part (1% or less) of an LC-peak volume will fill the cell. Obviously, this places a significant limitation on the obtainable sensitivity. Through the use of microbore LC columns, the volume of the flow cells and LC peaks can be made more compatible and, for that reason, micro-LC/IR is often preferred. Besides a reduced solvent consumption, microbore LC may also offer higher peak concentrations in the cell. At the same time, however, it should be noted that the sample capacity (both in mass and volume) of micro-LC columns is rather limited.\(^{(12)}\)

Two types of commercially available flow cells can be distinguished for LC/IR: transmission cells and attenuated total reflection (ATR) cells. The principle of a transmission flow cell is depicted in Figure 3. The basic part of the cell consists of an IR-transparent cavity or two IR-transparent windows separated by a metal spacer. The optical set is mounted into a metal body between flexible rings to prevent breakage. The LC effluent enters and exits the cell via capillary tubes connected to an assembly of universal fittings and the IR beam passes perpendicularly through the LC flow. Additional equipment can be purchased for operation at elevated temperature and pressure. Zero-dead-volume (ZDV) flow cells for IR detection have been developed to minimize the volumes needed to connect the cell to the column, which is very important when microbore LC is used. In this type of cell the LC effluent is directly led into the cavity of the optical element (Figure 4). Flow cells, micro-flow cells and ZDV-cells can be purchased in many variations, rigid and demountable, differing in window material, pathlength (0.001–0.5 mm) and internal volume (0.1–10 μL). Micro-cells are generally 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.

The second type of flow cell is based on the phenomenon of ATR and is called cylindrical internal reflectance cell or CIRCLE\(^{®}\) cell.\(^{(13)}\) The principle of the CIRCLE\(^{®}\) cell is depicted schematically in Figure 5. The cell consists of a cylindrically shaped IR-transparent rod crystal with cone shaped ends that is incorporated in a boat-type cell body made of stainless steel (SS) or glass. 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. The cell body fits into a small optical bench with special input and output optics. Several crystal materials can be used but ZnSe is commonly preferred because of its high IR transparency, high refractive properties and insolubility in water. CIRCLE\(^{®}\) cells can be equipped with heating or cooling jackets.
too. The cell design tends to involve a relatively large sample volume and efforts have been made to reduce the internal volume of CIRCLE® cells to 1–25 μL in order to allow their efficient application in LC. The effective pathlength of a CIRCLE® cell is defined by the number of reflections in the optical element and, therefore, sensitivity can be enhanced by using longer optical elements. 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 (typical 1–5 μm) and wavelength dependent. Special algorithms are used to compensate for this.

### 3.2 Use in Liquid Chromatography

As outlined in the previous sections, flow-cell IR detection is not the principal choice in LC. Yet, it is a valuable alternative and additional method to obtain specific quantitative and structural information on analytes. In view of the relatively poor sensitivity, IR detection is restricted to applications in which low detection limits are not required. In GPC, for example, column capacities and sample concentrations are usually high. Gel permeation chromatography/infrared (GPC/IR) is well suited for the characterization and quantification of compositional changes throughout a (bio)polymer mass distribution. Besides, the structural differences between the polymer components (usually homologues) often are small which implies that one or two spectral windows will suffice for specific detection.

Applications of on-line LC/IR, including automatic subtraction of the solvent background, had already been described in the mid-1970s. injected amounts at sub-microgram level were found to be feasible for detection in both NPLC and RPLC separations. In subsequent studies, Taylor et al. demonstrated that microbore LC (column i.d., less than 1 mm) offers improved sensitivity compared to conventional LC (column i.d., 4.6 mm) because of the higher sample concentration in the detector cell. Furthermore, use of halogenated hydrocarbons as eluent was shown to offer better spectral specificity owing to the higher IR-transparency of these solvents. A reasonable number of applications of flow cell LC/IR in a variety of disciplines has been developed since. Saunders and Taylor, for example, applied online GPC/IR with tetrahydrofuran (THF) as mobile phase for the determination of the nitrogen content of cellulose nitrates. A cylindrical micro flow cell with a pathlength of 1 mm and an internal volume of 4 μL was employed in combination with a beam condenser. The extinction of the O–N–O asymmetric stretching band was used for the quantification of primary and secondary carbon nitration. An improved resolution and quantification was achieved by spectral derivatization. On-line GPC/IR is also a viable analysis method in the polymer field. One method employs high-temperature GPC with flow-cell IR detection to characterize the molecular weight distribution of high-density polyethylenes. Tri- and dichlorobenzene were used as mobile phase as these solvents do not exhibit interfering absorption in the CH2-stretching band region (3000–2700 cm⁻¹). Furthermore, a quartz flow cell with a relative long pathlength of 1 cm could be used in order to enhance the sensitivity. The method compares favorably with gradient elution fractionation combined with 13C-NMR spectroscopic characterization.

The strong IR absorption of water, methanol and acetonitrile, limits the application of on-line reversed-phase liquid chromatography/infrared (RPLC/IR) to the analysis of samples with relatively high analyte concentrations, such as wines, beverages and cellulose solutions. Various examples of this type of analysis have been described in the literature. Recent applications are the identification and quantification of sucrose, glucose and fructose in soft drinks, and the specific analysis of monosaccharides, alcohols and organic acids in wine. In both studies, separations were achieved with ion-exchange columns using an aqueous eluent. IR detection was performed with a 25-μm pathlength flow cell and minimal identifiable concentrations of typically 1 g L⁻¹ were obtained.

