IDENTIFICATION OF POLAR MATERIAL VIA SUPERCRITICAL FLUID CHROMATOGRAPHY-FOURIER TRANSFORM INFRARED SPECTROMETRY

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INTRODUCTION

In the past several years, a great amount of interest has been generated in Supercritical Fluid Chromatography (SFC) among separation chemists. The physical properties of a supercritical fluid, namely low viscosity, high diffusivity, and good solubility, give the mobile phase characteristics intermediate to those demonstrated in high performance liquid chromatography (HPLC) and gas chromatography (GC).(1) This situation has allowed for the separation via SFC of additional compounds which are not separable by HPLC or GC. Thus, SFC offers much promise in the separation of certain complex mixtures.

Detectors that are used for HPLC and GC, ultraviolet absorption and flame ionization, are also successfully used for SFC detection. However, as in the past when dealing with more conventional chromatographic methods, the need for higher information detectors in SFC exists. The success of Fourier Transform Infrared spectrometric detection with GC(2) and HPLC(3) has led to the obvious extension of this technique to SFC. The most commonly used mobile phase in SFC is CO_2. It has relatively good solubility for many compounds, is nontoxic and inexpensive. Yet, both supercritical and subcritical CO_2 intensely absorb infrared radiation around 3600 cm^{-1} and 2400 cm^{-1}. Although these regions are opaque to any analyte which might absorb in the same region, excellent infrared spectra can be obtained in the regions of mobile phase IR transparency.

One problem in extending FTIR detection to SFC is insufficient detector sensitivity. An obvious means of improving the sensitivity in the IR is through an increase in the pathlength of the flow cell. This increase should enhance the signal-to-noise ratio of the spectra obtained. However, too great a pathlength may lead to a decrease in the width of the IR window region, a substantial drop in the IR thoughput, and/or a decrease in overall resolution and integrity of the on-line spectra. In this paper we present a comparison of two flow cells, differing in length and flow geometry and two mobile phases (CO_2, Freon 23) with UV-254 and FTIR on-line detection. In doing so, we show the eluting characteristics of basic nitrogen compounds on a packed bonded-phase silica column and compare retention characteristics of a polar model separation using two different supercritical mobile phases. A sensitivity study with a weak IR absorbing compound employing more nearly optimal chromatographic parameters than spectrometric parameters is discussed wherein the two cells are compared.

EXPERIMENTAL

A Hewlett-Packard (Avondale, PA) Model 1082B liquid chromatograph modified for supercritical fluid chromatography and equipped with a high-pressure Model 79875A variable wavelength UV detector was used in these investigations. Anaerobic grade CO_2 (Airco, Bluefield, WV) and Freon 23, CHF_3 (Dupont Co., Wilmington, DE) were used for the experiments. A Nicolet Model 6000C FTIR was used to collect time-resolved, 4 cm^{-1} resolution spectra. A Model 7010A narrow-band (5000 - 700 cm^{-1}) mercury-cadmium-telluride (MCT-A) detector was used in all experiments. Schematic diagrams of the flow cells employed in this study are shown in Figures 1 and 2. The internal surface of the 5 mm pathlength cell was a solid gold insert which had been polished longitudinally to give maximum infrared reflectivity. The internal cell dimensions were 1 mm ID x 5 mm pathlength. The cell windows were 13 mm diameter x

2 mm thick ZnSe disks, and were sealed in place with polyperfluorinated elastomer Kalrez O-rings (DuPont). The main body of the flow cell was 304 stainless steel with 0.010" ID x 1/16" OD SS inlet and outlet tubing silver-soldered in place. The second flow cell (Figure 2) was the high-pressure UV cell provided by Hewlett-Packard (1 mm ID x 10 mm). It was modified for FTIR by replacing the quartz windows used for UV detection with CaF_2 windows.

RESULTS AND DISCUSSIONS

Flow Cell Comparison

Each of the two cells has been evaluated in the same separation of a model mixture containing five basic nitrogen components. The optimum chromatographic conditions were determined via UV (254 nm) detection. The Gram-Schmidt reconstructed chromatogram arising from the optimized separation with the 5 mm cell showed only four components, while the UV trace (in-line after the FTIR) revealed all 5 components. Naturally with a UV detector, the elution order of a 5 component mixture could only be indentified by single injections of each material. Employing an FTIR, interpretation of individual file spectra afforded immediate identification. The GSR for the 10 mm cell showed the fifth component and over-all better signal-to-noise for the same amount injected than that for the shorter pathlength. In the same respect, the coadded file spectrum of each component was much sharper, and the match to the spectrum in the EPA vapor-phase library was improved.

