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Dramatic advances in on-line Fourier transform IR detection for capillary supercritical fluid chromatography

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ABSTRACT

We have shown that a flow cell Fourier transform (FT) IR detector for capillary supercritical fluid chromatography (cSFC) can give minimum detection limits which are much lower than any previously reported for on-line cSFC–FT-IR. The system was tested using a range of analytes. The following reproducible values were obtained, using supercritical CO_2 as the mobile phase: 2 ng for a polycyclic aromatic hydrocarbon (pyrene) —a rather weak infrared absorber, 95 pg for caffeine —a strong infrared absorber, and *ca.* 20 pg for the organometallic complex (mesitylene)chromium tricarbonyl —which has some intense infrared absorptions. These figures represent an improvement by a factor of at least 25 on comparable data in the literature. For caffeine a linear dynamic range of more than three orders of magnitude was established for this system.

INTRODUCTION

Supercritical fluids are fascinating and unique media. In the region of a phase diagram above the critical temperature and pressure the physical properties are in many ways intermediate between those of a gas and a liquid. In particular, the viscosity and diffusivity are gas-like, while the density and solvent strength approach those of liquids. This combination of properties has led to the use of supercritical fluids in chromatography (see, for example, ref. 1).

Supercritical fluid chromatography (SFC) has some of the characteristics of gas chromatography (GC) and some of high-performance liquid chromatography (HPLC), and these characteristics have ensured for SFC a particular niche, whereby a significant range of samples which are inaccessible to GC and HPLC can profitably be studied. Thus, the solvent properties of supercritical fluids, and the relatively low tempeatures at which SFC can be carried out, together with chromatographic efficiencies approaching those of GC, mean that high-resolution chromatography can be performed on high-molecular weight and/or thermally labile compounds (see, for example, ref. 2).

In many of the reported applications of SFC, the detection systems employed have been flame ionisation detection (FID) and UV detection, the latter usually at a fixed wavelength. Such detectors can be extremely sensitive, but they have one very serious drawback. They do not provide any direct information on the identities of the species present in any analyte mixture. All that can be achieved is comparison of retention times with those of standard substances, and this technique is only applicable when the likely identities of the analytes are known in advance.

Fourier transform (FT) IR spectroscopy is greatly superior to UV or FID detection in that a very high proportion of analytes possess characteristic infrared spectra, and therefore the spectroscopic identification of individual species is possible [3]. However, there are compensating disadvantages in the use of FT-IR detection. The chief among these

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is that the intensity of infrared absorption is very much less than that in the UV region, and this imposes severe restrictions on the design of any FT-IR detector. It has, however, been possible to make use of FT-IR detection in GC [4], although there remain problems in GC–FT-IR associated with the need to maintain the flow cell at a high enough temperature. HPLC–FT-IR is subject to very serious restrictions, because most of the available liquid mobile phases are themselves strong infrared absorbers, and the use of concentration–gradient elution methods makes effective subtraction of solvent spectra virtually impossible. Hence, on-line HPLC–FT-IR has a very limited potential.

Two very convenient supercritical fluids, however, CO_2 and xenon, leave regions of the infrared spectrum free of absorption. Indeed xenon possesses no infrared absorptions at all and therefore leaves the whole infrared range free [5]. There are two principal means by which FT-IR spectra can be obtained from SFC analyte mixtures: solvent-elimination and on-line methods. Both have been described in some detail by Taylor and Calvey [6]. The former technique, as the name implies, involves removal of the solvent and deposition of solid analyte on a moving substrate, which can then be subjected at leisure to FT-IR analysis [7]. The resulting spectra can then be matched to condensed-phase spectral library data [8,9] for species identification.

On-line SFC–FT-IR involves recording the spectrum of an analyte while still in the (supercritical) mobile phase, and here the chief problem is that the cell used to record the spectra must be of small volume compared to the volume of mobile phase containing a single chromatographic peak. For packed-column SFC this is not so difficult, but for capillary SFC (preferred in many cases for reasons to be discussed below) the technical problems in constructing an on-line flow cell for FT-IR detection are formidable. Nevertheless, there have been a number of published reports of such systems [10,11]. Good signal-to-noise ratios are attainable despite the limited number of FT-IR scans that can be carried out in the time taken to scan across a chromatographic peak. A possible modificatin of the on-line SFC–FT-IR method is to use a stopped-flow technique. The mobile phase can be switched from the FT-IR cell to a by-pass line and the spectrum recorded later [12].

