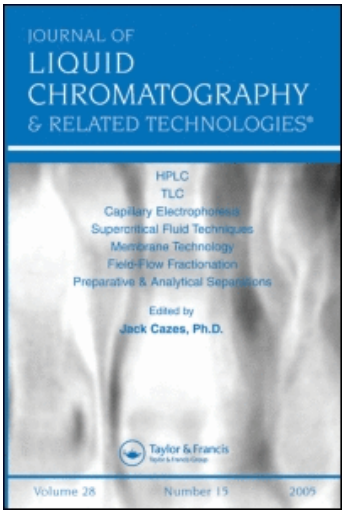

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PRELIMINARY STUDIES FOR INTERFACING COUNTERCURRENT CHROMATOGRAPHY (CCC) WITH FOURIER TRANSFORM INFRARED (FT-IR) SPECTROMETRY

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ABSTRACT

High speed countercurrent chromatography (CCC) and its use as an interface to a Fourier transform infrared (FT-IR) spectrometer are described. In this preliminary study the high solute-to-solvent ratios attainable with CCC are investigated, and infrared spectra are recorded of CCC fractions of aromatic components. The resulting infrared spectra of mg/mL concentration CCC fractions have very high signal-to-noise ratios, and no interference bands from the stationary phase (water/methanol) can be seen. Chloroform was used as the mobile phase. This study clearly demonstrates that a CCC/FT-IR spectrometry interface is feasible.

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INTRODUCTION

The development of LC/FT-IR spectrometry has lagged behind that of GC/FT-IR spectrometry because infrared absorption of the liquid chromatographic mobile phase is significant. This problem has encouraged the development of mechanically complex solvent removal methods (1-6), but systems employing flow-through cells have also been developed (7-14).

Griffiths and coworkers have developed systems that obtain diffuse reflectance spectra of liquid chromatographic eluates after solvent removal. Their first system (1,2) designed for normal phase HPLC used differential evaporation to increase the concentration of each peak by a factor of 10 before taking diffuse reflectance spectra. The detection limits obtained with this system (100 ng) are adequate for conventional HPLC where the column capacities are two orders of magnitude higher than these detection limits. The same research group has recently developed a solvent removal technique for reverse phase HPLC/FT-IR spectrometry (3). Since removing aqueous mobile phases has been a difficult task, the eluents are continuously extracted into CH_2Cl_2. The detection limit for this system is about 1 μg. The main problems associated with both the reverse and normal phase solvent removal systems are the large number of sample cells that must be loaded and the cumbersome instrumentation needed to deposit the eluent on the diffuse reflectance sample cups continuously.

Jinno and coworkers have developed a second solvent removal system using microbore LC columns (4-6), called the "buffer memory" method. In this system the solvent evaporates when it contacts a rotating salt plate, leaving the solutes on the crystal. Absorption spectra of the solutes are recorded. This technique is not as complex as those developed by Griffiths and coworkers; but its detection limits are two orders of magnitude higher than those obtained by diffuse reflectance.

Due to the complexity of solvent removal methods, some work has also been done with flow-through LC/FT-IR spectrometry. Vidrine and Mattson (7) have shown that size exclusion chromatography (SEC) is compatible with FT-IR spectrometry detection. Since in SEC the mobile phase is only used as a solvent for the mixture to be separated, solvents with large IR windows, such as chloroform and carbon tetrachloride, can be used. These workers (7) even had some success using tetrahydrofuran (THF) as the mobile phase by using relatively thin flow cells (0.1 - 0.3 mm) and high solute-to-solvent ratios (0.2% polybutyl acrylate and 0.8% polystyrene in 0.5 mL of injected THF).

Taylor and coworkers have developed microbore HPLC/FT-IR spectrometry (9-13). The most important advantage of this system is that microbore columns offer eluent concentrations up to 20 times those of conventional HPLC. This increase in eluent concentration is necessary for IR detection, where the sensitivity is lower than in UV detection. One disadvantage is that even with the higher solute concentrations possible with microbore HPLC it is necessary to sacrifice chromatographic resolution in order to achieve IR detection in some cases. The detection limit for this technique is about 1 µg.

An LC/FT-IR spectrometry system that employs high speed countercurrent chromatography (CCC) has been developed in this laboratory. With this system much higher solute-to-solvent ratios are achieved than with conventional HPLC or microbore HPLC. The objective of this study is to investigate the actual solute-to-solvent ratios produced by CCC and then to study the feasibility of interfacing CCC with FT-IR spectrometry.

