INFRARED SPECTROMETRY AS A HIGH PERFORMANCE LIQUID
CHROMATOGRAPHIC DETECTOR WITH APPLICATION TO
SOLVENT REFINED COAL PRODUCTS

by

Robert Scott Brown

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APPROVED:

L. I. Taylor, Chairman

J. G. Mason

H. C. Dorn

R. E. Dessy

P. J. Harris

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Infrared Spectrometry As A High Performance Liquid Chromatographic Detector with Application to Solvent Refined Coal Products

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(ABSTRACT)

The development of Infrared Spectrometry as a High Performance Liquid Chromatographic detector is presented. Early work with both a conventional dispersive instrument and a Fourier Transform Infrared (FTIR) Spectrometer is presented coupled via a flow cell to size exclusion chromatography. These were used for the analysis of the non-volatile components produced in the liquefaction of coal.

Additional work is presented for the coupling of FTIR to analytical scale normal phase chromatography via a flow cell technique. Analysis of both model mixtures as well as a complex process solvent used in the liquefaction process is discussed. Use of deuterochloroform as an improved IR transparent solvent is demonstrated.

Work with microbore (1 mm i.d.) columns coupled with on-line flow cell detection is presented. Modification of the flow cell design for microbore compatibility is shown as well as the benefits of microbore columns for flow cell FTIR. Detection limits as amount injected for both analytical and microbore scale HPLC-FTIR are shown.
Finally, detection limits as amount injected for a wide variety of chemical classes are presented for microbore HPLC-FTIR. The effects of various chromatographic conditions on detectability are presented and equations developed to describe them. Improvements in detectability through derivatization reactions are also explored.
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My association with Doug Hausler and our collaboration on some early work, both with FTIR and ICP-AES, was instrumental in laying the framework for later investigations. His willingness to share his experience, both while at VPI&SU and after he left, is greatly appreciated.

Thanks also should go to many of the rest of the members of our research group. Pat Amateis, Chuck Johnson and John Cooper who are involved with research similar to that presented in this dissertation, deserve thanks for their suggestions and comments regarding this work. Mike Spratt's help with the derivatization work is also greatly appreciated. A special thanks goes to John Hellgath whose tireless efforts at sample generation, preparation and pre-fractionation have made much of the analyses possible. Also, thanks to those who were not directly involved in this research, but who helped create the cooperative atmosphere which was so conducive to research.
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PREFACE

Some of the work presented in this dissertation has appeared previously in a series of papers which at this writing have either been (a) published, (b) accepted for publication or (c) are in the review process. The appropriate citation is included in the reference section in the rear of this dissertation. Portions of Chapters 2, 3, and 4 were published in Analytical Chemistry (Ref. 103, 62, 104) while portions of Chapter 5 and 6 have been accepted (100) and are in review (105) respectively by the same journal.
CHAPTER 1

INTRODUCTION

This work deals with the development of analytical techniques for the analysis of coal-derived products. The techniques reported here will deal specifically with the organic portion of solvent refined coal (SRC) and coal-derived process solvent although techniques for the inorganic portion (1) have also been developed.

First, what is the SRC process? The solvent refining of coal is one of several different processes being developed to convert coal into a more "petroleum like" product. A good overview of the various processes is presented by Farcasiu et al. (2). In its simplest form, coal is slurried with a solvent, pressurized with several thousand psi of hydrogen and reacted at ~800°F for various amounts of time, each variable affecting the product composition differently. The solvent has classically been tetralin which act as a hydrogen donor which can transfer hydrogen to fragments of coal that have been thermally cleaved during the reaction and which stabilizes these fragments.

From this reaction mixture, three main products are produced. The volatile product (solvent) is distilled off (up to 850°F) and is recycled back as the solvent for the next run. This solvent now consists of not only the original solvent, but also coal produced material and hydrogen poor forms of the original donor solvent (i.e. naphthalene from tetralin), as well as any breakdown products of the
solvent. This recycle solvent may then be upgraded through catalytic rehydrogenation or simply reused without any upgrading. The composition of this solvent system has been shown to be crucial in the production of desirable products.