When a high sensitivity or selectivity is required, conventional RPLC solvents cannot be used with IR flow cells. Therefore, alternative methods have been developed to circumvent the IR-opacity problems imposed by the aqueous mobile phase, while maintaining the advantages of on-line detection. One approach is the extraction of the analytes into a more IR-transparent solvent. Another approach is the use of deuterated solvents which virtually have the same chromatographic properties but do not absorb in the spectral region of interest. An example of the first method is the dynamic extraction of the analytes from the aqueous effluent into chloroform or carbon-tetrachloride. The on-line liquid–liquid extraction (LLE) is carried out in an extraction coil followed by continuous separation of the aqueous and organic phases by a hydrophobic membrane. Subsequently, the organic phase, carrying the extracted analytes, is monitored in a common IR flow cell. A basically different post-column extraction method has been developed by Messerschmidt. In this method, the RPLC effluent is diluted with water, and the analytes of interest are trapped on several small solid-phase extraction (SPE) columns. These columns are dried with a flush of nitrogen, and sequentially eluted with a small volume of tetrachloromethane into an IR flow cell allowing the
individual spectra of the analytes to be recorded. An additional advantage of this method is the improved minimal identifiable concentration as a result of analyte concentration on the SPE column. This technique has been successfully applied by DiNunzio\textsuperscript{21} for the separation and identification of active compounds and degradation products in pharmaceutical samples.

In on-line LC/IR, deuterated solvents can be attractive substitutes for conventional (hydrogenated) solvents. The absolute absorbance of deuterated solvents is usually smaller and, more importantly, their IR absorption bands are shifted to different spectral regions. Additionally, deuterated solvents and their hydrogenated counterparts show very similar elution properties. With respect to on-line extraction procedures, the use of deuterated eluents has advantages in terms of simplicity, speed and maintenance of the chromatographic resolution. A major drawback is their high price. A detailed study on the utility of deuterated eluents in micro-column RPLC, NPLC and GPC with flow-cell IR detection was carried out by Fujimoto et al.\textsuperscript{22} It follows that the problem of interfering solvent absorption bands can be (partially) solved by a deliberate choice of a deuterated eluent. Chen and Kou,\textsuperscript{23} for instance, used deuterated methanol and water instead of the non-deuterated solvents to overcome the strong interfering absorptions that hinder the effective detection and quantification of lipid fractions by on-line LC/IR. Remsen and Freeman analyzed proteins using on-line gel permeation chromatography/Fourier transform infrared (GPC/FTIR) with a deuterium oxide eluent to remove water and other detection-interfering low-molecular-weight compounds and to achieve a rapid hydrogen exchange.\textsuperscript{24} Amounts of 50 μg per protein could be detected in the amide-I region between 1600 and 1300 cm\textsuperscript{-1}, and important information on the protein conformation could be obtained.

### 3.3 Use in Flow-injection Analysis

FIA is a well-established analytical technique, based on the automated injection of a series of samples in a continuous carrier stream. In FIA various detectors are used, mainly for quantitative purposes. Amongst these detection modes, flow-cell IR is not a frequent choice because of the interfering solvent absorptions and the relative poor sensitivity compared to UV/VIS and fluorescence detection.\textsuperscript{25} In several cases, however, on-line flow-injection analysis/infrared (FIA/IR) is an appropriate alternative for the rapid determination and quantification of a specific analyte in a simple mixture. In FIA, there is no need for an eluting solvent and, therefore, in contrast with LC, a carrier solvent suitable for IR detection can be selected quite easily. In this respect, FIA/IR has a wider application range than LC/IR. Still, the flow cells used in FIA/IR are not different from the ones used in LC/IR and the resemblance in detection approach is evident. Commonly, FIA/IR is applied for single-component analysis but since IR spectra comprise a range of absorption frequencies, multi-component analysis can in principle be carried out as well. Obviously, the IR absorption bands of the carrier should not spectrally interfere with the marker band of each analyte.

Guzman et al.\textsuperscript{26} compared the characteristics of transmission and CIRCLE\textsuperscript{®} cell detection in FIA/IR for multicomponent analysis of ternary solvent mixtures. With tetrachloromethane as carrier, the transmission cell provided better sensitivity in the continuous-flow mode. The shorter effective pathlength of the CIRCLE\textsuperscript{®} cell was compensated for by applying the stopped-flow technique which allowed data averaging and, thus, enhancement of the S/N of the ATR spectra. Analyte concentrations of 1–2% (v/v) could still be measured.

The principles and applications of flow-cell FIA/IR have been studied extensively by the group of de la Guardia.\textsuperscript{27–33} The effects of flow rate, injection volume, cell volume and optical pathlength on the performance of the FIA/IR analysis of o-xylene in solutions of hexane, were investigated.\textsuperscript{29} Further optimization was achieved in the development of a method for the automated determination of benzene in gasoline.\textsuperscript{30} In a further study, incorporation of an analyte-enrichment step was proposed to improve the detection limits of on-line FIA/IR.\textsuperscript{31,32} Preconcentration was carried out by trapping the analytes on an SPE cartridge. After drying of the cartridge with nitrogen gas, the analytes were eluted with dichloromethane and detected by IR transmission spectroscopy using a micro flow cell. For aqueous solutions of the pesticide carbaryl and its major metabolite 1-naphthol, detection limits of 0.36 mg L\textsuperscript{-1} and 1.6 mg L\textsuperscript{-1}, respectively, were obtained after preconcentration of 100-mL samples. Quantification was achieved by specific detection of the bands at 1744 cm\textsuperscript{-1} (carbaryl C=O) and 1276 cm\textsuperscript{-1} (1-naphthol C–O) (Figure 6). FIA/IR is also a useful alternative for the quantitative analysis of mineral oil and grease.\textsuperscript{33} Analogous to the International Organization for Standardization (ISO) procedure for the quantitative analysis of mineral oil in water and soil, this method is based on measurement of the aromatic, olefinic and aliphatic C–H stretching bands in the 3200–2700 cm\textsuperscript{-1} region. The samples were extracted with tetrachloromethane by LLE or microwave-assisted extraction. Next, the extracts were analyzed by FIA/IR at a sampling frequency of 60 h\textsuperscript{-1} using a quartz flow cell with a 10-mm pathlength. The detection limit was 1 mg L\textsuperscript{-1} when 300 μL of the extract was injected.

