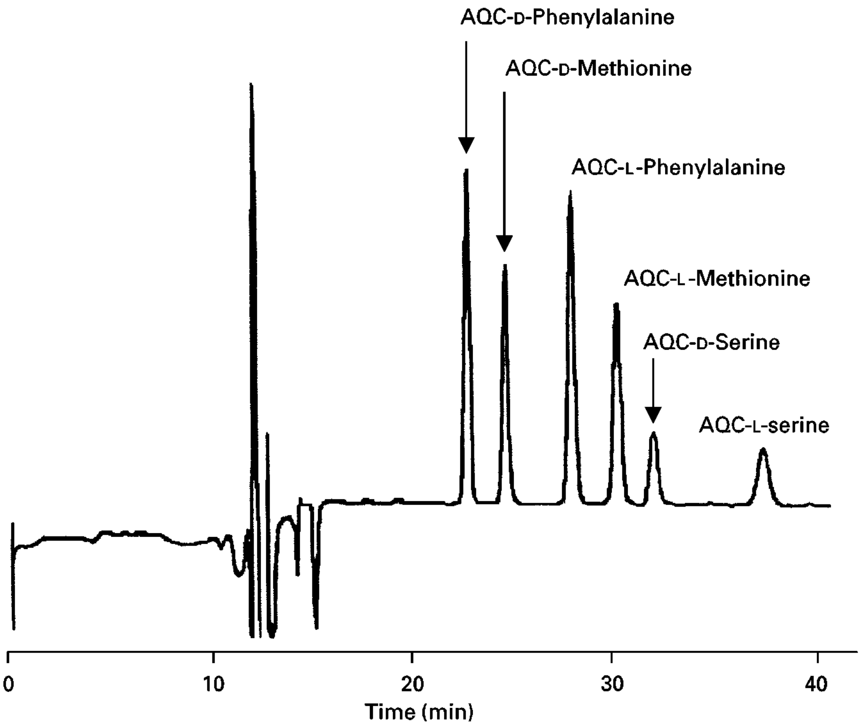

Figure 7 The separation of the enantiomers of the AQC fluorescent derivatives phenylalanine, methionine and serine. Courtesy of LC/GC. (T. L. Bereufer, *LC-GC*, Vol. 12 No. 10 (1994) 748).

Conclusion

Although relatively few substances are inherently fluorescent, most can be made to form fluorescent derivatives by relatively simple derivatization procedures. In addition, with the aid of a suitable laser, the natural high sensitivity of the device can be greatly enhanced. The main disadvantage of the fluorescence detector is its limited linear dynamic range but since in most LC analyses high sensitivity is usually required for trace analysis, in practice this limitation is not found to be so important.

See Colour Plate 22.

Further Reading

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Detectors: Infrared

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Introduction

Infrared (IR) light is the term given to electromagnetic radiation having a wavelength lying between 1 and 15 micron. In contrast to UV light, which is adsorbed when the light energy at a particular wavelength is equal to specific electronic transitions in the molecule, IR light is adsorbed when its energy is equal to changes in the vibrational and/or rotational energy of a molecule. A molecule can be considered as being made from a number of spheres (atoms) joined by springs (chemical bonds) and thus can vibrate in a very complex manner. As a general rule, a polyatomic molecule containing (n) atoms will exhibit ($3n - 6$) modes of vibration and a characteristic fundamental frequency (or wavelength) will be associated with each vibration mode. Both UV and IR spectra can be used for substance identification but, due to the many vibration modes that are possible, there is considerably more fine structure in IR spectra and consequently, IR spectra are generally more useful and give less ambiguous identification.

In general, IR adsorption is much weaker than UV adsorption and thus much larger samples are required. In addition, most of the solvents that are used in liquid chromatography adsorb strongly in the IR region and thus there have been very few IR based LC detectors developed, and none sufficiently effective for general use. In practice, the liquid chromatograph is usually combined with an IR spectrometer as a tandem system to provide spectra of the eluted solutes for identification purposes and not primarily as a detector. The basic construction of the traditional IR spectrometer is similar to that of the UV spectrometer except that the optical components must be transparent to IR light and thus must be made of appropriate materials. In addition, due to relatively low sensitivity, the interface is usually some form of transport device that also concentrates the sample as well as removing any interfering solvent. There are two forms of IR spectrometer, the grating dispersion IR spectrometer and the Fourier transform IR spectrometer (FTIR). The dispersion instrument is rarely used today in conjunction with the modern liquid chromatograph and the FTIR-LC tandem instrument now dominates the LC/IR field. Consequently only the FTIR instrument will be briefly described.