MATERIALS AND METHODS

Materials

Acetophenone was obtained from Fisher Scientific Co., Fairlawn, New Jersey, phenol from Baker and Adamson, Morristown,

New Jersey, and o-nitrophenol from Eastman Kodak Co., Rochester, New York. Doubly distilled water was used. Chloroform, UV spectral grade, was obtained from J.T. Baker Chemical Company.

High Speed CCC

Countercurrent Chromatography (CCC) has been described extensively in the literature (15-20), and a review will not be given here. The high speed CCC used has a 125 meter column with an i.d. of 1.68 mm and a total capacity of 270 mL. The column is multilayer and has a total of 10 layers of tubing. The column rotational speed (0-1000 rpm) is controlled by a speed control unit (Bodine Electric Co.). A model 11A Beckman reciprocating pump was used for the mobile phase, although less expensive and simpler metering pumps can be used since it usually takes less than 200 p.s.i. to push the mobile phase through the column. A VALCO six port injector was used. The injector was designed for 1/16" o.d. tubing, so it was necessary to fuse the wider column tubing to it. UV detection was achieved with an ISCO Model 1840 (Lincoln, Nebraska) variable wavelength UV-VIS absorbance monitor with a built-in chart recorder. The outlet tubing was fused to tubing of about 0.3 mm i.d., and a stainless steel fitting was used to avoid formation of air bubbles due to the pressure drop across the detector cell. A 5-mm analytical flow cell from ISCO with a 9.5-μL illuminated volume was used. Fractions were collected with an ISCO 1800 fraction collector.

The two-phase solvent system consisted of chloroform, water and methanol in a combination of 3:3:1, respectively. The two phases were equilibrated in a one-liter separatory funnel before being introduced into the column. The sample solutions were prepared from saturated solutions of acetophenone, phenol, o-nitrophenol and p-nitrophenol. All were dissolved in chloroform, except for the p-nitrophenol which was dissolved in a 4:1 mixture of chloroform and methanol. A 50-μL sample loop was used, and 3-4 mg

of each of the mixture components were injected except for p-nitrophenol (8 mg were injected).

The column was first filled with the stationary phase. The mobile phase was then pumped through while the column was spun at 600 rpm. The sample solution was injected, the eluate was monitored at 270 nm, and fractions were collected every 0.6 minute. Through the head-end of the column chloroform (heavier phase) was pumped. The tail-end of the column was connected to the detector.

Infrared Instrumentation

The FT-IR spectra were obtained with a Digilab FTS-20/E/D spectrometer (Digilab Division of Bio-Rad, Cambridge, MA). A Mercury-Cadmium Telluride (MCT) detector (Infrared Associates, New Brunswick, New Jersey) was used. Spectra were measured at 8 cm^{-1} resolution. A KBr cell with a 0.05-mm pathlength was used (Barnes Analytical Spectra-Tech, Stamford, CT).

RESULTS AND DISCUSSION

CCC Separation

Chloroform was chosen as the mobile phase because of its large IR spectral windows. The flow rate used for the separations described in this study was 4.0 mL/min, and the high speed CCC was run at 600 rpm. Under these conditions approximately 79% of the

TABLE 1

Partition Coefficients for CCC

Compound	Partition Coefficient
Acetophenone	0.030
o-Nitrophenol	0.022
p-Nitrophenol	0.26
Phenol	0.40

stationary phase was retained. Several CCC separations were run using the described conditions. Simple three-component mixtures were separated, and the overall results of the separations are presented in Table 1. Acetophenone and o-nitrophenol were not run in the same separation because they coelute, as can be seen from the partition coefficients in Table 1. The column produced about 950 theoretical plates.

IR Detection

As mentioned above, the development of LC/FT-IR spectrometry has been plagued by the fact that solvents necesary for chromatographic separations usually exist in concentrations too great to permit identification of the solute in flow-cell systems. Results presented here indicate that spectra of very high signal-to-noise ratios can be obtained from high speed CCC fractions, without removing the solvent. Figures 1 and 2 show spectra of 1 mg/mL fractions of acetophenone and o-nitrophenol. These spectra can readily be compared with standard reference spectra, and the comparison indicates the quality of the spectra is very high. The high signal-to-noise ratio in Figures 1 and 2 indicates that the detection limit is at least ten times lower than the concentrations used to record these spectra.