Miller et al.\textsuperscript{34} used FIA/IR for the simultaneous detection of succinylcholine chloride and bethanochloride,
The IR flow cell (pathlength, 49 mm) was used to monitor the aqueous flow from the stainless steel tubing. Hydroponic solutions, fruit juices and soft drinks. However, for routine application, for example in blood analysis, further improvement of the reproducibility is still required. The first method aimed for the determination of glucose and urea after enzymatic digestion to gluconic acid and ammonium carbonate, respectively. A 25-μm pathlength transmission cell was used to monitor the aqueous flow from the enzyme detector. The performance of the system was satisfactory for relative clean samples such as standard solutions, fruit juices and soft drinks. However, for routine application, for example in blood analysis, further improvement of the reproducibility is still required. The second FIA/IR method was used to determine the amyloglucosidase activity during starch hydrolysis processes. The IR flow cell (pathlength, 49 μm) was constructed of two different window materials in order to compromise between high transparency (ZnSe) and low reflectivity (calcium fluoride). Because of the high viscosity of the starch solutions, a stopped-flow method was used to obtain maximum reproducibility and sensitivity. The changes in the IR-spectra of the process mixture appeared to be directly correlated with the enzyme activity.

Figure 6 Absorbance spectra of carbaryl (—) and 1-naphthol (— - -) dissolved in CHCl₃ showing peaks at (A) 1741 cm⁻¹ and (B) 1276 cm⁻¹. (Reproduced by permission of Elsevier Science from Y. Dagbouche, S. Garrigues, M. de la Guardia, Anal. Chim. Acta, 314, 203–212, © 1995.)

4 SOLVENT-ELIMINATION TECHNIQUES

4.1 Deposition Substrates and Infrared Detection Modes

The solvent-elimination approach in LC/IR involves the use of an eluent-evaporation interface that deposits the LC-separated compounds onto an IR-compatible substrate. With most described set-ups, after immobilization of one or more chromatograms, the substrate is transferred to the IR spectrometer where spectra from the deposited spots are recorded. The deposited traces on the substrate may be moved (stepwise) through the interrogating IR beam so that, when scanning is complete, continuous IR chromatograms can be reconstructed by computer software. In some designs, IR detection is executed during the immobilization process, within 5–20 s after deposition, allowing spectra to be obtained in real time. Obviously, such a design requires a dedicated detector set-up. Dependent on the type of substrate used (see below) and/or size of the deposited spots, special optics, such as a (diffuse) reflectance unit, a beam condenser or an IR microscope, are used to scan the deposited compounds.

Basically three types of substrates and corresponding IR modes are used in solvent-elimination LC/IR: powder substrates for diffuse reflectance Fourier transform infrared (DRIFT) detection; metallic mirrors for reflection/absorption (R/A) spectrometry; and IR-transparent windows for transmission measurements. In early solvent-elimination LC/IR designs DRIFT detection of analytes on potassium chloride (KCl) powder was used, but today other, more convenient, detection modes are preferred. DRIFT as such is one of the most sensitive IR modes and sub-microgram identification limits can be achieved when the residual solvent is 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. This will limit the sensitivity, because the effective penetration depth of a DRIFT measurement is not more than 100 μm. To overcome this problem, diffuse transmittance spectrometry has been applied instead of DRIFT, using a layer of KCl powder on an IR-transparent substrate. The main limitations of DRIFT detection in LC/IR, show up during application. Reproducibility is hard to control since 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 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 aluminum mirrors, which are suitable for R/A detection, are compatible with aqueous eluents and are relatively easy to handle. Compound deposition on this type of substrate requires efficient solvent-elimination interfaces because residual solvent will easily spread over the hard and smooth reflective surface. The band intensities in the R/A spectroscopy are largely governed by a double-pass transmittance mechanism, so that spectral data analogous to transmission data are obtained. Some useful results of solvent-elimination LC/IR using mirrors have been reported, although several authors\(^{[38-40]}\) 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 affect the shape and intensity of R/A spectra obtained from analytes on aluminum mirrors. The use of an IR-transparent germanium disc with a reflective backing has been proposed in order to reduce spectral distortions. This type of disc is used in the commercially available LC-Transform™ LC/IR interfaces.\(^{[10]}\) The cleaning of aluminum mirrors in between analyses is quite delicate: the thin metal layer is fragile and can be damaged easily by rubbing.

Most favorable results in solvent-elimination LC/IR are obtained with IR-transparent deposition substrates that allow straightforward transmission measurements. So far mainly KBr and ZnSe windows have been applied in experimental LC/IR set-ups. These substrates have a hard and smooth surface and, therefore, eluent elimination has to be fast to achieve proper depositions. ZnSe is a water-resistant, inert material, while KBr usually cannot be used in combination with RPLC. Deposited compounds on ZnSe can be removed simply, with water or alcohol, so that one window can be used repeatedly. With ZnSe, good-quality IR spectra with symmetrical band profiles can be recorded from deposited spots. When the size of the sample spots is small and microscopic optics are used for measurement, the sensitivity of ZnSe transmission measurements is higher than the sensitivity of DRIFT measurements.\(^{[40]}\) ZnSe windows also cause fewer spectral artifacts than mirror substrates for R/A detection.\(^{[39]}\) Many LC/IR studies demonstrated that spectra obtained using ZnSe, closely resemble conventional KBr-disk transmission spectra. Consequently, existing spectral libraries and search programs can be used for identification purposes, which is very important for the acceptance of IR as a valuable detection technique.