The Fourier transform IR (FTIR) spectrometer involves relatively simple instrumentation but quite complicated data processing. Due to its basic design, the spectrometer monitors all the wavelengths coincidentally, and consequently, the FTIR spectrometer can scan samples much faster than the dispersive instrument. This feature alone, makes the FTIR spectrometer highly suitable for tandem operation. A diagram of the basic system is shown in **Figure 1**.

Light from a broad band infrared source is collimated and passes into an optical system where it strikes a beam splitter consisting of a very thin film of germanium. 50% of the light passes through the film and is reflected back along its original path by a fixed mirror, where half of the light intensity (25% of the original light intensity) is reflected by the same beam splitter, through the sample cell, to the infrared sensor. The remaining 50% of the incident light is reflected at right angles to its incident path onto an axially movable mirror. Light from the moving mirror returns along its original path and again, half of the light intensity is transmitted through the beam splitter, through the sample cell, to the infrared sensor. As a result of this optical system, a quarter of the original collimated incident light from the source reaches the sensor from the fixed mirror and a quarter from the movable mirror. Because the path length of the two light beams striking the sensor will differ, there will be destructive and constructive interference, the system constituting a Michelson interferometer. As the movable mirror traverses its programmed path, it will produce a series of maxima and minima as all the different wavelengths generated by the source pass through conditions of constructive and destructive interference. These maxima and minima are continuously monitored by the sensor and recorded. It should be noted that the frequency of the waveform is controlled by the velocity of the moving mirror which will be selectable.

The interferometer actually takes a Fourier transform of the incoming signal. An example of an

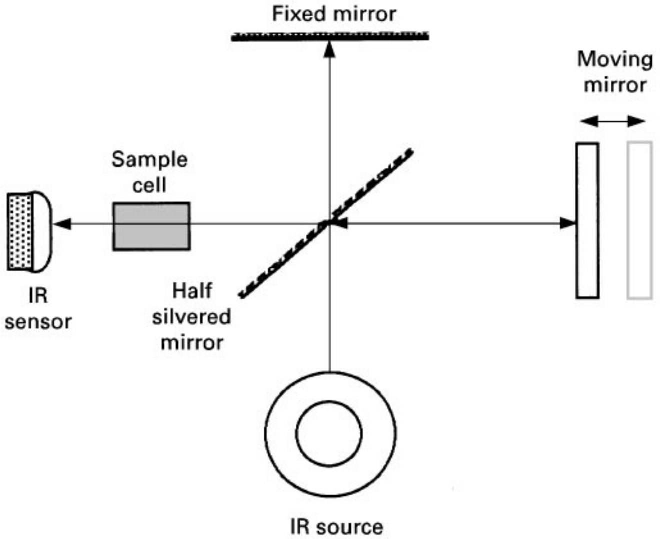

Figure 1 The elementary FTIR instrument. Courtesy of Nicolet Inc., Madison, Wisconsin, USA.

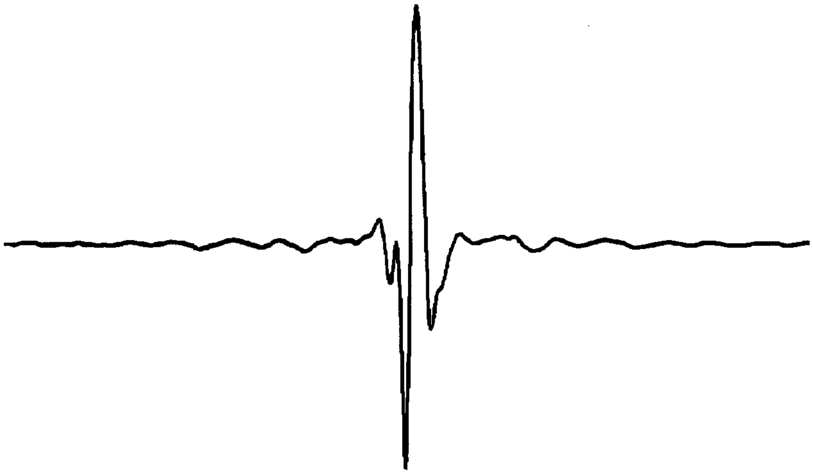

Figure 2 A typical interferogram. Courtesy of Nicolet Inc., Madison, Wisconsin, USA.

interferogram obtained from the FTIR is shown in **Figure 2**.

