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Packed column supercritical fluid chromatography–mobile phase elimination Fourier transform infrared spectrometry employing modified fluids

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Abstract

A mobile phase elimination interface originally designed for liquid chromatography–infrared spectrometry (LC–Transform) has been shown to perform exceedingly well for packed column supercritical fluid chromatography–infrared spectrometry (SFC–FTIR). The separation of Irganox 1076 with 5% methanol modified CO_2 is demonstrated. High quality spectral data were obtained throughout the 4000–700 cm^{-1} range with no interference from methanol. The only modification to the commercially available interface for SFC–FTIR was the insertion of a fixed integral restrictor into the spray nozzle. The modified CO_2 mobile phase is evaporated during the spray process, and the resulting chromatogram is deposited as a continuous track on an IR transparent (e.g., usually germanium) flat surface (e.g., the sample collection disk). This track can then be scanned in a spectrometer to obtain spectra of the discrete sample components.

Keywords: Mobile phase elimination interface; Fourier-transform infrared spectrometry; Detection, SFC; Interfaces, SFC–FTIR

1. Introduction

The use of modifiers in supercritical fluid chromatography (SFC) increases the solvating power of the chromatographic mobile phase. In order to use modifiers with Fourier transform infrared (FTIR) detection, mobile phase elimination interfaces would be desirable since they make available a greater amount of spectral information. The most common modifier is methanol, which absorbs strongly in the IR region.

SFC–FTIR mobile phase elimination was introduced in 1984 [1], but this technique has been slow to develop. Two primary groups, P. Griffiths and M. Raynor, have shown detection limits to be as low as or better than those for liquid chromatography (LC)–FTIR. KCl, KBr and ZnSe have been principally used as the solid substrate. In the majority of the cases cited, FTIR microscopes were used to match the IR beam size with that of the SFC deposit [1–5]. For example, Raynor et al. achieved low nanogram detection limits for two polymer additives using SFC and 100% CO_2 [6]. The spectra were collected with a microscope and consisted of 1000 co-added scans. Tapered capillary fixed restrictors were used to deposit the analytes onto a window that was stepped

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after each analyte was deposited. Heat, 150°C, was applied to the capillary restrictor to avoid plugging by the analyte.

To further advance the mobile phase SFC–FTIR technique, efforts must be made in adapting and optimizing existing instrumentation. In this regard, Norton and Griffiths [7] have described real time direct deposition SFC–FTIR using a slightly modified gas chromatography–FTIR interface. An open tubular column (100 μm, I.D.) was used along with 2% methanol modified CO_2 and the maximum decompress CO_2 flow-rate was 5 ml/min. At the moment, the future of SFC appears to rest more with packed columns as a complement to LC. For SFC to become a plausible substitution or complimentary technique for LC, the instrumentation should be easily adaptable and as efficient for SFC as LC. In this regard, the original application of the LC–Transform, a liquid mobile phase elimination device, was designed for coupling normal-phase chromatography and FTIR. Since the instruments inception, it has proven capable of a number of hyphenation modalities. Different separation methods, such as other LC modes, temperature rising elution fractionation and field flow fractionation have been employed with the LC–Transform. In a like manner, the instrument has been successfully interfaced to diverse analytical instrumentation such as raman spectrometers, UV/Visible spectrometers and mass spectrometers [8]. We report here that the LC–Transform can be readily interfaced to packed column SFC with methanol modified CO_2 at relatively high decompressed flow-rates.

The study reported here was not meant to fully optimize the parameters of the interface and FTIR for supercritical fluid uses but to present various aspects that will be important for getting good deposits from SFC as well as getting equally good spectra from the deposits. The goals of the study were to determine the initial parameters needed to use this interface for SFC and to test its general feasibility. These include sheath gas flows, temperature, restrictor position and the need for a post-column split. We also wish to demonstrate the universality of a high-performance liquid chromatography (HPLC) interface for packed column SFC.

2. Experimental

The Suprex MPS/225 system (Pittsburgh, PA, USA) was used and set up for chromatography only. A Valco 6-port injection valve with external sample loop (Valco, Houston, TX, USA) was used in place of the traditional four port valve (with internal volume rotor) supplied on the instrument. The external sample loops were used for the initial studies because of our uncertainty in the volume/mass of sample that would have to be used to get suitable deposits on the disk. The LC–Transform (Marlborough, MA, USA) was a series 100.