Although FT-IR alone is very useful for identifying unknown analyte spectra, no single detection technique can give enough information for unambiguous speciation in all possible analytical mixtures. It is, therefore, our aim to construct a "multiply-hyphenated" analytical system, with sequential UV, FT-IR, FID and/or mass spectrometric detection [13]. The resultant data would be vastly more informative than results obtainable from any single detection technique. It has been decided to use capillary SFC (cSFC) as the method of choice, despite the more stringent volume requirements for the FT-IR cell, as pressure-programming is much easier than with packed-column SFC. Capillary columns also enable very high chromatographic efficiencies to be achieved, and the flow-rates are to be preferred when using expensive and/or toxic mobile phases.

The first stage in developing such a system has been to construct an on-line cSFC–FT-IR system, with a flow-cell FT-IR detector. In order that the analytical system should be compatible with standard chromatographic equipment, care was taken to design the cell so that it could be used in existing commercial FT-IR spectrometers. The cell development has been described in detail [5], together with some preliminary results [14], showing that excellent FT-IR spectra and chromatograms (reconstructed from measurements of total infrared absorption overtime) can be obtained.

We wish now to report some further significant developments with this apparatus: (1) determination of minimum detection limits with on-line cSFC–FT-IR for several types of analyte; and (ii) establishment of the linear dynamic range [15] of amounts of material which can be determined by cSFC–FT-IR.

RESULTS AND DISCUSSION

Experimental

The cSFC–FT-IR system, see Fig. 1, used a Brownlee Microgradient pump (Anachem) for delivery and pressure programming of the mobile phase. The outlet of the pump was connected via a length of stainless steel tubing (1.6 mm O.D. × 0.25 mm I.D.) to the injection valve. This was a Valco C14W two-position switching valve (Anachem) with an internal loop of 100-nl volume and was lo-

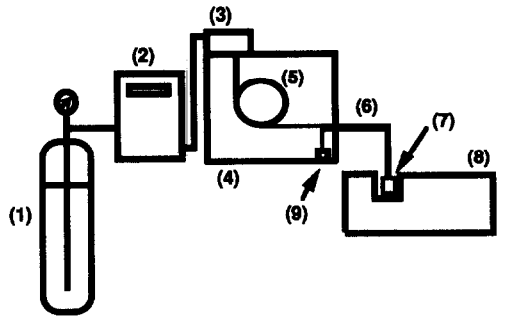

Fig. 1. A schematic diagram of the cSFC–FT-IR system. 1 = Mobile phase cylinder, 2 = syringe pump, 3 = injection valve, 4 = GC oven, 5 = capillary column, 6 = capillary transfer line, 7 = FT-IR flow cell, 8 = FT-IR spectrometer, 9 = flame ionisation detector.

cated on the door of an 8500 GC-oven (Perkin-Elmer). The SFC capillary column (Lee Scientific, from Dionex, Camberley, UK), was installed inside the GC oven and connected directly to the valve. Samples of 100 nl were introduced onto the column by direct injection. The column outlet was connected to a 50 cm × 50 μm I.D. uncoated, deactivated fused-silica capillary transfer line (S.G.E., Milton Keynes, UK) using a zero dead-volume butt-connector (S.G.E.) which passed through the oven wall and was connected to the FT-IR flow-cell.

Two differnt flow cells were used in the experiments described in this paper. Their volumes were 980 nl (path length 5 mm) and 500 nl (path length 4.5 mm), respectively. The cell in use was installed in the sample compartment of a 1760-X FT-IR spectrometer (Perkin-Elmer) fitted with an mercury cadmium telluride detector. After the flow cell a second transfer line was used to return eluent to the GC-oven. This transfer line was butt-connected to a frit restrictor (Lee Scientific) which was then interfaced to the flame ionisation detector of the GC. Following injection IR data were collected using the standard GC–IR software of the spectrometer.

The mobile phase was SFC grade CO_2 (Air Products, Rotherham, UK). Caffeine (Aldrich, Gillingham, UK) and pyrene (B.D.H., Poole, UK) were both of 99% purity, and used without further purification.