The resolution of the interferogram does not appear to be very good. However, this is misleading as a one second scan from an FTIR instrument gives equivalent resolution to that of a dispersive instrument scanning for 10 to 15 min. In any event, the FTIR resolution can be improved still further by using repetitive scans and processing the sum. The resolution is increased proportionally to the square root of the number of accumulated scans, e.g. 16 accumulated scans would increase the resolution by a factor of four. Spectra accumulation also increases sensitivity which is a distinct advantage in tandem systems, as the available sample may be severely limited. A single scan may take about a second, thus scanning for four seconds would double the sensitivity. Tandem systems involving IR measurements would not be successful without the introduction of the Fourier transform IR spectrometer.

LC/IR Transport Interfaces

The first transport system to be used as a liquid chromatograph–spectrometer interface was introduced by Scott *et al.* for a liquid chromatography/mass spectrometer tandem system. Eventually, the transport concept was extended to LC/IR tandem instruments and the most effective LC/FTIR interface commercially available incorporated a solvent transport interface. One of the first LC/IR transport systems was developed by Kuehl and Griffiths. Initially, moving ribbon devices were used in a similar manner to that of Scott *et al.*, but were eventually discarded in favour of a rather crude, but effective, rotating disc transport system. Their final model consisted of a cup carousel containing potassium chloride that actually acted as a fraction collector and thus was hardly an in-line interface. Depending on the speed of the transport process, many transport interfaces could be considered as automated off-line monitoring devices. Fast moving transport systems such as a wire or belt transport however, give the impression of being in-line devices although in principle, they are not. The LC/IR carousel had 32 cups fitted with a fine mesh screen and filled with potassium chloride powder. Carousel position was controlled automatically in three positions, where specific sampling activities took place. In position (1) the eluent passed onto the potassium chloride until the halide powder was saturated with mobile phase. In position (2) a stream of air was drawn through the packing to evaporate the solvent. In position (3) infrared light was directed through the dry halide, and the spectrum was taken. The carousel interface concentrated the solute and increased the sensitivity of the LC/IR combination. Unfortunately, with modern LC columns, many peaks can be eluted in a few seconds and so intermittent sample collection is unsuitable. The carousel interface primarily acts as a chromatographic ‘memory’; all the eluted solutes are stored as a ‘physical’ chromatogram as localized masses, deposited on the transport medium. The first chromatographic memory was introduced by Karmen, who used a wire transport detector to accumulate each eluted solute onto the wire surface, which was then stored on a reel. Subsequently, the wire was passed continuously through the flame of an FID, to produce a record of the separation. The most effective LC/IR interfaces are directly or indirectly based on this principle.

Jino and Fujimoto employed a potassium bromide plate as the transport system which was used as the sample holder for the IR measurement. Plate rotation was actuated by the detector signal and, at the start of a peak, the plate was moved to a new collection position. The disc moved on when elution was complete so that the sample was isolated at a specific position on the plate perimeter. A small bore column was used (flow rate 5 μL min^{-1}) so the eluent fell onto the plate and the solvent either evaporated under ambient conditions or with the aid of an infrared heater. After the separation was complete a spectrum was taken by measuring the light transmitted through the dry deposit in the usual manner. Obviously, because of the solubility of the halide in water, aqueous solvents could not be used.

Gagel and Bieman employed an aluminium disc, on top of which was cemented a circular glass mirror to form a transporter with a reflective surface; it was used in conjunction with a simple nebulizer that deposited the sample on the surface. Their basic apparatus is shown diagramatically in **Figure 3**.

The disc rotated continuously during separation, leaving a spiral trail of solid deposits on the surface of the reflective plate. Evaporation was accomplished by the nebulizer. The column eluent passed into a T, one limb of which carried a flow of nitrogen gas. The gas and eluent passed out *via* a narrow nozzle in the third limb, which directed the spray onto the disc surface. After separation, the disc was placed in a modified total reflectance IR accessory. The disc was rotated, the surface scanned by the IR spectrometer, and the reflectance–absorbence spectra continuously collected. This LC/FTIR interface appeared successful, and functioned without significant peak dispersion or loss of chromatographic resolution. The minimum mass needed to provide a satisfactory spectrum varied with the characteristic absorbence of the substances being monitored. However, it was shown that between 50 and 100 ng of sample could provide a recognizable spectrum.