The second and major product is the SRC itself. This depolymerized coal product is classically defined as the pyridine soluble, non-volatile product and constitutes generally 70-80% of the mineral matter free coal by weight when employing the most common solvent (tetralin) and conditions. The final portion of the product is the pyridine insoluble portion which represents mineral matter, unreacted coal and products of regressive reactions. This too, is sometimes recycled to the next run in certain processes and can improve the overall conversion to soluble products.

Since the solvent system composition is critical in order to produce a soluble product with good conversion efficiency, a method for its analysis is important in order to aid in understanding the complex chemistries involved in the coal conversion process. Similarly, if the SRC material is to be employed as a fuel, its composition is important from an environmental standpoint. The ability to monitor what types of products are produced with a given set of reaction conditions is also desirable. Also, catalytic upgrading to distillable fuels is being employed in some processes and a good understanding of the starting material composition is therefore necessary.

The detailed analysis of these products is hampered by several factors. The composition of coal-derived materials is not only
extremely complex but extremely heterogeneous as to component classes. Unlike petroleum products, most of the coal derived product is of relatively high molecular weight and has a large amount of heteroatom (N, O) content. Also, the major portion of the product is non-distillable and does not allow the use of many of the procedures developed for the analysis of crude oils. Due to the sample's complexity, many workers have reported methods to separate the products into simpler fractions for subsequent analysis. These will be briefly reviewed.

These fractionation schemes can be broken down into two areas: (1) wet chemical methods, and (2) preparative liquid chromatography (LC) methods. The earliest and perhaps most widely used methods were based on solvent extraction procedures. These tended to vary somewhat from lab to lab depending upon the extraction solvents that were employed. A representative modern method (3) was described by Bockrath et al. The major classifications of the fractions include oils (hexane soluble compounds), asphaltenes (hexane insoluble-benzene soluble compounds) and asphaltols (benzene insoluble-pyridine soluble compounds). As one might expect, there is much spill over between fractions of similar compound classes. Nevertheless, this is still a widely used procedure.

A second type of fractionation procedure takes advantage of the acidic or basic nature of certain chemical classes. These procedures vary according to the lab but are referred to as acid, base, netural separations. A good procedure was given by Hausler et al. (5). By treatment of a THF solution of the sample with HCl gas, a precipitate is
formed which is filtered. By treatment of the precipitate with an aqueous NaOH/NaCl solution and extraction with THF, the base fraction is generated. The original THF solution from which bases have been removed is now treated with an aqueous NaOH/NaCl solution which precipitates out the acid salts. Both the aqueous solution and precipitate are acidified to pH 2 with 12N HCl and extracted with THF to produce the acid fraction. The remaining original THF solution now contains only the neutral fraction.

This fractionation scheme gives a better separation as to chemical classes than the extraction procedures. However, there is still compound spill over due to multiple functionalities for a given compound, amphoteric behavior and steric hindrances of some of the functional groups.

The most recent fractionation procedures have been based on preparative liquid chromatographic separations. With the growth of LC over the past decade, more and more use of this technique has been seen. Due to the non-volatility of the SRC product, an alternative to gas chromatographic (GC) analysis (so widely used in the petroleum industry) was needed. LC is extremely well suited for coal-derived materials. The proliferation of LC based fractionation methods attests to this fact. Many workers have employed a wide variety of schemes to separate particular chemical classes using preparative LC. The two most popular supports for this work have been silica (7,9,12) and alumina (6,8,10,11); while, ion exchange has also been used (13). The above procedures employed open column LC. These separations provided fractions which
were rich in a variety of chemical classes (e.g., polyaromatic hydrocarbons, polynuclear aromatics, simple and polyphenols, etc.) for analysis by a wide variety of additional techniques (Mass Spectrometry, High Performance Liquid Chromatography, Nuclear Magnetic Resonance and Infrared Spectrometry to name a few). The LC schemes appear to provide the best fractionation to date of coal-derived materials for subsequent analysis.