A detection limit study was carried-out by varying the amount of N-methylaniline injected under the same chromatographic and spectrometric conditions used for the five component separation. The IR band of interest was 1608 cm^{-1}. For each cell 4 scans/file (scan time = 0.45 sec/scan) were collected, and coaddition over 1.4σ of the Gram Schmidt Reconstructed peak was performed. The injected minimum detectable quantity (IMDQ) of N-methylaniline using the 5 mm pathlength cell was found to be 156 ng ($3xN_{rms}$).

Mobile Phase Comparison

Certain fluorocarbons possess rather moderate critical pressures and temperatures. Their use as a relatively IR transparent mobile phase in HPLC is well documented.(4) Freon 23 (trifluoromethane) which has a critical temperature of 25.9°C and a critical pressure of 701.4 psi, has been suggested to exhibit similar eluting characteristics as CO_2.(5) The IR windows of CO_2 have been found to complement very well the IR windows of Freon 23. A seven component mixture of phenols, alcohols and esters has been separated on a bonded-phase phenyl derivatized silica packed column under identical chromatographic conditions using supercritical CO_2 and Freon 23. Initially the separations were optimized with UV-254 nm detection. Afterwards sequential FTIR-UV-254 nm detection was employed. Injection of approximately 30 µg/each component yielded the Gram Schmidt reconstructed chromatograms shown in Figure 3. The solvent in each case was carbon tetrachloride which consistently eluted first. With UV-254 detection this peak is a minor one, however, with FTIR detection the peak is quite intense which reflects the high infrared absorbance of CCl_4. The seven components eluted in the same order regardless of the mobile phase. The solvent strength of CO_2 appears to be slightly greater since each component has a lower retention time. This is notably the situation with benzyl benzoate, phenol, phenyl acetate and benzyl alcohol, the latter of which never eluted over a reasonable time period with Freon 23.

Examination of the on-line IR spectrum of several common components from the CO_2 and Freon 23 separations illustrates the complementary nature of IR information obtainable. For example while the O-H stretching mode of 2,6-di-tert-butyl phenol is obscured with CO_2, it is readily observable with Freon 23. Although the remainder of the Freon 23 spectra is useless, it is those regions that are

transparent with CO_2. Consequently continuous information is available through the mid-infrared region except for the triple-bonded absorbances around 2100 cm^{-1}. The on-the-fly spectra of o-nitrophenol in CO_2 and Freon 23 are shown in Figure 4. A relatively weak, broad bonded O-H stretching mode is observed in both mobile phases for o-nitrophenol. There is a loss in sensitivity at this frequency when using Freon 23 relative to CO_2 due to decreased IR throughput to the detector windows.

In summary, both lightpipe cells offer very good S/N spectra obtained on-line during a chromatographic run. Although CO_2 seems to be an adequate mobile phase for SFC-FTIR, the regions of total absorbance may hinder the identification of a component in a complex mixture. Freon 23, although relatively opaque in the IR, does have reasonable transparency from 4000 cm^{-1} to 2500 cm^{-1}. The seemingly similar elution charcteristics to CO_2 make method development with Freon 23 fairly easy and inexpensive. Thus CO_2 and Freon 23 make a good mobile phase pair for SFC-FTIR allowing for more complete identification of analyte species.