Gagel and Bieman modified the nebulizer to improve the deposition, to make it amenable to aqueous solvents by reducing spreading, and to concentrate the material into a smaller area. The modified jet design involved the use of two nitrogen streams. The column eluent was mixed in a high pressure mixing T with nitrogen under pressure and directed through a syringe needle to the deposition surface. The needle

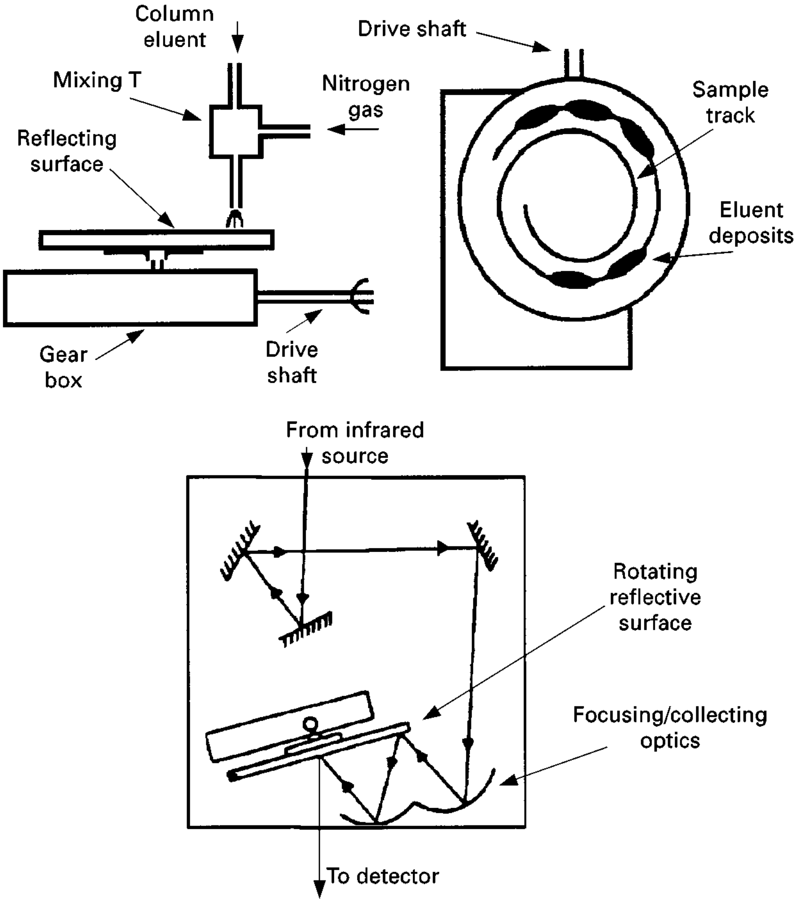

Figure 3 The layout of the transport LC/FTIR apparatus developed by Gagel and Bieman. (Reproduced with permission from Gagel and Bieman, 1986.)

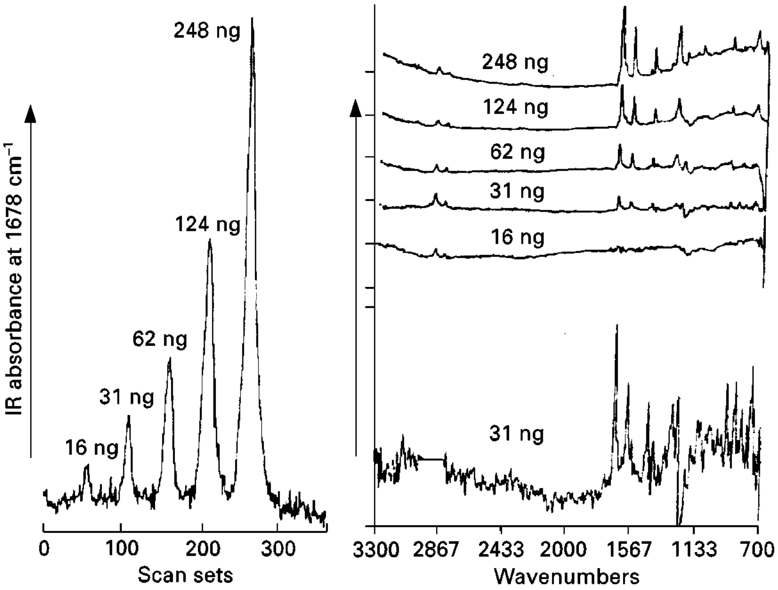

Figure 4 Results from the modified LC/FTIR interface demonstrating the overall sensitivity of the tandem instrument. (Reproduced with permission from Gagel JJ and Bieman K (1987) *Anal. Chem.*, 59(9): 1267.)