The injector was connected directly to the column via a piece of 75 mm I.D. deactivated fused-silica (SGE, Austin, TX, USA). SFC columns were a 10 cm×1 mm Deltabond cyano, 5 μm particles, for the 100% CO_2 work and a 15 cm×2 mm Deltabond cyano column (Keystone Scientific, Bellefonte, PA, USA) for the modified fluids work. The larger bore and longer column used in the methanol modified experiments provided excess separation capability in order that the analyte peak did not elute on top of the solvent peak. Methanol (5%) modified CO_2 was purchased from Scott Specialty Gases (Plumsteadville, PA, USA).

Linear, integral and tapered restrictors were connected directly to the exit of the column. Tapered restrictors were used only in determining decompressed flow-rates since they were not rugged enough to be used with the FTIR interface. For FTIR detection, a linear or integral restrictor was fed through a hole drilled into the oven wall and inserted directly into the nozzle of the interface. Initially, a piece of 15 μm I.D. deactivated fused-silica (e.g., approximately 13 cm long) linear restrictor was used to deliver the slow flows that were tested. In all subsequent experiments the flow-rates were controlled with integral restrictors made on-site.

In order to determine the decompressed flow-rate used, tapered restrictors were pulled to give linear velocities that were slow, optimum and fast as calculated by the retention time of a dichloromethane peak. The flow-rates at several pressures were measured in order to approximate a working range of flows for a 10 cm×1 mm column. From these data it

was decided that the interface would be tested at both 35 ml/min and 130 ml/min decompressed flow.

The restrictors were fed directly into the "t" at the top of the LC–Transform nozzle, Fig. 1. No split was used because small packed columns for SFC deliver slow flows. It is possible that at the flows used for larger columns (I.D. 4.6 mm) it would be necessary to use a split. If analytical scale columns had been necessary, the existing interface splitter mechanism could not have been used because of its inability to withstand the supercritical pressures. A single analyte, Irganox 1076 (provided by Paul Seemuth, Dupont, Chattanooga, TN, USA), was used in this feasibility study. Solutions were made in concentrations of 1, 0.5, 0.125 and 0.005 mg/ml. Injection volumes of 20, 10 and 5 μl were made to give injection and thus deposition masses on the disk of 10, 5, 2.5, 1.25, 0.625, 0.3 and 0.05 μg.

After the chromatographic eluent was deposited, the disk was removed from the portion of the interface and placed on a rotating optics mount in the FTIR. The FTIR was a Nicolet (Madison, WI, USA) Magna-550 equipped with a deuterated triglycine sulfate (DTGS) detector. The FTIR was run at 16 scans per spectrum with a resolution of 8 cm^{-1}. The disk was rotated for IR analysis at the same rate as the rotation rate for sample deposition. From the generated Gram–Schmidt reconstruction (GSR), individual peak spectra were generated by noting the data file closest to the peak maximum in the GSR (e.g., one 16 scan spectrum at the peak maximum, no co-addition of multiple 16 scan spectra).

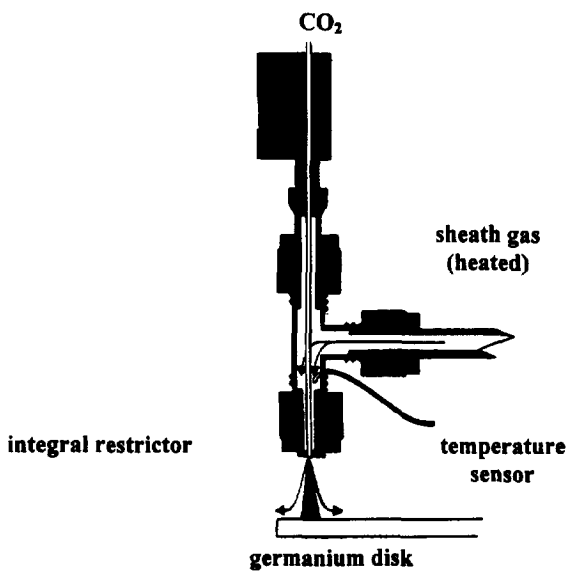

Fig. 1. Configuration of nozzle assembly and integral restrictor.