Detection limits with cSFC–FT-IR

The sensitivity of any detection technique can be defined in a number of ways, but a convenient concept is that of the injected minimum detectable quantity (IMDQ), as defined originally by Griffiths [16]. The IMDQ for any substrate is defined as that quantity which gives a detector response with a signal-to-noise (S/N) ratio of approximately 3, which can be regarded as the minimum acceptable value for clear identification of a peak.

Among detection techniques that have been used for SFC, the following are the best reported values for IMDQ: (i) scanning UV detection, approximately 3 pg for naphthalene and anthracene [17]; (ii) fluorescence detection, also approximately 3 pg for pyrene [18]; and (iii) electron capture detection, in favourable cases the most sensitive of the techniques reported, 270–640 fg for several polychlorinated biphenyls [19]. Such figures, however, are only attainable in favourable cases for each of these techniques and is important to be able extend the number of detection methods so as to encompass the widest possible range of analytes.

As FT-IR is generally regarded as a less sensitive chromatographic detection technique than, for example, UV, it is important to determine the minimum detection limits for analytes by this method and to show that these are small enough to enable FT-IR detection to play a significant role in SFC detection. As indicated in the Introduction, there have been two distinct approaches to interfacing FT-IR with SFC: on-line flow-cell detection [10], and solvent elimination [6]. The latter is clearly inappropriate for "multi-hyphenation" of detection methods, although in principle it can be made more sensitive, as it is possible to carry out a very large number of FT-IR scans to improve signal-to-noise ratios. One report [7] of such an experiment showed that 1.4 ng of indole-3-acetic acid, after 1000 scans, gave a clear spectrum, with a signal-to-noise ratio for the carbonyl stretching mode (νC=O) of approximately 20.

In order to achieve our goal of "multiple-hyphenation", however, it is necessary to use on-line FT-IR detection. This is, of course, restricted to rather a small number of scans, as the spectrum has to be recorded during the time it takes for a chromatographic peak to pass through the cell. Under such conditions, the lowest reported value for the injected minimum detectable quantity (IMDQ) for strong infrared absorber is 2.5 ng for caffeine, where the strongest νC=O band had a signal-to-noise ratio of approximately 3 [11].

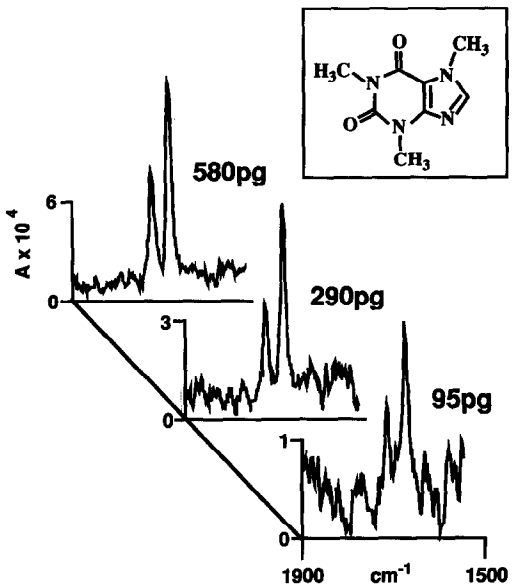

Fig. 2. FT-IR spectra of the $\nu C{=}O$ region for caffeine in supercritical CO_2. The injected quantities of caffeine are shown for each spectrum. Experimental conditions: column: 10 m × 50 μm I.D.; stationary phase: 5% phenyl-substituted polymethylsiloxane; temperature: 100°C; mobile phase: CO_2; program: linear pressure ramp from 90 to 400 atm at 10 atm/min; injection: 100 nl direct, solution in dichloromethane; detection: FT-IR flow cell (500 nl) at 25°C.

Using the Nottingham on-line cSFC–FT-IR apparatus, we have recorded the FT-IR spectrum of caffeine in the $\nu C{=}O$ region (1500–1900 cm^{-1}) for a range of injected quantities. Fig. 2 shows the spectra obtained for injected amounts of 580, 290 and 95 pg of caffeine in supercritical CO_2 as the mobile phase. In each case the background CO_2 spectrum had been subtracted, and the resulting spectra show clearly the expected two $\nu C{=}O$ bands of caffeine. Witj an injected quantity of 95 pg, the signal-to-noise ratio is reaching the lowest acceptable limits, and so in this experiment we can regard 95 pg as the IMDQ for caffeine. This represents a dramatic improvement on any published results (*i.e.* by a factor of more than 25).