was situated inside another nozzle through which heated nitrogen was flowing. The new arrangement functioned well with aqueous solvent mixtures and the overall sensitivity of the apparatus was significantly increased. The sensitivity of the modified interface is demonstrated in **Figure 4**.

The peaks from the injection of different masses of phenanthraquinone are shown on the left. The peaks are curves relating the IR absorbance at 1678 cm^{-1} to scan number for samples deposited from 29% water in methanol. The ultimate sensitivity, defined as the mass of solute that would provide a signal to noise ratio of 2, was about 16 ng.

Solvent elimination is relatively easy with nonaqueous mobile phases but the majority of LC separations employ reversed phase columns and require mobile phases with a high water content. Poor volatility of such solvent mixtures, causes the deposits to be smeared into one another. This seriously impairs the separation. Water in the mobile phase also restricts the choice of the transport medium as it must be water resistant. A considerable amount of work has been carried out on nebulizer design to improve solute deposition and focus the material onto a smaller spot. Techniques that have been tried include thermospray and hydrodynamic focusing that employs a concentric gas flow to reduce the jet diameter by the Bernoulli effect. Different transport media have also been explored, including potassium chloride layers on the surface of a zinc-selenium metallic stage using diffuse transmission spectroscopy to obtain the spectrum of the deposited material. The deposition of the eluent from a narrow bore reversed phase column, onto the surface of a linearly moving substrate, using a jet spray assembly as an interface, has also been developed. The immobilized chromatogram (actually a chromatographic memory) is analysed by moving the substrate linearly under an FT-IR microscope while collecting the spectra. Zinc selenide was found to be preferable to an aluminized reflective surface as a disc transport. An example of the disc system used to display a reversed-phase separation of some polynuclear hydrocarbons is shown in three-dimensional form in **Figure 5**. The sensitivity to pyrene at a signal to noise ratio of 2 was 13 ng.

The In-Line Flow Sensor

The alternative to a transport interface is an in-line flow-through cell, and in 1983 a micro IR cell, 3.2 μL in volume, that fitted directly into the IR spectrometer was described by Brown and Taylor. By using a small-bore column they achieved an overall increase in mass sensitivity of about two orders of magnitude, relative to that obtained from the standard 4.6 mm i.d. column. An FTIR spectrometer was used, but the actual sensitivity improvement was confused as the length of the small bore column differed significantly from that of the standard column. Consequently, the true sensitivity in terms of minimum sample mass that would provide an acceptable spectrum, could not be assessed accurately.

A different cell design cell for use with LC microbore columns interfaced with an FTIR spectrometer

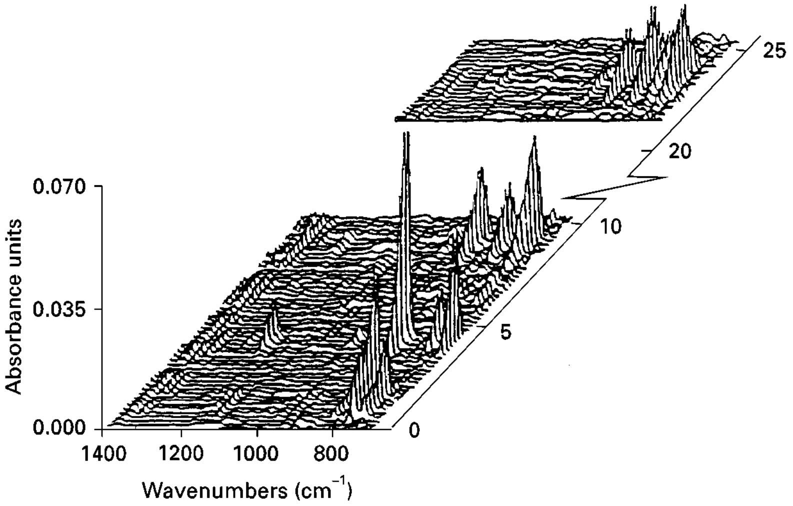

Figure 5 A three-dimensional reversed phase LC/IR plot of the separation of some polynuclear aromatic hydrocarbons. (Reproduced with permission from Conroy and Griffiths, 1984.)

was described by Johnson and Taylor. It was claimed that the cell would reduce the detection limit (the minimum mass required to produce a useful IR spectrum) to about 50 ng. The flow cell design is shown in **Figure 6**.