3. Results and discussion

As in its other applications, the LC–Transform delivers the separation process eluents via a spray nozzle. The carrying solvents are evaporated during the spray process, and the resulting chromatogram is deposited as a continuous track on an IR transparent flat surface (e.g., the sample collection disc). This track can then be scanned in a spectrometer to obtain spectra of the discrete sample components. We have found that the LC interface is particularly effective when coupled to SFC. Output from a supercritical fluid instrument flows through a capillary fixed restrictor. The CO_2 flashes as it enters the terminal low pressure region, and the solutes spray out of the capillary tip and deposit directly on the germanium sample collection disc. As with any HPLC run, sheath gas is used. Its primary function here however is to prevent the CO_2 from freezing into a solid plug in the capillary tip rather than to assist in evaporation of the mobile phase. The solute deposit from SFC is well focused and quite compact; typically the deposit is less than 1 mm in diameter. During SFC, if a peak has a broad low intensity elution band, the rotation of the sample collection disc under the nozzle may be stopped during peak elution. This concentrates all the eluate as one spot, rather than as a broad band, and its spectral intensity increases accordingly. The methodology is non-destructive, and deposited peaks can be recovered from the collection disc for other purposes or assays such as mass spectroscopy.

Preliminary experiments were conducted using a 10 μg injection mass and no sheath temperature, sheath flow, or nebulizer flow for deposition. It was, however, quickly determined that the interface temperature had to be increased so that the tip of the restrictor would not freeze and form a plug as a result of Joule–Thompson cooling caused by expansion of the supercritical fluid (SF). The sheath gas

(35°C) was therefore, introduced at a sheath flow of approximately 2 l/min.

Chromatographic injections of Irganox 1076 at 270 atm CO_2 and 100°C were made to give deposits ranging from 10 μg to 50 ng. Fig. 2 shows the GSR for six replicate 50 ng deposits. Also shown are six Chemigram (Nicolet) traces for the C–H region (3040–2700 cm^{-1}) which takes into account only the high analyte absorbance region and does not include regions of excess low end noise which can cause distortion in the reconstruction. The corresponding spectrum (16 scans) from one of the deposits is shown in Fig. 3. Peak intensities are above three times the standard deviation in the noise (especially for the C–H and C=O regions). It is therefore perceived that in collecting data via the on-line spectrum approach (e.g., disk rotates), the limit of detection for this method would fall in the 10–25 ng range. It is important however to take into consideration that this limit could be furthered lowered by collecting a larger number of scans on a single deposit.

The absorbancies of several of the primary bands (e.g., measured in triplicate) in the spectrum of Irganox 1076 were plotted as a function of analyte mass collected (Fig. 4). As one can see these plots do not give rise to a linear relationship, especially at the higher mass loadings. Injection volumes ranged from 5–20 μl. The optics on the interface sample stage are set up to condense the IR beam to 2 mm