The quality and appearance of spectra obtained with solvent-elimination LC/IR will be influenced by the morphology of the deposited analytes. The morphology will depend primarily 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. Also, some analytes will deposit as a smooth film, whereas others may form irregular clusters. When the spot thickness exceeds a certain level, the effect of light scattering may become apparent. A compound may also exhibit polymorphism so that mutually (slightly) different spectra can be obtained for the same compound. In general, IR detection of deposited compounds on IR-transparent substrates does not pose serious problems. However, analyte morphology should always be taken into consideration during spectral interpretation.

In solvent-elimination LC/IR the identification limits usually improve when the width of the analyte spots is decreased. A prerequisite for this gain 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 these deposits, the focus of a conventional beam condensor is too large and the use of an IR microscope is indicated. Frequently, the sensitivity enhancement is rationalized by considering the relatively increased spot thickness only (Beer’s law!), but this approach is too simple. From more complete S/N considerations it follows that the good sensitivity of IR microscopic detection essentially results from the low noise level of the IR detectors in IR microscopes.\(^{[4]}\) 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, 100–200 μm). Of course, as with any IR experiment, the S/N ratio also can be improved by increasing the measurement time (signal averaging). As outlined in section 2.2, this advantage can be exploited to its full extent in solvent-elimination LC/IR, although at the cost of an increased time of analysis.

4.2 Early Interfaces

The solvent-elimination systems that were developed in an early stage, generally used KCl-powder substrates for DRIFT detection or flat KBr windows for transmission detection. The first working interface for the coupling of NPLC and IR was designed by Kuehl and Griffiths.\(^{[3,41]}\) 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. A carousel rotated the cups into the IR spectrometer where identifiable spectra could be recorded for sub-microgram 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.\(^{42}\) For extracted compounds good-quality spectra were obtained. The carousel–DRIFT method was also adopted for narrow-bore NPLC (use of 1-mm i.d. column) by reducing the size of the KCl cups and by omitting the concentrator tube.\(^{43}\) Using a similar set-up, Kalasinsky et al.\(^{44,45}\) coupled both narrow-bore NPLC 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 (DMP). The identification limits of these systems were 1–3 \(\mu\)g, typically.

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 et al.\(^{46–47}\) proposed the use of micro-LC columns (i.d., 0.3 mm) in solvent-elimination LC/IR in order to alleviate the problem of the evaporation of large eluent volumes. The effluent (5\(\mu\)L min\(^{-1}\)) from either a GPC or an NPLC 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 \(\times\) beam condensor. The potential of the approach (termed “buffer-memory” technique) was illustrated by the analysis of a mixture of dithiocarbamate metal complexes.\(^{48}\) In a modified set-up, Jinno\(^{47}\) replaced the rotating KBr plate by an IR reflectance cell. The immobilized chromatogram was monitored continuously and full IR spectra were recorded. In order to permit the use of RPLC, a SS wire net (WN) was used instead of an KBr window.\(^{49}\) 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 \(\mu\)g 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.

### 4.3 Spray-type Interfaces

When using the “buffer-memory” technique for compound deposition on flat substrates, it is not possible to eliminate organic or aqueous eluents at flow rates higher than about 5 \(\mu\)L min\(^{-1}\) without spreading the compounds over a large area of the substrate surface. To achieve a more viable coupling of LC and IR, the use of interfaces with enhanced evaporation power is essential. The solvent-elimination interfaces developed in the last decade all use some kind of spraying to induce rapid eluent evaporation. Heat, gas, electric potential and/or ultrasonic vibrations are used to break up the LC eluent stream into small droplets. Some designs incorporate existing (commercial) equipment, while others have been built from scratch.

In the thermospray (TSP) interface, originally developed for liquid chromatography/mass spectrometry (LC/MS), a directly heated tube evaporates part of the column effluent to an expanding vapor causing nebulization of the remaining effluent. As a result, a mist of desolvating droplets emerges from the tube. In the TSP-based LC/IR systems, nebulization is performed at atmospheric pressure and the spray is directed towards a deposition substrate. Eluent flow rates of up to 1 mL min\(^{-1}\) can be handled. Griffiths and Conroy\(^{50}\) reported preliminary results on the use of TSP for LC/IR, but the first really working interface was introduced by Jansen.\(^{51}\) With a home-made TSP, the LC effluent was sprayed on a SS IR-reflective tape which moved through an optical accessory in the IR spectrometer (Figure 7). Most of the eluent was eliminated directly by the TSP and residual solvent was evaporated off the tape by heating. The immobilized chromatogram was monitored continuously and full IR spectra were recorded. Several simple polymer samples (20–80 \(\mu\)g injected) were analyzed by GPC/IR using an organic eluent at a flow rate of 0.5–1 mL min\(^{-1}\), but some low-molecular-weight monomers were too volatile to be deposited. The characterization of two Irganox-type polymer additives (100 \(\mu\)g each), which were separated by RPLC using an eluent

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**Figure 7** Schematic of TSP–moving belt interface for LC/IR; 1, moving SS tape; 2, TSP; 3, heater; 4, IR reflectance cell mounted in the IR spectrometer.
Robertson et al. further optimized the TSP-moving belt interface by studying the effect of the TSP temperature and distance to the substrate. The system was used for the analysis of amino acids, saccharides, carboxylic acids, antioxidants and polymers, and analyte identification could be achieved down to concentrations of 50 µg mL⁻¹ or about 2.5 µg injected.