The cell was formed crystalline calcium fluoride or potassium bromide in the form of a block $10 \times 10 \times 6$ mm. A hole 0.75 mm i.d. was drilled through it to carry the mobile phase from the column through the block and out to waste. The collimated IR beam passed through the block, normal to the cylindrical aperture and, in doing so, transversed a section of the exiting eluent. A beam condenser was used to reduce the focal diameter of the beam to that of the hole. It was noted that the maximum signal-to-noise was obtained by summing the spectra from scans taken across the peak, between $\pm 1.53\,\sigma$ of the Gaussian profile, as it passed through the cell. As a practical point of interest, it was found easier to modify optically the size of the IR beam to match the flow cell, than to construct a cell that would accurately match the dimensions of the IR beam.

Sabo *et al.* developed an attenuated total reflectance cell for both normal- and reversed-phase

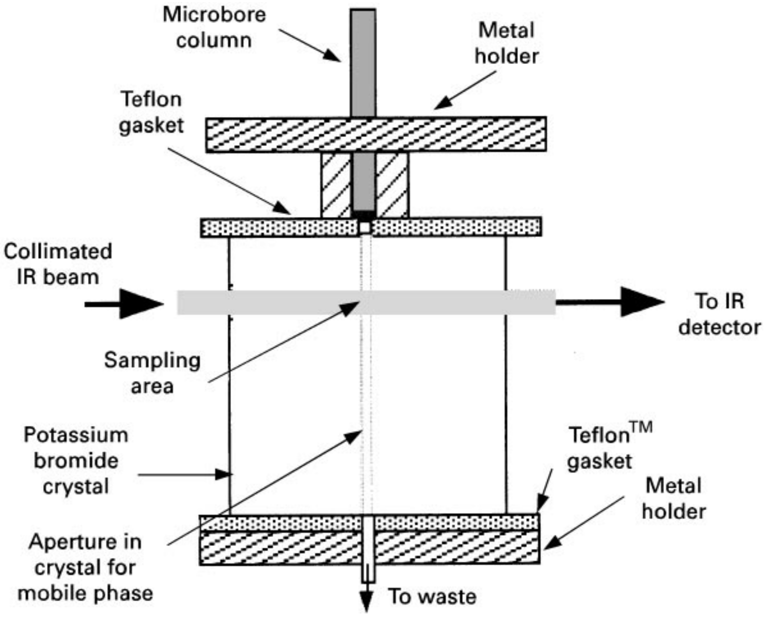

Figure 6 Zero dead volume micro IR cell.

chromatography. The cell was made with cone shaped ends, from a cylindrical shaped zinc selenide crystal, and mounted in stainless steel. The crystal was blazed at 45° and consequently gave ten reflections during passage of the IR beam down its length. The incident beam was focused onto the cone face and the radiation leaving the crystal was focused onto the IR sensor. The cell volume was large, ca. 24 μL and thus would adversely affect the resolution of a small-bore column. Clear, identifiable spectra were obtained from a 100 μL sample, containing 2% of acetophenone and ethyl benzoate and 1% of nitrobenzene from on-the-fly spectra. However, this was not a very sensitive device compared with other LC/FTIR systems.

A rather complicated solvent extraction system was developed by Conroy and Griffiths for use with an LC/FTIR tandem instrument. It involved a process that continually extracted the solute from the column eluent into dichloromethane. The solution is dichloromethane was concentrated and dispersed onto a plug of potassium chloride powder. The residual solvent was evaporated, the sample scanned and a spectrum taken. This process is somewhat clumsy but it introduces a new concept for constructing LC/IR interfaces. Employing the same basic principle Johnson *et al.* constructed a rather unique extraction cell for use with an LC/IR tandem system by introducing the technique of segmented flow. The aqueous eluent from a reversed phase column was mixed with chloroform (with which the column eluent was immiscible) producing segmented flow. The extraction solvent (chloroform) was then separated from the segmented flow by means of a ‘hydrophobic’ (dispersive) membrane. There were two pumps, one for the mobile phase and the other for the extraction solvent, which could be either chloroform or carbon tetrachloride. The two streams were mixed at a T junction (post column) and formed the segmented flow. The segmented flow then passed through an extraction coil and then to a separator. The separator was made of stainless steel with a membrane having pores about 0.2 μm in diameter dividing its length into half and its general layout is shown in **Figure 7**.