Various spectroscopic methods have been employed to characterize samples which had been separated by different means into class fractions. Kershaw (14) employed fluorescence spectroscopy to analyze for polyaromatic hydrocarbons in coal product extracts. Several ring systems were identified. Bodzek et al. (15) employed Field Ionization Mass Spectrometry (FIMS) for the analysis of acid, base, neutral fractions. Proton magnetic resonance ($^1$H NMR) and dispersive infrared were also employed to determine overall composition of fractions.

Both Anbar et al. (16) and Swansiger et al. (17) also employed mass spectrometry (FIMS) to determine molecular weight profiles of whole products as well as polyaromatic hydrocarbons (PAH) types. NMR ($^1$H and $^{13}$C) has been widely used also to study the aromatic and aliphatic content of soluble SRC material (18,19) as well as solids at elevated temperatures (20). Finally, Fourier Transform Infrared (FITR) spectrometry (21) has been employed to study the functional groups present in chromatographic fractions of SRC products produced under different reaction conditions. All of these spectroscopic techniques have aided greatly in understanding the composition of the bulk SRC product.
Other workers have concentrated on further characterization of SRC fractions by chromatographic means. This has involved liquid chromatography since only a portion of the recycle solvent is amenable to gas chromatography. The rapid development of high performance liquid chromatography (HPLC) over the last 10 years has greatly enhanced this work (12,27,28,29). However, given the simplified fractions generated by previous separation schemes, the individual fractions remain very complex when subjected to additional chromatographic analysis. The most popular detectors currently in use in HPLC today (ultraviolet and refractive index) give little more than chromatographic retention data. With such complex mixtures as SRC products, complete resolution of all (or even most) components by HPLC is not possible. Therefore, compound identification based solely on retention time is suspect. Even were this not so, the wide range of possible components in SRC makes trying to model all unknowns virtually impossible. HPLC, however, gives us the best separations of these complex mixtures. Various spectroscopic techniques provide detailed structural information but suffer from not being able to distinguish individual components. Clearly, the coupling of HPLC separations directly with highly information specific spectroscopic detectors on-line would provide the potential for the identification of many of the components of these complex mixtures.

This coupling of two (or more) analytical techniques together has been termed "hyphenated" techniques and stems from the great success of Gas Chromatograph-Mass Spectrometry GC-MS. An extremely good review (22) is given by Hirschfeld of the many combinations that are currently
being worked on or have potential for the future. Depending on the functionalities of interest, a variety of combination techniques can be envisioned. With the great variety of compound types present in SRC material, many hyphenated techniques could be employed to aid in analysis. For example, work by Dark (25) and Dark et al. (26) has employed LC-MS for SRC analysis.

Heteroatom containing components have been shown to be important in coal conversion processes (23,24,26,10). Infrared spectrometry (IR) provides characteristic and often specific absorbances for many organic functionalities and particularly many of the heteroatom containing compounds which need to be analyzed. IR seems a very logical choice as a detector for the HPLC separation of SRC materials. Some work has been done by other workers employing LC-IR which will be outlined in detail in the following chapters. However, several problems exist in adapting IR as an effective HPLC detector and this has limited its use. The forthcoming chapters deal with the development of the potential of IR (especially FTIR) as a general HPLC detector. Specific applications will address the analysis of SRC products.
CHAPTER 2

CONVENTIONAL IR DETECTION FOR HPLC

2.1 Background

Several workers have published on the use of dispersive IR as a HPLC detector. Since most HPLC solvents are strong IR absorbers, this has limited its use and has necessitated short pathlengths which are typically 1 mm or less depending on the solvent and wavelength monitored. This is especially detrimental since IR is inherently less sensitive by several orders of magnitude compared to UV detection. The absorbing nature of the solvent also makes gradient elution difficult. Despite these drawbacks, the information content of IR spectrometry makes it an extremely attractive detector for compound identification.