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

The particle beam (PB) interface was modified for LC/IR by de Haseth et al. and Wood. 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 travel straight through the skimmer cone, while the gas and vapour are pumped away. For IR detection an IR-transparent substrate is placed in the particle-beam path to collect the analytes of interest (Figure 8) and after deposition, the substrate is transferred to the IR spectrometer for analysis. Until now stationary substrates have been used in liquid chromatography/particle beam/infrared (LC/PB/IR), that is, no complete chromatograms, only fractions, were analyzed. The particle beam/infrared (PB/IR) interface can effect the elimination of aqueous eluents at flow rates of up to 0.3 mL min⁻¹ as was demonstrated by the analysis of erythrosin B and p-nitroaniline (50 µg each) by RPLC/IR. Since the PB interface has strong eluent-elimination capacities, it was believed that interference caused by buffers might be small. The interface indeed appeared to be able to process a 0.3 mL min⁻¹ flow of buffered eluent, but 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 IR spectra recorded from microgram amounts of analyte. When phthalate or phosphate buffers were used, the analyte spectra were completely dominated by absorption bands of the buffer salts. Spectral subtraction procedures could be used to recover spectra from 130-µg depositions but were unsuccessful at the 13-µg level. PB/IR has been used as a tool for the determination of protein structures. For β-lactoglobulin and lysozyme it was shown that their structural integrity is maintained during the PB desolvation process and the subsequent deposition on the substrate. In addition, lysozyme appeared to retain its biological activity. The sample loads in these experiments generally were quite high (5–500 µg).

The PB interface can effectively remove both organic and aqueous solvents. However, relevant applications in LC/IR would still require the construction of a device that allows the continuous deposition of a complete chromatogram on a moving substrate. The PB/IR analysis of compounds at the nanogram level has been indicated, but the reported sample quantities mainly are in the (high) microgram 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.

The potential of electrospray (ESP) nebulization for micro-LC/IR was studied by Raynor and co-workers. A high electrical potential is used to form a spray of charged droplets at the end of a capillary filled with flowing liquid. As a result of charge density, the initial droplets break up into smaller droplets which facilitate solvent evaporation. Use of low flow rates (typically 1–20 µL min⁻¹) is indicated in order to obtain a stable ESP. The ESP is formed under atmospheric conditions and a sheath flow of nitrogen gas is applied to enhance eluent evaporation (Figure 9). The ESP interface was used to deposit the effluent from a micro-RPLC column onto a ZnSe plate. After LC separation, 20-ng amounts of caffeine and barbital could be analyzed.
successfully using an IR microscope. Identification of 2 ng caffeine appeared to be possible, 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 electrospray/infrared (ESP/IR) detection in LC.

During pneumatic nebulization a high-speed gas flow is used to disrupt the liquid surface and to form small, fast-moving droplets. Organic solvents can be rapidly evaporated by pneumatic nebulization, while removal of aqueous solvents is possible when the nebulizer gas is heated. Pneumatic nebulizers have been used in several solvent-elimination LC/IR designs, among which are the most successful so far. One of the first nebulizer-based LC/IR methods involved the continuous spraying of the effluent from a narrow-bore NPLC column on a rotating IR-reflective disk (Figure 10). The effluent was mixed with nitrogen gas to form a fine spray and the immobilized chromatogram was analyzed by rotating the disk through a 3 × condensed IR beam while recording R/A spectra. The system was tested with polycyclic aromatic compounds (200–800 ng each) which were separated using hexane–dichloromethane as eluent (30 μL min⁻¹). Good-intensity spectra were obtained, although some spectral deviations were observed. This pneumatic nebulizer design was improved to accomplish elimination of aqueous solvents. A heated nitrogen flow served as an evaporation-enhancing and spray-focusing sheath gas. Eluents containing up to 55% water could be handled at 30 μL min⁻¹ and a number of isomeric naphthalenediols (500 ng each) were separated and identified. Again the recorded R/A spectra showed anomalies. These spectral problems could be partially solved by using an IR-transparent germanium disk with a rear surface of aluminum as substrate. This pneumatic nebulizer
LC/IR design is commercialized by Lab Connections. The instrument consists of a sample-collection module and an optics module for R/A analysis. So far the commercial interface has been applied mainly in the field of GPC/IR. It was also used for the identification of triclosan, an antibacterial agent, in toothpaste.

A simple but effective concentric flow nebulizer (CFN) for the coupling of narrow-bore LC and IR spectrometry was constructed by Lange et al. The interface consists of two concentric fused-silica capillaries. The LC column effluent is led through the inner capillary and heated helium gas through the outer capillary (Figure 11a). The hot gas facilitates the evaporation of the solvent and the focusing of the spray emerging from the inner tube. To enhance the elimination of aqueous eluents, the CFN and the ZnSe substrate were placed in a vacuum chamber (Figure 11b). IR microscopy was used for optimum detection. The CFN can handle eluents with up to 100% water at a flow-rate of 50 μL min⁻¹ and identifiable spectra of analytes can be recorded down to the low-nanogram range. The CFN was also 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⁻¹) could be continuously deposited on the moving substrate. After immobilization of the chromatogram, spectral data could be collected without the need to transport the substrate from the Chromatograph to the IR spectrometer. To further improve the on-line character of the system, a modified CFN was installed on the optical bench of a Tracer (Biorad, Düsseldorf, Germany) gas chromatography–IR interface which allows spectral acquisition in real time. So far, only some preliminary results with this on-line LC/IR system have been reported. Unfortunately, proper analyte spectra cannot be obtained with the CFN/IR system when using non-volatile buffers, because of strong co-deposition of buffer salts. However, if sufficient vacuum pump capacity is applied, 1 mM ammonium acetate buffer can be completely eliminated, although higher ammonium acetate concentrations cause interference.