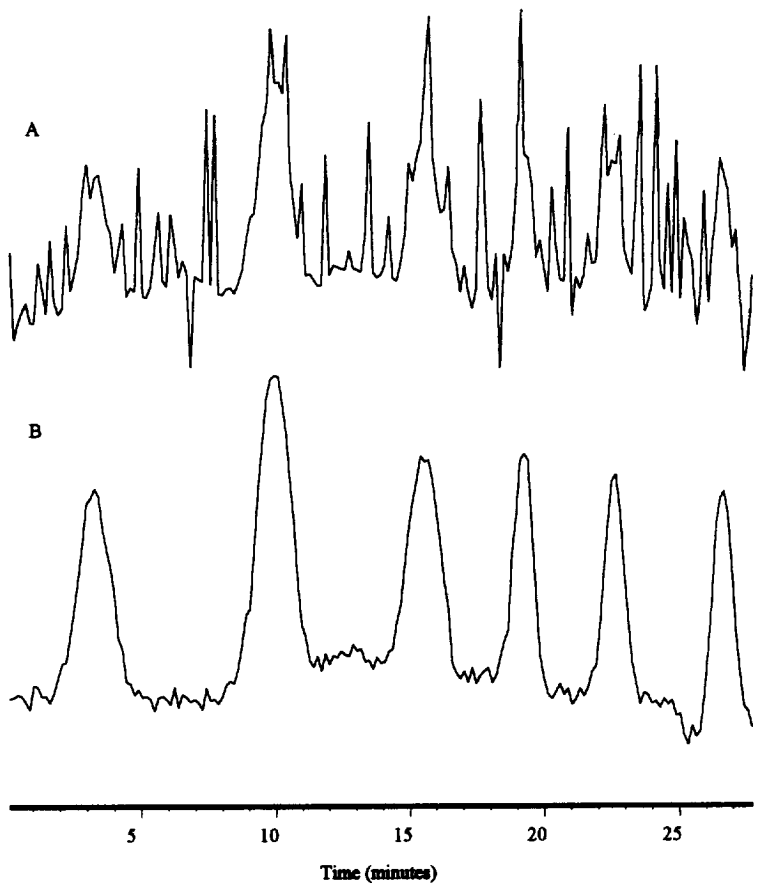

Fig. 2. (A) Gram–Schmidt reconstruction and (B) Chemigram for the 3040–2700 cm^{-1} region for six replicate injections of 50 ng Irganox 1076 deposited via SFC–FTIR mobile phase elimination using 100% CO_2. Chromatography: 10 cm×1 mm cyano column, 100% CO_2, 32 ml/min decompressed flow.

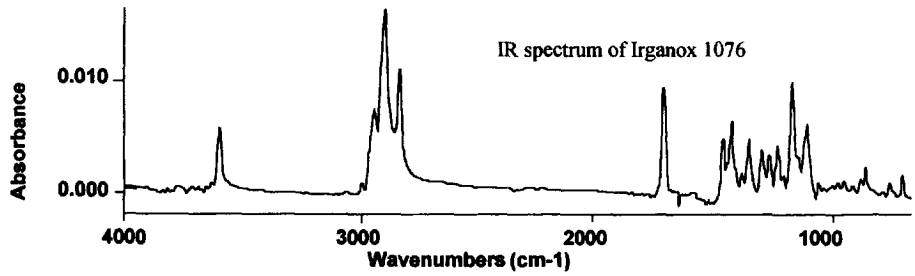

Fig. 3. FTIR spectrum for 50 ng of Irganox 1076 collected via SFC–FTIR mobile phase elimination: 16 scans.

which is the approximate size of an LC deposit. The deposits that have been seen to this point using SFC are much smaller, <1 mm. The non-linearity in the plots may be due to this deviation in the spot size to beam width ratio. In actuality, the linearity in these data up to the 5 mg level is much better than had been expected given this factor. In order to optimize the signal obtained from these SFC deposits, it would be necessary to re-focus the beam to a smaller width or use an FTIR microscope for higher efficiency. If quantification were being performed using an absorbance vs. analyte mass curve, an area of overlapping peaks obviously should not be used without a curve fitting routine. One should realize that quantification is more easily accomplished using a flame ionization detector. With modified fluids, however, this becomes problematical, therefore, FTIR can be used both for identification and quantification.

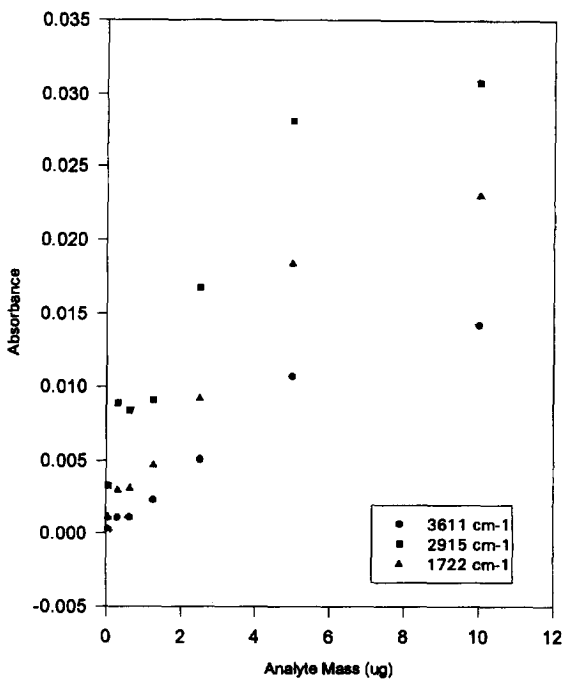

Fig. 4. Irganox 1076 FTIR peak intensities vs. mass collected at 5 deg/min disk rotation and 16 scans per spectrum.