Ettre (30) has reviewed selective detectors for chromatography including IR. These can be divided into on-line and off-line techniques. In the on-line method, standard transmission is used to monitor a single pre-selected wavelength corresponding to a particular functionality as a function of time. Off-line techniques are more varied and either employ stop-flow methods to record parts of the IR spectrum of the effluent, usually by transmission, or the actual collection of fractions for subsequent analysis via standard infrared methods. The latter method allows the elimination of solvent interference; yet, it does not allow continuous monitoring and involves complicated sample preparation.
Stop-flow and fraction collection techniques (off-line) have been employed by some workers. Mirabella et al. (31) used a standard dispersive IR coupled via a flow cell as a detector for size exclusion chromatography (SEC) of polymers. A stop-flow approach was used where the pump was stopped and several microns were scanned in the IR at various points in the chromatographic run. The IR served to monitor the polymer elution profile. Similarly, Bartick (32) studied co-polymer composition by SEC-IR. He employed both flow cell and fraction collection methods. A semi-automatic fraction collection device has been described by Jinno (33,34) for coupling with IR. Normal phase chromatography (silica) was employed and the effluent was deposited onto KBr plates with the solvent being evaporated. These were then brought into the KBr beam automatically. Segmented single wavelength chromatograms were then produced. Alternately, the system could be stopped and the IR scanned over a particular spectral region. The second of Jinno's papers is essentially using the same interface with the dispersive optics removed, thus monitoring "total" IR absorption.

Continuous flow cell methods (on-line) with dispersive IR as single wavelength monitors seem to be more numerous. Size exclusion chromatography (SEC) of polymers is perhaps the most popular use with good IR solvents such as CHCl₃ (35,36). The analysis of insecticides (37) was accomplished employing a silica column and CC1₄ as the mobile phase while monitor single characteristic IR absorbance bands. Glycerides were separated (37) and quantified using gradient elution HPLC (Whatman PAC bonded phase) with single wavelength IR detection. A
tertiary solvent system was employed and noticeable, but not severe, background changes occurred. Parris has employed both isocratic (38) and gradient (39) non-aqueous reversed phase LC separations (Dupont ODS) with dispersive IR detection. For the isocratic work, methylene chloride-acetonitrile (40:60) was used while for the gradient elution work, solvents used for the binary gradient were themselves binary mixtures of solvents such that the absorption at the wavelength of interest was matched for both solvent systems causing only minimal background changes during the solvent gradient.

The limited number of reports dealing with LC-IR show its promise as an LC detector for certain samples and separations. Early efforts at characterizing the solid SRC product employed SEC due to the wide diversity of chemical classes present and the desire to characterize as large a portion of the material as possible. Since SEC is not dependent on the mobile phase employed but rather the effective molecular size of the components, we were able to employ a reasonably transparent IR solvent which had good solvating properties for SRC (i.e. CHCl₃). The following represents our early efforts at using a variable filter IR detector directly coupled with SEC for the analysis of the non-volatile portion of the SRC product.

2.2 Experimental

Samples

A synthetic mixture (1% w/w) containing 7 components thought to model SRC was prepared by dissolving equal weights of squalane (Microtek), benzophenone (Fisher), dibenzo furan (Aldrich), anthracene
(Eastman), 2-tert-butyl-4-methyl phenol (Aldrich), aniline (Theta Corp.) and cyclohexanol (Fisher) in chloroform.

SRC samples derived from Amax feed stock were obtained from a Southern Services Inc. pilot plant (Wilsonville, AL) funded by Electric Power Research Institute and operated by Catalytic Inc. Two preliminary separation schemes were employed to obtain simpler fractions of the SRC material for subsequent LC-IR. Scheme 1 (Figure 1) consisted of a continuous hexane extraction of SRC (40:1) followed by evaporation of the hexane to yield an "oils" fraction (40). Scheme 2 (Figure 2) is a modification of the Sequential Elution Selected Solvent Chromatography (SESC) procedure developed by Farcasiu et al. (4,5). SRC material is sequentially eluted from silica gel by a pseudo-step gradient. Two of these fractions, SESG #3 and #4 (designated polar aromatics and simple phenols, respectively) were chosen for analysis in this feasibility study.