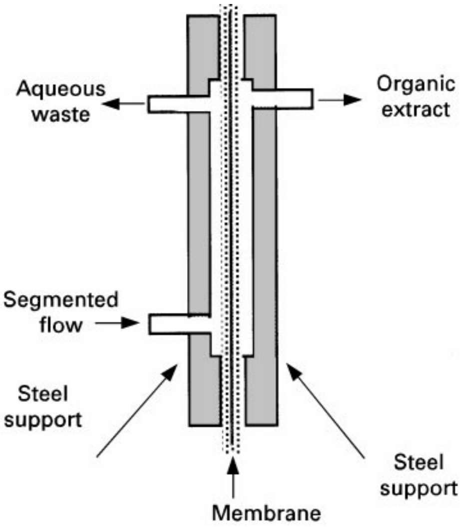

Figure 7 An extraction interface for LC/IR.

The volume on either side of the membrane was about 16 μL and the amount of solvent passing through the membrane was controlled by the differential pressure across the membrane. Obviously this device could cause serious peak dispersion and would be unsuitable for use with high-efficiency of small-bore columns. Samples containing at least 300 μg of material were necessary to produce a satisfactory spectrum, indicating a relatively poor sensitivity.

The segmented flow interface was developed further by Hellgeth and Taylor, who improved both the segmentation and the extraction efficiency. The segmented flow generator was made from 1/16 in. Swagelok T union, drilled out to contain 1/16 in. tubes the ends of which were only 0.45 mm apart and the general design is shown in **Figure 8**.

The column eluent and extraction solvent passed into the mixing T through tubes 0.020 in. i.d. The exiting segmented flow passed through an extraction conduit consisting of a Teflon^{TM} tube, 75 cm long and 0.8 mm i.d. The membrane separator was constructed from two stainless steel plates with grooves in each surface, and a triple-layer membrane of Gore-Tex^{TM} sheet. The membrane was made from two materials. The inner layer comprised an unsupported 1 μm pore Teflon^{TM} sheet which was sandwiched between two outer sheets of 1 μm pore Teflon^{TM}. These sheets were supported by non-woven polypropylene membranes which were located on the outer surfaces. The infrared cell was a modified Spectra-Tech Inc. demountable flow cell fitted with windows of either calcium fluoride or zinc selenide. The system appeared to function reasonably well; satisfactory spectra were obtained from 100 μg of material. Although a considerable improvement, the sensitivity was still relatively poor compared with that obtained with the rotating disc transport interfaces.

Further work by Somsen *et al.* has resulted in a segmented flow concentrator with significantly reduced band dispersion. A conventional liquid chromatograph was employed. It included a pump, pulse damper, injection valve and column. The column eluent entered a T piece where it was joined by an immiscible extraction solvent, usually methylene dichloride, supplied from another pump and pulse damper.

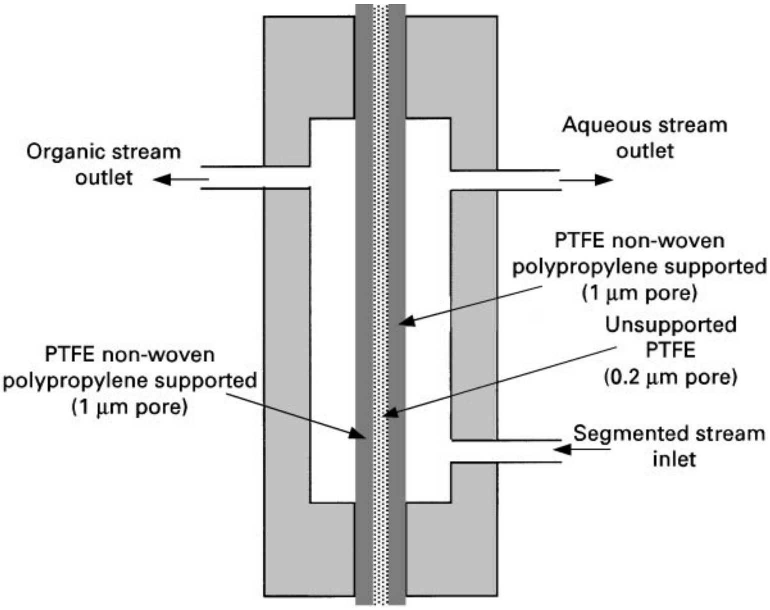

Figure 8 Diagram of a phase separator.