The single beam spectra of fractions from high speed CCC peaks were ratioed against fractions that contained only the mobile phase. In this manner chloroform and methanol bands were eliminated, so the spectrum of the pure eluate was obtained. Nevertheless, discontinuities in the spectra of the eluates occurred where strong bands of chloroform were ratioed. In the acetophenone spectrum these discontinuities can be seen at 2400, 1200, and 800 cm^{-1}. In these regions the solvent is opaque, and no eluate spectral response can be measured.

It is obvious that these spectra are not adversely affected by the use of water and methanol in the solvent system. Even though

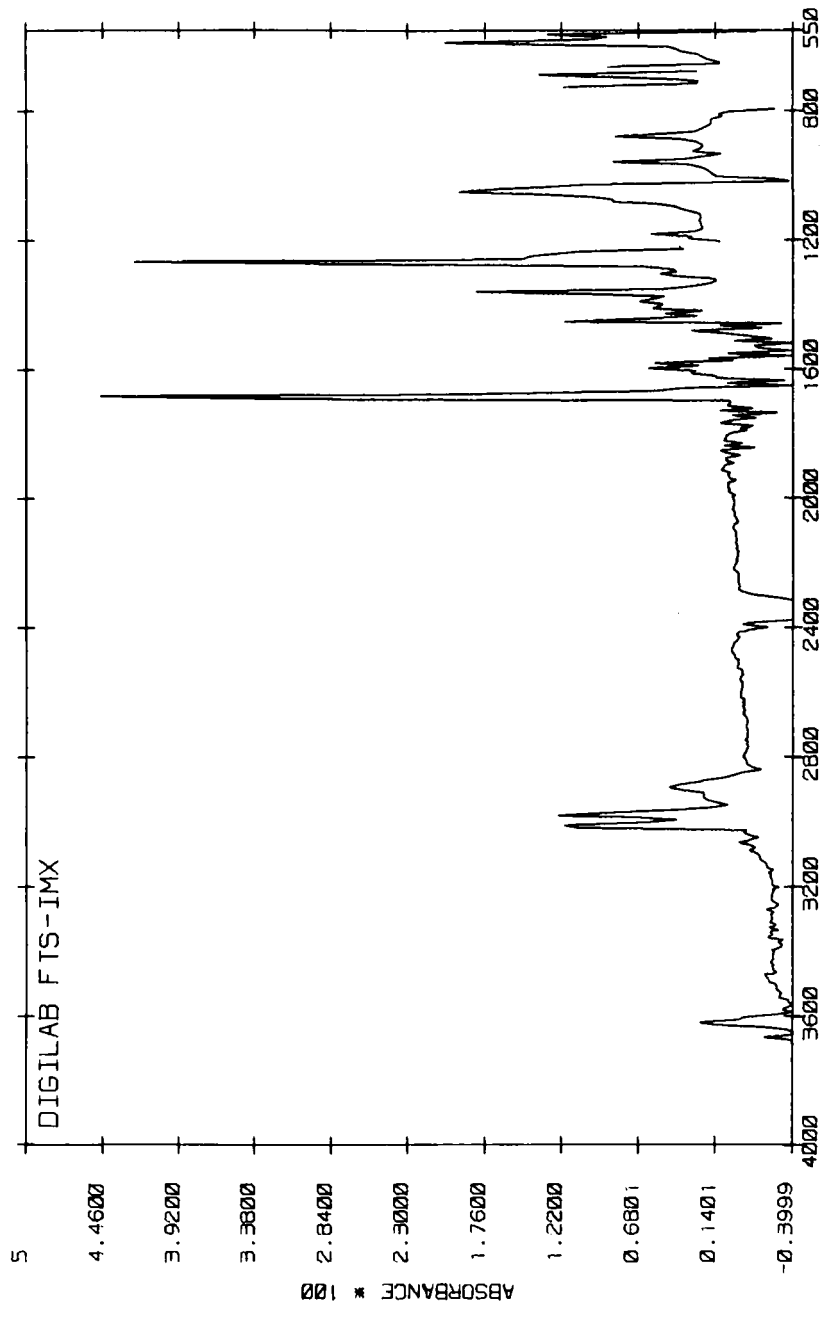

Figure 1. Spectrum of acetophenone in a 0.05-mm pathlength cell.