**Chromatography.** Chromatography in the size exclusion mode was carried out with a Waters 6000A reciprocating piston pump. A Varian TSK-1000 (originally packed in THF but for this study equilibrated in CHCl₃) column (3/8 inch x 25 cm) was employed for the separations. The TSK-1000 column had ~6000 plates/m as determined by an injection of benzene. The lower than normal plate count was due to bed volume changes arising from the solvent change. Flow rates of 1 mL/min of chloroform were employed. A differential refractometer (LDC Model 1107) served as an auxiliary detector for monitoring the separation.

A variable wavelength IR spectrometer (Miran-IA, Foxboro
Figure 1. Oil-Asphaltene-Asphaltol Separation Scheme.
Figure 2. Modified Sequential Elution Selected Solvent Chromatography (SESC) Separation Scheme.
Analytical) was chosen as a conventional "on-the-fly" IR detector. It employed a variable filter system to select wavelengths between 2.5 to 14.5 μm with a resolution of 0.05 μm at 3 μm; 0.12 μm at 6 μm and 0.25 μm at 11 μm. The flow cell consisted of either a conventional KBr IR cell adapted with O-rings for a flowing system with a 0.1-mm (30 μL) or a 1-mm (300 μL) spacer or a NaCl μ-flow (1.5 mm, 4.5 μL) cell available from Foxboro Analytical. Multiple flow cell pathlengths were required to obtain useable solvent transparency which is dependent both on the wavelength monitored and the cell pathlength.

2.3 SEC of Model System

A synthetic mixture containing many of the functionalities suspected to reside in SRC was prepared for subsequent size exclusion chromatography. Prior to their separation, individual components were independently chromatographed to determine retention volumes ($V_R$) (see Table I). Figure 3 shows the SEC separation of the mixture as monitored by refractive index (RI). The RI trace yields 3 resolved peaks and a shoulder for the 7 component mixture. This is due to the fact that the RI detector responds to both positive and negative RI changes which can and do have a net canceling effect in the chromatograms. However, even if all components were resolved in the RI trace, no information as to the nature of the compound other than its relative molecular size is provided.

By employing an IR detector with the same column, chromatograms (Figure 4) are obtained which characterize the functionality of each component in the mixture. The chromatogram produced by detection at
<table>
<thead>
<tr>
<th>Component</th>
<th>Elution Volume, mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>squalane</td>
<td>3.92</td>
</tr>
<tr>
<td>benzophenone</td>
<td>5.24</td>
</tr>
<tr>
<td>cyclohexanol</td>
<td>6.10</td>
</tr>
<tr>
<td>dibenzofuran</td>
<td>6.28</td>
</tr>
<tr>
<td>2-tert-butyl-4-methylphenol</td>
<td>6.40</td>
</tr>
<tr>
<td>anthracene</td>
<td>6.70</td>
</tr>
<tr>
<td>aniline</td>
<td>7.04</td>
</tr>
</tbody>
</table>
Figure 3. Chromatogram of Model Mixture with Differential Refractive Index (RI) Detection.
Figure 4. Size Exclusion Chromatograms of 7-Component Model Mixture as Detected at Various Characteristic IR Frequencies.
2.75 μm shows the previously incompletely resolved separation of the "phenol" component and "alcoholic" component now detected free of interferences. Monitoring the 2.95 μm band yields a chromatogram which shows a main peak for the characteristic absorbance of the -NH₂ functionality in aniline with a small peak due to "bonded" -OH functionality. The synthetic mixture contains only one ether component (dibenzofuran) and only one peak is observed in the 8.4 μm chromatogram. An identical situation is expected for 5.9 μm (carbonyl) detection, but a weak additional band is observed which suggests that a second component of the mixture weakly absorbs IR radiation in this region. Three components contain aliphatic hydrogen detected at 3.4 μm but only two peaks are resolved. The peak at Kd (Kd=V_R /V_E-V_p; where V_R= retention volume, V_E=totally excluded volume and V_P=totally permeated volume) closest to zero is attributed to squalane, while the other peak is due to both alkylated phenol and alcohol based on independently measured retention volumes. It appears that 2.75 μm is a more selective wavelength for the detection of alkyl phenols/alcobols than the 3.4 μm band. This demonstrates the ability to select the most useful wavelength for the compound being separated. Five aromatic materials appear in the synthetic mixture; however, the 6.25 μm wavelength detects what appears to be only three components. The largest "sized" fraction is assigned to benzophenone. The middle peak is due to dibenzofuran and the substituted phenol, while aniline and anthracene elute near the totally permeated region. Absorbance by CHCl₃ in the aromatic C-H stretching region (3.2 μm) precluded our monitoring this wavelength. In
summary, we have been able to observe selectively all components of the
synthetic mixture except in those cases where the chromatography is not
sufficient to separate common functionalities.