It was also decided to investigate the IMDQ for a species which is a much weaker infrared absorber than caffeine, and a polycyclic aromatic hydrocarbon (PAH) was chosen, specifically pyrene. The only report of an estimated IMDQ for on-line cSFC–FT-IR detection of PAHs [10] suggested that the value was approximately 100 ng. The FT-IR spectra of a range of injected quantities of pyrene are shown in Fig. 3, with a characteristic peak, due to an out-of-plane C–H deformation of the hydrocarbon, at 847 cm^{-1}. With the accepted definition of IMDQ, *i.e.* S/N not less than 3, this figure shows that 3 ng of pyrene can be detected by cSFC–FT-IR with supercritical CO_2 as the mobile phase. Although approximately 30 times better than previous results by this technique for PAHs, this quantity is still some three orders of magnitude worse than can be achieved by scanning UV detection for such a molecule [17], and FT-IR will not be the method of choice for detection of low levels of PAHs. However, use of supercritical xenon as the mobile phase enables strong infrared bands of PAHs *ca.* 700 cm^{-1}, which are highly characteristic of individual species, to be detected in cSFC–FT-IR [14], and this will be of use in differentiating PAHs with similar UV spectra.

Many transition-metal carbonyl and organometallic complexes find use as catalyst materials, and the detection of residues of such compounds is likely to become an analytical priority. In order to test the feasibility of detecting small quantities of an organometallic complex, we have determined the IMDQ for (η-mesitylene)chromium tricarbonyl, $(1,3,5\text{-}C_6H_3Me_3)Cr(CO)_3$. In common with all other transition metal carbonyl complexes, this possesses very strong infrared absorptions in the $\nu{\equiv}O$ range (1800–2000 cm^{-1}), and Fig. 4 shows the cSFC-FTIR spectra from injected quantities of

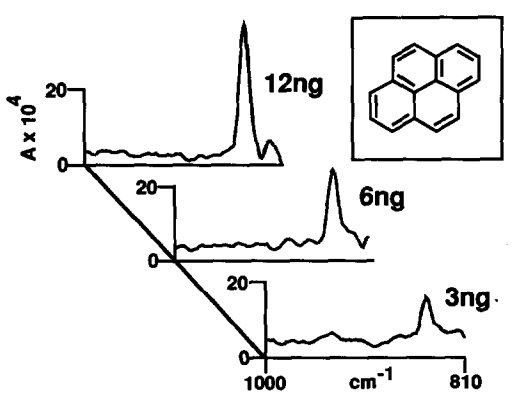

Fig. 3. FT-IR spectra of pyrene in the out-of-plane C–H deformation region. The injected quantities are shown for each spectrum. Experimental conditions as for Fig. 2.

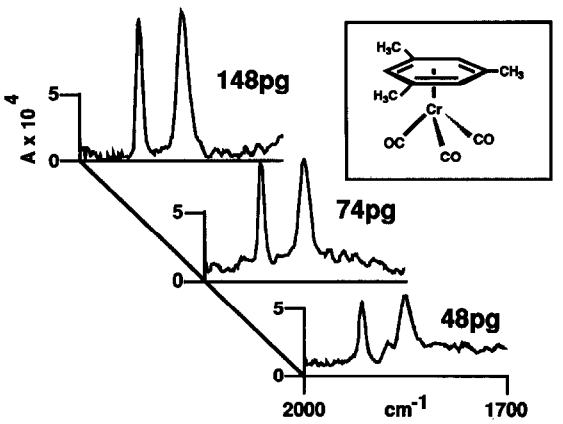

Fig. 4. FT-IR spectra of (mesitylene)chromium tricarbonyl in the carbonyl stretching region. The injected quantities are shown for each spectrum. Experimental conditions as for Fig. 2.

mesitylenechromium tricarbonyl down to 48 pg. The spectrum of the 48 pg sample has a S/N ratio of about 6, and so in this case we can estimate the IMDQ at approximately 20–25 pg. Thus cSFC–FT-IR is potentially a very powerful method for the detection of small residual amounts of transition metal carbonyl complexes.