The extraction solvent flowed through a second column situated prior to the T piece to provide more pulse damping. The segmented mixture then passed through an extraction coil which provided the necessary time for the solutes to diffuse from the aqueous phase into the solvent. There is no parabolic velocity profile in segmented flow, and thus little or no peak dispersion can occur.

The segmented flow entered a phase separator and the separated solvent then passed through a UV absorption detector and into a spray jet assembly. Heated nitrogen was used in the spray jet assembly, to aid in the nebulization process. The chromatograms obtained indicate that very little peak dispersion occurs and that the column resolution is not significantly degraded. The finite volume of the extraction tube, however, produced a significant retention delay (about 3.5 min), which varied with both the flow rate and volume of the extraction system. Providing the solvents were reasonably volatile, they were completely removed in the nebulizing process. However, the percentage of organic solvent in the mobile phase must not be large enough to make it miscible with the methylene dichloride and prevent the formation of segmented flow. It follows that the choice of mobile phase was somewhat restricted.

Conclusion

Although considerable work has been applied to the development of LC/IR, it remains the least effective of all the LC tandem instruments. However, the IR spectrum does provide unique information not readily available from other spectroscopic techniques and consequently, for the elucidation of certain molecular structures, can be extremely useful, if not essential. It would appear that, at this time, the transport interface will provide the highest sensitivity and the best spectra if sample availability is limited.

See also: **II/Chromatography: Supercritical Fluid:** Fourier Transform Infrared Spectrometry Detection.

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Detectors: Mass Spectrometry

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Introduction

Liquid chromatography (LC) can often separate complex mixtures but simple detectors (e.g. ultraviolet-visible UV/Vis) do not allow identification of the individual components. Comparison of retention data and spiking with known standards is normally required to provide evidence of composition but this may lead to erroneous results as absolute identification is not possible. Development of diode array detection has somewhat alleviated the problem but not removed it entirely. Absorbance requires the presence of a chromophore in the molecule and, as such, UV/vis spectra do not enable absolute identification but are frequently used to confirm identity through comparison of recorded spectra with reference spectra.

Mass spectrometry (MS) provides a unique means of determining the presence of a compound in a mixture by producing a mass spectrum which will aid or confirm its identification. The relative molar mass (RMM) and/or structurally important information may also be obtained from the mass spectrum.

The combination of a separation technique with MS provides a powerful instrumental method for the analytical scientist. Modern gas chromatography–mass spectrometry (GC-MS) instrumentation, having overcome the obstacles associated with coupling them to each other, has matured into an easy-to-use benchtop technique. The interfacing of high performance liquid chromatography (HPLC) with a mass spectrometric detector (LC-MS) poses many problems, not least the different sample requirements of the respective instruments, i.e. liquid and vapour. The purpose of this article is to describe those interfaces that are most routinely used in LC-MS applications and, as such, will cover aspects of ionization methods and, to a lesser extent, mass analysers.

Background

The combination of HPLC and MS can be used as an offline technique, that is, fractions are collected and then a mass spectrum of each obtained. Much greater sensitivity, however, may be achieved by having an online interface, but this is much more difficult to achieve than with GC. The vapour flow in HPLC is much greater than in GC and there may be problems with electrical breakdown in high voltage instruments. HPLC may be operated in either normal or reversed-phase modes and the mobile-phase composition may be either isocratic or gradient. Different-sized columns are available (analytical, microbore and capillary), leading to a wide range of operational flow rates. The various possible configurations complicate the interfacing to MS.

The development of LC-MS has a history of more than 20 years and many interfaces have been reported, although only a small number have become commercially successful. Each method has its advantages and disadvantages (i.e. there is no universal interface). All facilitate the transition of analyte from solution into the gas phase with either simultaneous or sequential ionization. Those interfaces that have stood the test of time and are (or have been)