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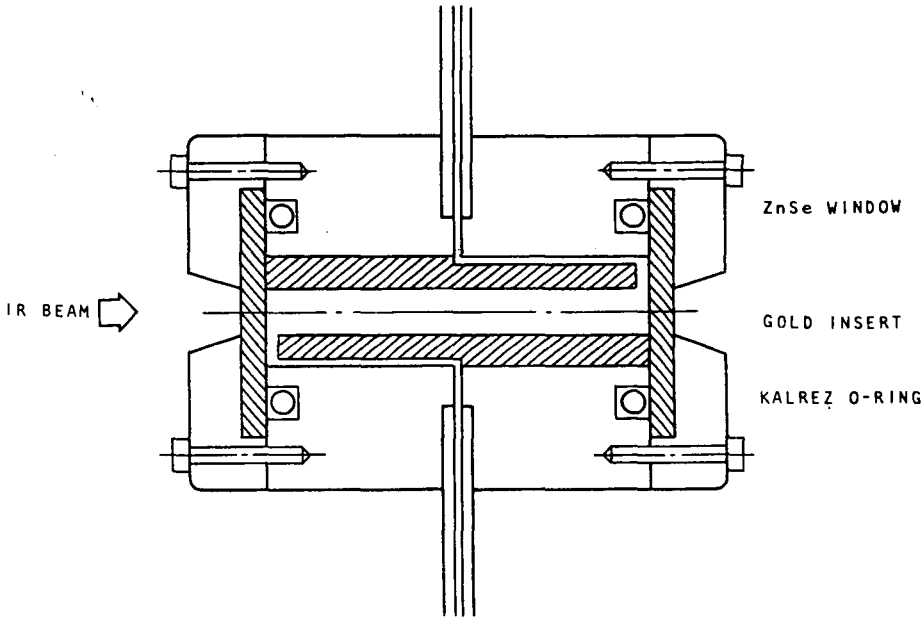
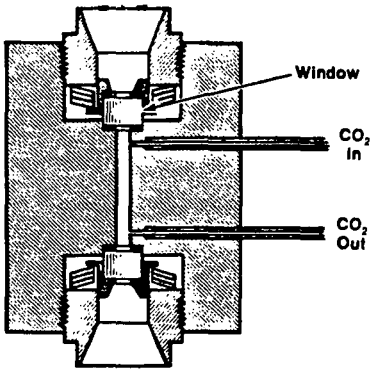