2.4 SEC-IR of SRC "Oils" Fraction

Employing the same column and detector characteristics, SRC "oils"
(hexane solubles) have been "sized" separated. The SEC chromatogram of
the "oils" fraction (Figure 5) employing differential RI detection
provides only the limited information that material is eluting over the
entire range (K_d=0 to 1). Upon examining the chromatograms of the
"oils" at various IR detected wavelengths, we see significant
absorbances for heteroatom functionalities in what has typically been
thought to be a coal-derived fraction with little or no heteroatom
content (40). Figure 6 illustrates a series of chromatograms describing
the SEC separation of SRC "oils" employing sequential 8.2 to 9.2 μm
detection. The chromatograms show a shift in response from large (K_d=0)
to small (K_d=1) molecular size as the wavelength of detection decreases.
The region from 9.2-8.8 μm is characteristic of aliphatic ethers, and
absorbances in this region appear to be concentrated in the large
molecular "sized" fraction. Absorbances in the 8.6 to 8.2 μm region
(lactones and esters) are observed and are concentrated in the smaller
molecular "sized" region.

Further evidence for the presence of small molecular "sized"
lactones can be seen by noting Figure 7. Seven IR wavelengths which
are characteristic of various "carbonyl-like" environments have been
independently monitored (5.3-5.9 μm). Relatively strong absorbance is
Figure 5. Chromatogram of Amax SRC Oils Fraction with Differential Refractive Index (RI) Detection
Figure 6. Size Exclusion Chromatograms of Amax SRC Oils Fraction with IR Detection in the "-C-O-C-" Region.
Figure 7. Size Exclusion Chromatograms of Amax SRC Oils Fraction with IR Detection in the "C=O" Region.
observed for the small "sized" material at 5.7-5.4 µm. This spectral region is characteristic of lactones and its elution profile "size-wise" is similar to that of the ether chromatogram (Figure 6). These assignments are not conclusive because alicyclic ethers absorb over the entire 9.2-8.2 µm region being monitored. The 5.7 to 5.9 µm region region represents ketone and ester functionalities which are present in small amounts in the larger "sized" fraction. Detection at 2.75-3.10 µm (Figure 8) reveals the presence of a broad distribution over the entire molecular "sized" range of O-H and/or N-H functionalities. Absorption, however, at 2.8 µm (characteristic of phenolic and nonbonded O-H) predominates.

The detection of ethereal- and carbonyl-containing species in SRC derived "oils" has not been reported to our knowledge. Although their concentration is low, their presence is nevertheless surprising since "oils" are normally considered to be simple aliphatic and aromatic hydrocarbons. Our sample of Amax SRC "oils" has not been protected from the atmosphere; therefore, it is possible that selective oxidation of certain hydrocarbons has occurred. On the other hand, the sensitivity and selectivity of LC-IR may have provided the clearest picture to date of trace species in the "oils" fraction of a coal-derived product. Naturally, better preserved and more highly documented SRC "oils" must be investigated via this technique if definitive answers are to be obtained regarding the chemistry of coal liquefaction. Suffice it to say that the LC-conventional IR detection approach appears promising for elucidating molecular composition information on these materials.
Figure 8. Size Exclusion Chromatograms of Amax SRC Oils Fraction with IR Detection in the Bonded -OH and -NH- and Nonbonded -OH region.
2.5 SEC-IR of SESC Fractions