Somsen et al. proposed a spray-jet interface for the coupling of narrow-bore RPLC and IR spectrometry. In this interface a heated nitrogen flow provides pneumatic nebulization of the column effluent (20 μL min⁻¹) which is led through a SS needle that protrudes through a spray nozzle (Figure 12). With ZnSe as substrate and IR microscopy for detection, identification limits in the 10–20-ng range were achieved for quinones and polycyclic hydrocarbons. The narrow-bore RPLC/IR system was used for the impurity profiling of a steroids, for the isomer-specific characterization of chlorinated pyrenes, and for the identification of additives in polymer samples. Furthermore, the suitability of the interface for GPC/IR was demonstrated by analyzing polystyrene oligomers. When RPLC is applied, the spray-jet LC/IR system is limited with regard to the LC flow rate, the water percentage of the eluent and the handling of buffered eluents. In order to take away these limitations, a post-column LLE module (phase segmentor, extraction coil and phase separator), was inserted on-line and the organic phase, carrying the extracted analytes, was sent to the evaporation interface. The resulting liquid chromatography/liquid–liquid extraction/infrared (LC/LLE/IR) system can handle eluents with high water percentages (20–100 vol%) at flow rates up to 0.2 mL min⁻¹ and provides identification of compounds at the sub-milligram per liter level. Since the salts are not extracted, non-volatile buffers can be used without
causing interference. The detectability of the analytes was further improved by incorporation of on-line SPE for analyte enrichment (Figure 13). With such a system, triazine herbicides, including several isomers, could be identified at the low-microgram per liter level in river water (Figure 14).\(^{(76)}\)

In an alternative approach to improve the compatibility of the spray-jet interface with RPLC, the eluent flow rate was reduced to 2\(\mu\)L min\(^{-1}\).\(^{(77)}\) To obtain a useful spray, a make-up liquid (20\(\mu\)L min\(^{-1}\) of methanol) was added to the micro-LC effluent. As a consequence, the performance of the interface became independent of the water content of the eluent, so that gradient elution was possible. A micro-precolumn for on-line trace enrichment was applied to improve detection limits. With a 40-\(\mu\)L sample volume, good-quality IR chromatograms and analyte spectra were recorded at the low-milligram per liter level.

In an ultrasonic nebulizer a spray is formed by depositing the LC effluent on a transducer that is vibrating at ultrasonic frequencies. For LC/IR purposes, the spray is directed towards a substrate by a carrier gas. Castles et al.\(^{(78)}\) used ultrasonic nebulization for the deposition of compounds separated by narrow-bore RPLC on a diamond-powder substrate suitable for DRIFT detection. Spectra of satisfactory quality were obtained for injections of 3\(\mu\)g 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 diamond powder. Dekmezian and Morioka developed an interface for high-temperature GPC/IR which involved an ultrasonic nebulizer.\(^{(79)}\) The nebulizer was placed in a vacuum chamber and sprayed the column effluent on a set...
Figure 14 SPE and LC/LLE/IR chromatograms of river Meuse water samples spiked with five triazines: (a) 20 mL (30 µg L⁻¹), (b) 50 mL (6 µg L⁻¹) and (c) 100 mL (2 µg L⁻¹). 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.

of heated KBr discs, which were subsequently analyzed by IR transmission spectrometry. The system was applied to the determination of compositional changes of ethylene–propylene rubbers. An interface comprising an ultrasonic nebulizer in a vacuum chamber is used by Lab Connections in their LC-Transform™ 300 Series. This commercial device sprays the chromatographic effluent on a rotating germanium collection disk suited for R/A analysis (see above). The system was used for the quantitative analysis of copolymers by GPC/IR and for steroid analysis by RPLC/IR.

5 STATE OF THE ART

In the contemporary practice of LC/IR both flow-cell and solvent-elimination approaches are applied.
Since the flow-cell procedure cannot get around the detection limitations imposed by the LC eluent, it has developed into a special-purpose method with restricted applicability. The IR absorptions of any solvent invariably take up parts of the mid-IR spectral region and, therefore, the main power of IR spectroscopy, i.e. the reliable identification of compounds, cannot be fully exploited in flow-cell IR detection. Nevertheless, making use of the spectral windows of the eluent, flow-cell IR spectroscopy can serve as a moderately sensitive, compound-specific detection technique. Occasionally, it is used as a universal, fast and low-cost method to obtain quantitative and structural information on major constituents of samples. Various types of flow cells are commercially available, and the experimental set-up and practice of flow-cell LC/IR is relatively simple and well-suited for routine applications.

When the objective of IR detection in LC is the unambiguous identification of (low-level) constituents of mixtures, coupling via solvent elimination should be the approach of choice. Solvent elimination procedures offer the possibility

- to record spectra over the entire mid-IR region without interference from the eluent;
- to perform signal averaging in order to improve the S/N of the spectra; and
- to contain a relatively large part of the chromatographic peak within the IR beam.

As a result, the solvent-elimination approach provides a set-up which features increased sensitivity and enhanced spectral quality, two important conditions for effective analyte identification. The most recent commercial LC/IR systems which are presently available\(^{(10,11)}\) are solvent-elimination devices. Interestingly, also in gas chromatography and supercritical-fluid chromatography analyte-deposition-based IR detection has proven to be more sensitive and versatile than flow-cell-based techniques. On the other hand, one should realize that the vibrational information obtained after solvent elimination is different from the vibrational information obtained with flow-cell detection (condensed-phase spectra against solution-phase spectra). Recently, this difference was used to determine subtle molecular features of drug metabolites which were analyzed by both flow-cell and solvent-elimination LC/IR.\(^{82}\)

Today, the vast majority of LC separations is carried out by means of RPLC and, not surprisingly, research in the field of LC/IR has concentrated on the development of interfaces that are suitable for the elimination of aqueous eluents. Table 2 summarizes the characteristics of the various solvent-elimination reversed-phase liquid chromatography/Fourier transform infrared (RPLC/FTIR) systems which have been developed during the last years. The systems based on TSP, PB and ultrasonic nebulization can handle relatively high flows of aqueous eluents and allow the use of conventional-size LC, which evidently is an advantage. However, these systems often exhibit moderate, or even unfavorable identification limits and, therefore, their analytical applicability is limited. Until now, the most favorable results have been obtained with pneumatic nebulizers which essentially represent the state of the art in solvent-elimination LC/IR.