Figure 1



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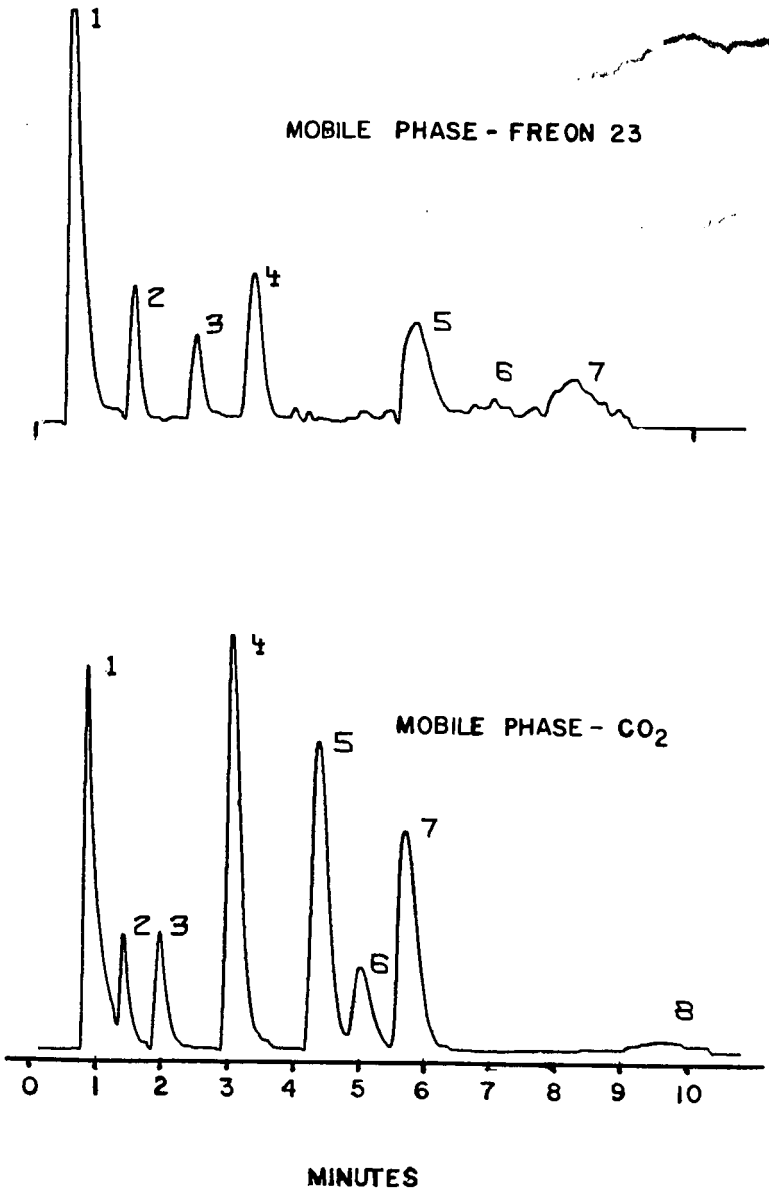
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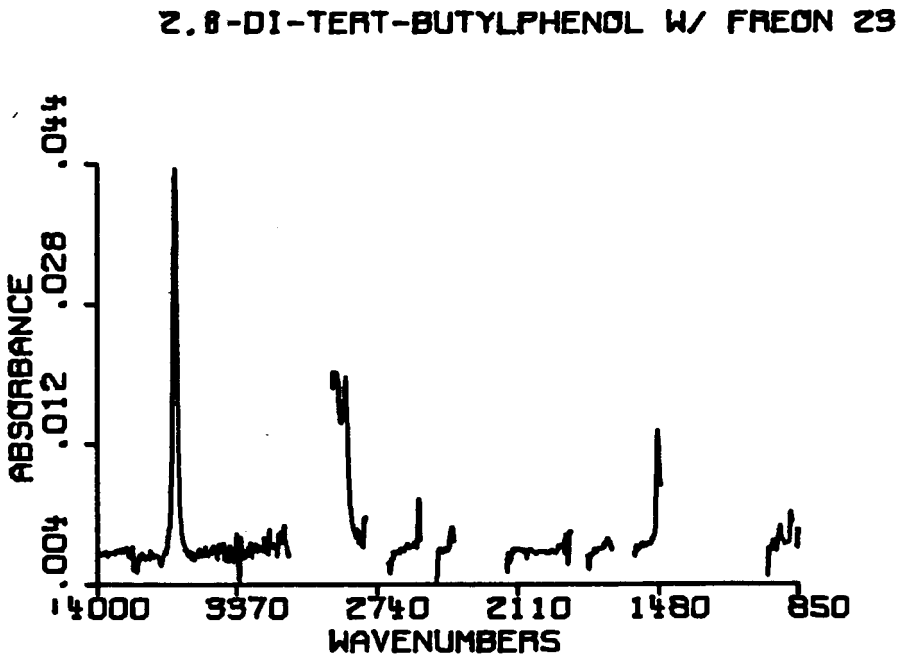
1
2
3
4
5
6
7
MOBILE PHASE - CO_2
1
2
3
4
5
6
7
8
0
1
2
3
4
5
6
7
8
9
10
MINUTES

Figure 3


2,8-DI-TERT-BUTYLPHENOL W/ FREON 23
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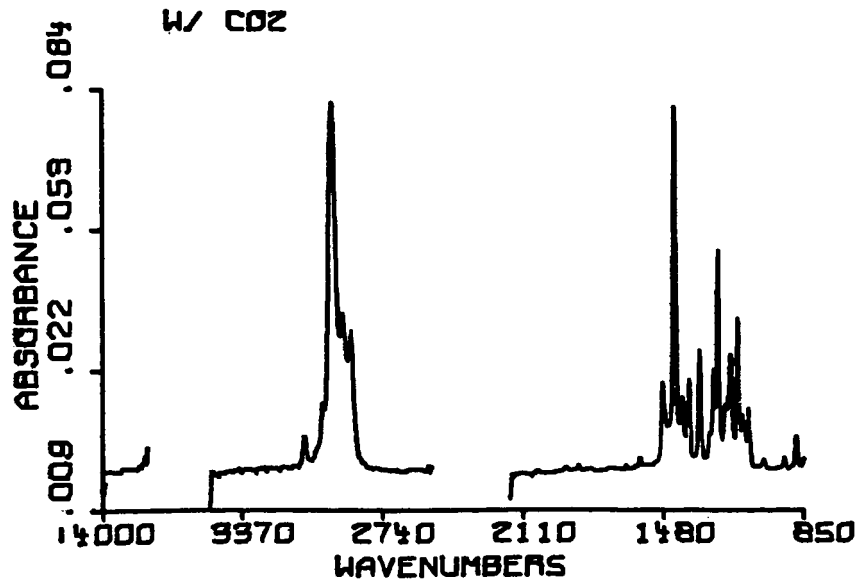
.012
.028
.044
14000
3370
2740
2110
1480
850
WAVENUMBERS