One of the goals of this study was to determine how the position of the restrictor would affect the nature of the deposit. The above experiments utilized a restrictor that was at the same position as the HPLC deposition needle. Injections of 1.25 μg of material were made with the restrictor in this position and then at restrictor positions of 1 cm and 3 cm above the tip of the needle. The data collected at restrictor positions of 0 cm and 1 cm above the tip of the nozzle were the same. Sheath flows up to 6 l/min could be used at 35°C; but as the flow was increased the intensity of the GSR peak decreased. Apparently high sheath flows blow much of the analyte off of the disk. At a temperature of 40°C, only a flow of 2 ml/min could be used to obtain a deposit, and even at this low flow the reconstruction peak was small and misshaped.

Next, the restrictor was pulled 3 cm above the needle tip. At 35°C, sheath flows of 2 to 5 l/min were used to obtain analyte deposits. The GSR peaks were low in intensity and the reconstructions had an increased amount of noise between peaks. It is believed that at this restrictor height the analyte is depositing on the inside of the needle upon decompression. Some of the analyte was immediately deposited and some slowly moved from the walls of the needle to the disk which could account for the increased noise between primary GSR peaks. Given

these data, it was determined that the conditions that were used originally were probably optimum for obtaining SFC–FTIR deposits.

A higher decompressed CO_2 flow-rate (130 ml/min) was also tested using the same injection masses as the low decompressed flow (30 ml/min) experiments. The same sheath flow and temperature were used. The fused-silica that was used to make integral restrictors for the larger flows had a larger outer diameter than the linear 15 μm restrictor. In order to position this restrictor similarly, the "t" in the Lab Connections LC–Transform interface had to be bored out to a larger I.D. and the needle that resided within the nozzle was taken out. The restrictor was then fed through the nozzle to protrude slightly past the exit which is positioned where the needle usually sits for LC–FTIR. The resulting deposit size at 210 atm CO_2 and 100°C was approximately the same and results with respect to peak heights at various disk rotation speeds 3, 5 and 10 deg/min were also the same as with the 35 ml/min decompressed flow.

3.1. Methanol modified CO_2

Pre-mixed methanol (5%) modified CO_2 was used to determine the feasibility of the LC–Transform interface for supercritical fluids work. It was expected that the optimum conditions for depositing analytes using a modified fluid would differ because of the decreased volatility of the mobile phase. To determine the sheath flow and sheath temperature needed, 2.5 μg of Irganox was deposited (methanol modified CO_2 200 atm, 70°C) at varying sheath conditions and at a CO_2 decompressed mobile phase flow-rate of approximately 120 ml/min. Temperatures of 35, 40, 50 and 60°C were used. At each of these temperatures three injections were made at sheath flows of 3, 4, 5, 6 and 8 l/min. A sheath flow of 2 l/min was not sufficient in eliminating the methanol. At temperatures of 35 and 40°C, all of the methanol was also not removed; therefore, peak shapes were not as good as with the 50°C data. At 60°C, however, the peaks were again not "clean". At temperatures this high, the methanol is most likely starting to evaporate prior to exiting the restrictor. Some of the analyte, therefore, can deposit on the inside of the restrictor forming a partial plug. This plug will ultimately be removed from the restrictor by the mobile phase; however, the analyte will not be deposited in a narrow band on the disk. Temperatures close to the boiling point of the solvent can cause analyte to condense on the tip of the restrictor. In this case, methanol will form a large droplet which will fall onto the disk and cause peak/deposit distortion. The optimum sheath conditions for depositing Irganox 1076 with methanol modified CO_2 were, therefore, determined to be 50°C at 4–6 l/min.

It should be stressed that only the minimal parameters needed to accomplish deposition should be used. Similar spectral results were acquired using 5% methanol as were obtained with 100% CO_2 (e.g., no spectral evidence of methanol, see Fig. 3). Also, the size of the deposits acquired using modifier was slightly larger than those with pure CO_2 but still smaller than LC deposits (1–1.5 mm). The results obtained here were very similar to those solvent elimination FTIR interface results previously cited. In all cases, low to mid nanogram detection limits were easily obtained. It was found in our study that the small deposit size did not necessitate the use of a microscope, although it is expected that the linearity of response would increase with an optics adjustment. Consideration should be given to using a restrictor heater to maintain the critical temperature as long as possible. In summary, the main goal of easily adapting the LC–FTIR interface to SFC was successfully accomplished.

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