The pneumatic interfaces combine rapid solvent elimination with a relatively narrow spray. This implies

### Table 2 Characteristics of solvent-elimination RPLC/IR systems\(^{a}\)

<table>
<thead>
<tr>
<th>Type of interfacing</th>
<th>IR mode</th>
<th>Substrate</th>
<th>Eluent flow rate(^{b}) (µL min(^{-1}))</th>
<th>Limit of identification(^{b})</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrator after LLE</td>
<td>DRIFT</td>
<td>KCl powder</td>
<td>800</td>
<td>100 10</td>
<td>42</td>
</tr>
<tr>
<td>Deposition after DMP</td>
<td>DRIFT</td>
<td>KCl powder</td>
<td>50</td>
<td>1000 1000</td>
<td>44</td>
</tr>
<tr>
<td>Buffer memory</td>
<td>trans</td>
<td>SSWN</td>
<td>4</td>
<td>10000 10000</td>
<td>49</td>
</tr>
<tr>
<td>TSP</td>
<td>R/A</td>
<td>SS tape</td>
<td>500</td>
<td>10000 –</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>R/A</td>
<td>SS tape</td>
<td>1000</td>
<td>10000 25</td>
<td>52</td>
</tr>
<tr>
<td>PB</td>
<td>trans</td>
<td>KBr window</td>
<td>300</td>
<td>1000 200</td>
<td>56, 58</td>
</tr>
<tr>
<td>ESP</td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>4</td>
<td>1 10</td>
<td>62</td>
</tr>
<tr>
<td>Pneumatic nebulizer</td>
<td>R/A</td>
<td>Al mirror</td>
<td>30</td>
<td>30 30</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>50</td>
<td>1 17</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>20</td>
<td>5 3</td>
<td>39, 73</td>
</tr>
<tr>
<td>... after LLE</td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>200</td>
<td>30 0.2</td>
<td>75</td>
</tr>
<tr>
<td>... after SPE + LLE</td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>200</td>
<td>50 0.001</td>
<td>76</td>
</tr>
<tr>
<td>... after SPE + make-up</td>
<td>trans-micr</td>
<td>ZnSe</td>
<td>20</td>
<td>20 0.02</td>
<td>77</td>
</tr>
<tr>
<td>Ultrasonic nebulizer</td>
<td>DRIFT</td>
<td>Diamond powder</td>
<td>40</td>
<td>1000 1000</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>R/A</td>
<td>Ge disk</td>
<td>500</td>
<td>100 20</td>
<td>81</td>
</tr>
</tbody>
</table>

\(^{a}\) DMP, on-line reaction with DMP; make-up, addition of excess methanol; trans, transmission; trans-micr, transmission with IR microscope; –, concentration and injection volume not stated.

\(^{b}\) Typical values.
that analytes can be deposited, for example on ZnSe, in a narrow track of spots and IR microscopy can be applied effectively to achieve mass sensitivities in the low-nanogram range. Bearing in mind that often only part of the injected amount of analyte is actually analyzed, this means that the mass detectability of these LC/IR systems approaches a level close to the minimum that can be identified by IR spectroscopy. The systems based on pneumatic nebulization are limited with regard to flow rate (2–5 μL min⁻¹) and water percentage of the eluent. The tolerable water content of the eluent depends on the flow rate. Flow rates of 2–5 μL min⁻¹ of even pure water can be eliminated, but for 20–50 μL min⁻¹ flows of aqueous eluents, enhancement of the solvent evaporation efficiency is required, for example by mixing the effluent with nitrogen gas or by placing both the nebulizer and the deposition substrate inside a vacuum chamber. The tedious evaporation of water can also be circumvented by applying on-line LLE of the aqueous effluent with an organic solvent. Such a system allows much higher flow rates (0.2 mL min⁻¹) and percentages of water (up to 100 vol%) of the solvent, the spray droplets are cooled considerably.

The identification limits in terms of concentration units of the pneumatic nebulizer-based systems therefore seem to be technical rather than a fundamental problem. In other words, the development of an overall effective and routinely applicable interface probably is limited with regard to simultaneous eluent evaporation and analyte deposition, i.e. the difficulty of solvent-elimination LC/IR, i.e. interfaces have been used only by their designers. Nevertheless, the development of coupling techniques proceeds, and still proceeds, quite slowly and until now most interfaces have been used only by their designers. Nevertheless, the difficulty of solvent-elimination LC/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 is 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.

6 PERSPECTIVE AND FUTURE DEVELOPMENTS

In the last fifteen years, LC/IR has emerged as a potentially powerful tool for the specific identification of major components (flow-cell approach) or for the identification of (minor) constituents of complex mixtures (solvent-elimination approach). With respect to common LC detection techniques such as UV/VIS absorption detection, the sensitivity of flow-cell IR detection is rather poor and its merits therefore mainly lie in the ability to quantitatively monitor absorptions that are specific for the analyte or for a certain functional group. Since the limitations of flow-cell LC/IR are inherent in the technique, no vast improvements can be anticipated in the future. Some gain in performance may be achieved by optimization of the flow-cell design and use of advanced FTIR spectrometers, but these improvements will be modest and not essentially change the applicability of flow-cell IR detection. Employment of chemometrical techniques, however, may be useful particularly when the signal or spectrum is the result of the absorbance of two or more substances.