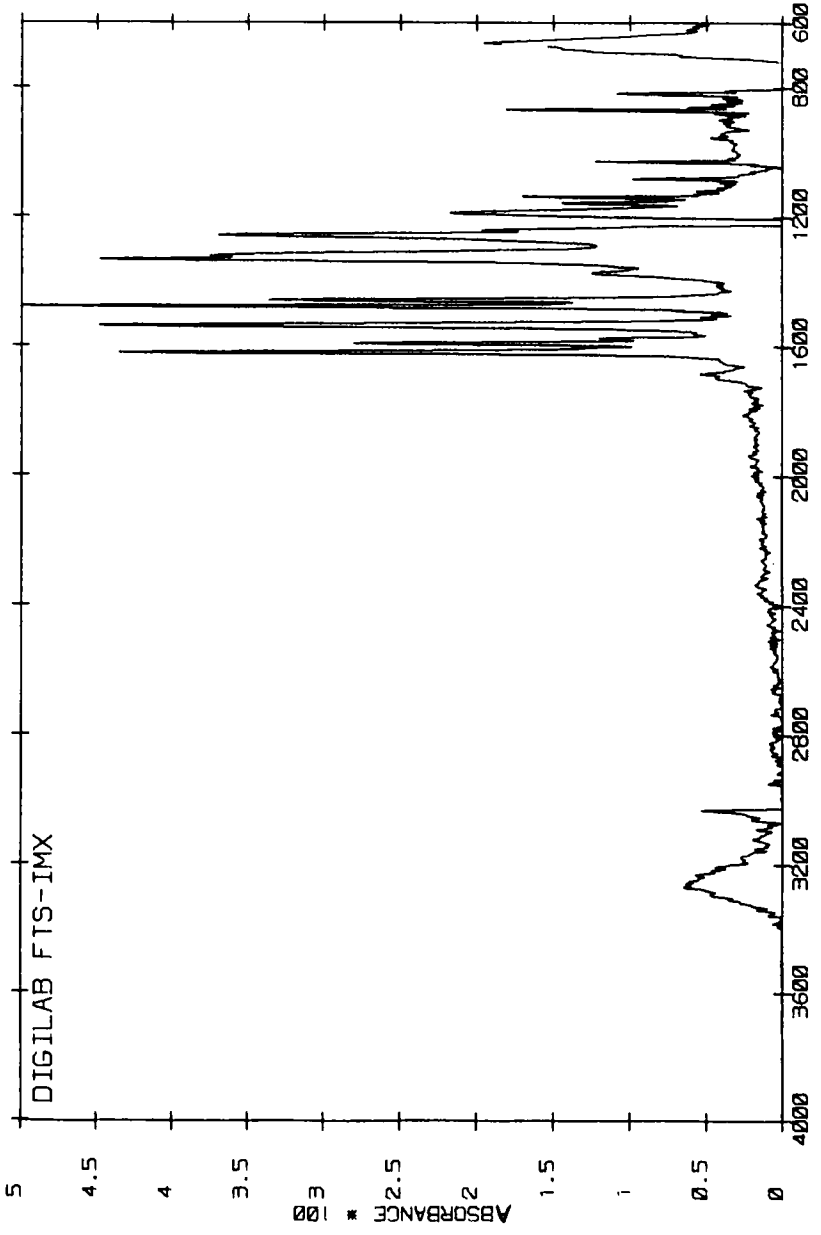

Figure 2. Spectrum of o-nitrophenol fraction with a pathlength of 0.05 mm.

water is slightly soluble in chloroform and the use of methanol increases its solubility, the water bands are very weak. The concentrations of water and methanol in the mobile phase are sufficiently low that they can be eliminated without causing any distortions in the spectrum. The cell pathlength of only 0.05 mm could be increased to improve the adsorption of the eluates. It should be possible then to obtain spectra of lower concentration eluates and still achieve good signal-to-noise ratio spectra. The disadvantage of increasing the pathlength is the decrease in the width of solvent windows, hence more of the solute is masked. Nonetheless, this is a rather small effect.

CONCLUSION

High-speed CCC can be used as a liquid chromatographic separation technique for interfacing to an FT-IR spectrometer. Although this study did not show spectra recorded on-line, high signal-to-noise ratio spectra were obtained from CCC fractions. Only small regions of the spectra were eliminated due to the infrared opacity of the mobile phase. The CCC system presented was capable of separating very high sample concentrations, but it may not be necessary to use such a high sample loading to produce quality FT-IR spectra. The results clearly demonstrate the feasibility of interfacing high-speed CCC with an FT-IR spectrometer. Future studies will involve the optimizations of CCC conditions and flow cell design for an interfaced high speed CCC/FT-IR spectrometry system.

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