W/ CO2
ABSORBANCE
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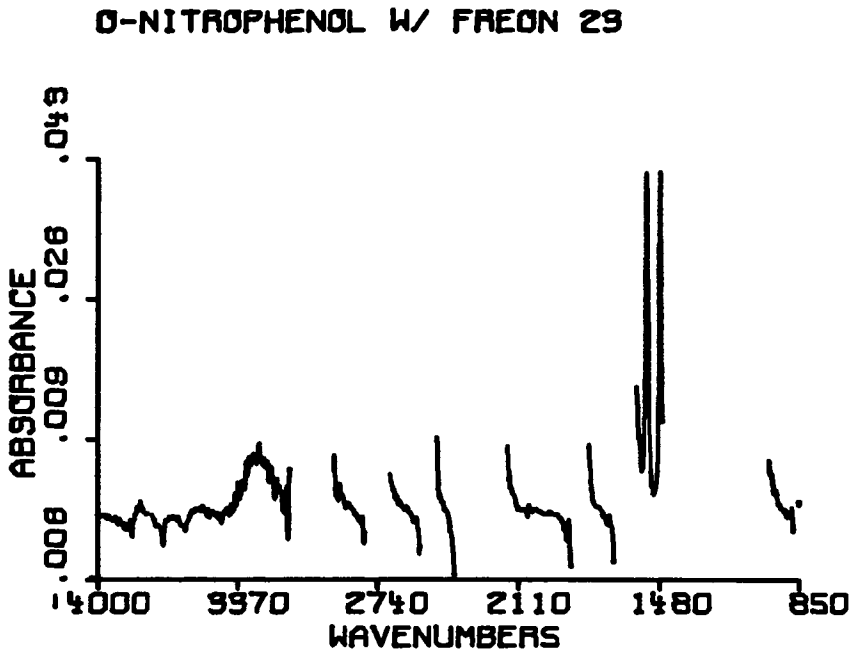
.022
.059
.084
14000
3370
2740
2110
1480
850
WAVENUMBERS

Figure 4


O-NITROPHENOL W/ FREON 23
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3370
2740
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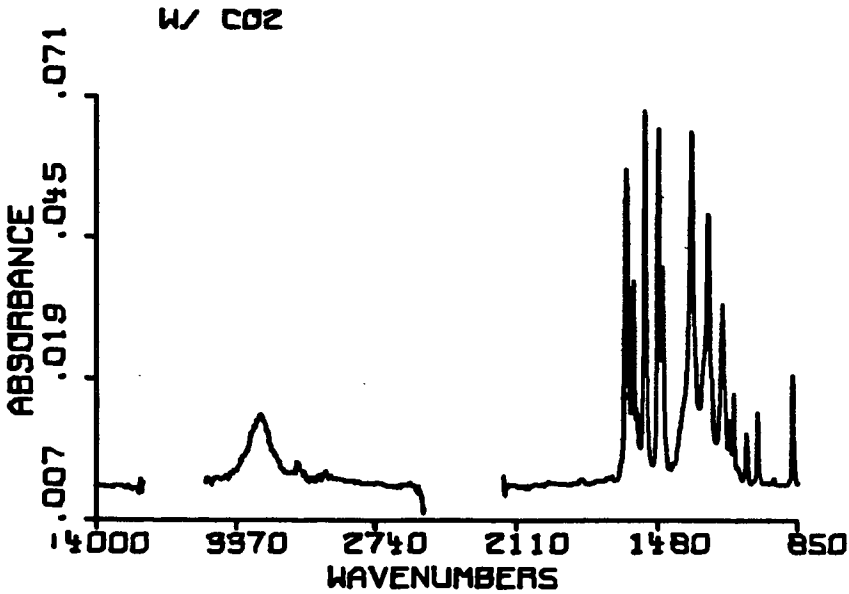
1480
850
WAVENUMBERS


W/ CO2
ABSORBANCE
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.019
.045
.071
4000
3370
2740
2110
1480
850
WAVENUMBERS

Figure 5