The usefulness of solvent-elimination LC/IR to provide structural information and/or identification of unknowns has been demonstrated convincingly since 1990. Unfortunately, the development of coupling techniques proceeded, and still proceeds, quite slowly and until now most interfaces have been used only by their designers. Nevertheless, the difficulty of solvent-elimination LC/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 is 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.
a matter of time, effort and technological innovations. Of course, solvent-elimination LC/IR has to compete with other identification techniques of which today online LC/MS undoubtedly is one of the most important. Quite a number of LC/MS interfaces have been developed and commercialized, but still each interface has its specific limitations. Furthermore, MS techniques cannot discriminate between isomers. Hence, even with adequate LC/MS techniques available, there is a need for alternative and complementary detection techniques which independently confirm MS-based identifications and differentiate between structurally highly similar compounds. A recent study demonstrated that LC/IR can make a viable contribution to identification analysis in a research setting that includes MS and NMR.\(^{82}\) Prerequisite for the successful implementation of IR detection was a good understanding of the relative strengths and weaknesses of each technique, and the integration of analysis in the total research program.

In order to enhance the acceptance of solvent-elimination LC/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\(^{72–74,76}\) indicate that LC/IR can indeed be used for the characterization and unambiguous identification of target and unknown compounds. LC/IR is particularly useful for the distinction of isomeric compounds\(^{77–79}\) which cannot be distinguished by LC/MS. Another item of attention is the development and use of appropriate online sample-treatment procedures to improve analyte detectability. Despite the low-nanogram identification limits, the detectability in concentration units of even the best LC/IR systems will not be sufficient to meet current demands in bio- and environmental analysis. Online SPE can improve the concentration detectability by one or two orders of magnitude. Obviously, such an improvement is unlikely to be obtained by optimization of interfacing and/or IR detection only. The first studies indicating this advantage in both flow-cell and solvent-elimination IR-detection have already been reported\(^{31,32,76,77}\).

Concerning the viability of solvent-elimination LC/IR, the availability and use of commercial interfaces also is essential. The LC-Transform™ interface (Lab Connections) has been available now for several years, but unfortunately few applications have been reported. Because this solvent-elimination system uses a mirror substrate and standard IR equipment, both the IR sensitivity and spectral quality are limited. In a more viable approach an IR-transparent substrate should be used together with microscopic IR detection. Such a configuration is used by the Infrared Chromatograph™ interface (Bourne Scientific). In this commercial and automated design the LC column effluent is deposited on a moving ZnSe window which instantaneously passes through the focused beam of the IR spectrometer allowing spectra and IR chromatograms to be recorded in real time. The placement of the chromatograms on the substrate is controlled by computer which also keeps a record of the position of deposited compounds. The IR Chromatograph™ seems promising but, as it has been introduced only recently, it is still too early to assess its merits. The handling of the obtained spectral data also is a matter of concern in solvent-elimination LC/IR. 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 spectral interpretation are important. 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 should be noted that the solvent-elimination approach in LC is not restricted to IR detection but can, in principle, be applied to any spectrometric technique which requires the compounds of interest to be present as deposits.\(^{83}\) 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/IR systems.

**ABBREVIATIONS AND ACRONYMS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ATR</td>
<td>Attenuated Total Reflection</td>
</tr>
<tr>
<td>CFN</td>
<td>Concentric Flow Nebulizer</td>
</tr>
<tr>
<td>DRIFT</td>
<td>Diffuse Reflectance Fourier Transform Infrared</td>
</tr>
<tr>
<td>DMP</td>
<td>Dimethoxypropane</td>
</tr>
<tr>
<td>ESP</td>
<td>Electrospray</td>
</tr>
<tr>
<td>ESP/IR</td>
<td>Electrospray/Infrared</td>
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<tr>
<td>FIA</td>
<td>Flow-injection Analysis</td>
</tr>
<tr>
<td>FIA/IR</td>
<td>Flow-injection Analysis/Infrared</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>GPC/FTIR</td>
<td>Gel Permeation Chromatography/Fourier Transform Infrared</td>
</tr>
<tr>
<td>GPC/IR</td>
<td>Gel Permeation Chromatography/Infrared</td>
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<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>ISO</td>
<td>International Organization for</td>
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<td></td>
<td>Standardization</td>
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<tr>
<td>LC</td>
<td>Liquid Chromatography</td>
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<tr>
<td>LC/IR</td>
<td>Liquid Chromatography/Infrared</td>
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<tr>
<td>Spectroscopy</td>
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ISO International Organization for Standardization

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**References:**

1. Bourne Scientific.
2. In this commercial and automated distinction of isomeric compounds unknown compounds. LC/IR is particularly useful for the characterization and unambiguous identification of target and unknown compounds. LC/IR can indeed be used for the characterization and unambiguous identification of target and unknown compounds. LC/IR is particularly useful for the distinction of isomeric compounds which cannot be distinguished by LC/MS. Another item of attention is the development and use of appropriate online sample-treatment procedures to improve analyte detectability. Despite the low-nanogram identification limits, the detectability in concentration units of even the best LC/IR systems will not be sufficient to meet current demands in bio- and environmental analysis. Online SPE can improve the concentration detectability by one or two orders of magnitude. Obviously, such an improvement is unlikely to be obtained by optimization of interfacing and/or IR detection only. The first studies indicating this advantage in both flow-cell and solvent-elimination IR-detection have already been reported.

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**Figure:**

- Figure of LC/IR system with ZnSe window.
- Figure of IR Chromatograph™ interface.
- Figure of matrix-assisted laser desorption/ionization (MALDI) MS.
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