Our results show that, with careful design of an FT-IR flow cell, it is possible to make on-line FT-IR a very versatile and sufficiently sensitive detection method for cSFC. In addition, as has already been shown [14,20], and further discussed below, the resulting FT-IR spectra are invaluable for identification of unknown species in an analyte mixture.

Linear dynamic range of cSFC–FT-IR

In order to be of use in the quantitative determination of analytes, any detection system used in chromatography should have a wide linear dynamic range, *i.e.*, the detector should respond linearly to concentration changes for a given species. Infrared absorption is subject to the Lambert-Beer Law, *i.e.*, the absorbance is directly proportional to the concentration of the absorbing substance and the path length of the cell. The later is fixed in our cell, and so absorbance at any given wavenumber corresponding to a vibrational mode of the analyte should depend linearly on the concentration.

Shah *et al.* [11] showed that the Lambert-Beer law was obeyed for caffeine in supercritical CO_2 for injected amounts of 2.5 to 50 ng, for a range of just over one order of magnitude. As part of the same series of experiments which was reported in the previous section, to determine the IMDQ value for caffeine using our FT-IR flow cell, we injected amounts of caffeine from 95 pg to 170 ng, *i.e.*, over a range of more than three orders of magnitude. Fig. 5 is a Lambert-Beer law plot for the $\nu C{=}O$ peak of caffeine at 1675 cm^{-1}, showing that the linear dynamic range available for this FT-IR detection method covers at least three orders of magnitude. Extension to significantly larger amounts of material is limited only by the capacity of the chromatographic column employed, and by moving to packed capillary or even packed columns for the chromatographic separation the linear dynamic range will be extendable by several further orders of magnitude.

Quantitative determination of amounts of analyte is clearly possible using a cSFC–FT-IR system, for analytes which possess at least one strong absorption band in the infrared range. The present experiment used supercritical CO_2 as the mobile phase, for which some parts of the infrared spectrum are inaccessible due to CO_2 absorption. As we

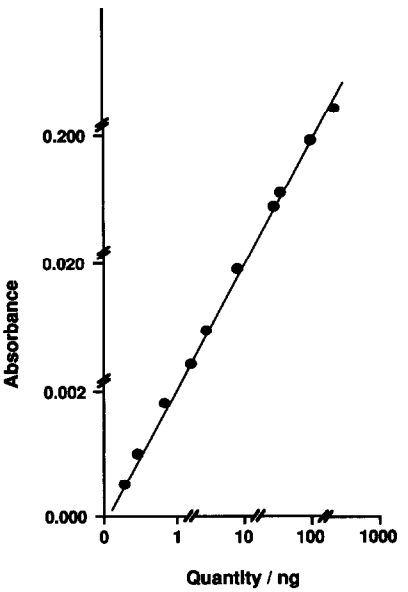

Fig. 5. Plot of the absorbance of the 1675 cm^{-1} ($\nu C{=}O$) band of caffeine *versus* quantity injected for cSFC analysis over the range 95 pg to 170 ng.

have shown previously [2], supercritical xenon is a superior mobile phase from this point of view, as it has no infrared absorptions, leaving the whole range available for observation of solute peaks.

CONCLUSION

We have demonstrated that on-line FT-IR detection following cSFC can lead to very much lower minimum detectable quantities than had previously been reported. These were 2 ng for pyrene, less than 100 pg for caffeine, and approximately 20 pg for (mesitylene)chromium tricarbonyl. In addition, the FT-IR detection has a linear dynamic range of at least three orders of magnitude, limited for larger amounts only by the column capacity.

Our results show that cSFC–FT-IR, with its very great potential for the identification of large numbers of individual analytes, is limited very much less by sensitivity constraints than had been thought. It is proposed in future work to extend the system in two principal ways. The first is to include a variable-wavelength UV detector, to obtain UV spectra of the individual components, and a mass spectrometer, to produce a "multiply-hyphenated" analytical system, cSFC–UV–FT-IR–MS. Secondly, it will be possible to use the wealth of spectroscopic information that will be generated by this sequence, in conjunction with the appropriate spectral libraries. To identify components for a very wide range of analyte mixtures. In the long term, the development of an expert system will covert such a procedure into an analytical technique of unprecedented power and generality.

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