Extension of this SEC separation-IR detection technique to the size separation of selected crude SRC fractions from a silica gel column was accomplished. We chose to examine the SESC #3 and SESC #4 fractions which have been previously designated, based on the elution pattern of model compounds, polar aromatics (non basic N,O,S) and simple phenols respectively. Figure 9 compares the "OH-NH-" region for the SEC-IR separation of SESC #3 and #4. From these chromatograms, we can see that small molecular size material in SESC #4 has a strong absorbance in the phenolic-nonbonded -OH region (2.80-2.85 μm) which would be expected of simple phenols. The bonded OH, -NH- region (3.0-3.05 μm) shows maximum absorbance at large molecular size. In SESC #3 chromatograms, bonded -OH, -NH- functionality is also contained in large molecular size material; while little preference is shown for phenolic nonbonded -OH absorbances as far as molecular size is concerned. It also should be noted that the absorbances in this region are much less intense for SESC #3 than for SESC #4 which gives credence to the simple phenol designation for SESC #4. Some caution is required in making this interpretation because SESC #4 SEC-IR separations were performed with tetrahydrofuran eluent rather than CHCl₃. SESC #4 has limited solubility in CHCl₃ and the lack of suitable "windows" in THF precludes monitoring of ether/carbonyl wavelengths.

Figure 10 shows that some of the remaining heteroatom content in SESC #3 is carbonyl and ether species but with different distributions than were observed in SRC "oils" (Figure 6 and 7). The carbonyl
Figure 9. Size Exclusion Chromatograms of Amax SRC SESC #3 and #4 Fractions with IR Detection in the Nonbonded -OH Region and Bonded -OH, -NH- Region.
Figure 10. Size Exclusion Chromatograms of SESC #3 and #4 Fractions with Carbonyl and Ether Detection.
absorbance characteristic of ketones, aldehydes, and esters is more intense at moderate to large molecular sizes, and there is an absence of the small molecular size spike (attributable to lactones) seen previously in the "oils" chromatograms. The ether region chromatogram (9.0-8.9 µm) mimics the chromatograms obtained by monitoring the aliphatic C-H region (~3.4 µm) rather than the phenyl region (~6.25 µm) which would suggest that aliphatic ethers are present rather than aromatic ethers.

2.6 Conclusions

The use of a variable filter IR spectrometer as an LC detector provides some unique advantages as compared to HPLC detectors based on refractive index and ultraviolet spectrometry. The use of this relatively low cost (~$3,000) IR detector has shown that we can effectively monitor the elution of various functionalities by IR. Although this is promising especially for complex SRC materials, drawbacks with this detector system remain.

The variable filter spectrometer only allows us to monitor selected wavelengths sequentially. Therefore, we must repeat the chromatographic run in order to monitor the different absorbance bands of interest. This is a tedious process and very wasteful of time, sample and solvent. With this system, we are not truly realizing the full benefits of IR detection. Also, the filter based dispersion system does not allow a high degree of spectroscopic resolution. This results in an inability to resolve close IR bands and causes interference when trying to monitor a "specific" band. This could be improved with a more expensive
dispersive system but only at the loss of sensitivity due to the loss in energy throughput. Finally, the sensitivity of the system is approximately equivalent to a refractive index system, but is far less sensitive than a UV detector. For complex mixtures, an increase in sensitivity is important, especially if other chromatographic separation modes are to be used which have lower sample capacity. Fourier Transform Infrared Spectrometry (FTIR) addresses all of these problems to various extents and is described in the following chapters.
CHAPTER 3

SIZE EXCLUSION CHROMATOGRAPHY-FOURIER TRANSFORM
INFRARED SPECTROMETRY

3.1 Background

Fourier Transform Infrared Spectrometry (FTIR) offers many advantages over conventional dispersive IR spectrometers. Hirschfeld (41) has outlined these and other properties of FTIR spectroscopy very extensively. Briefly, four advantages can be cited as unique to the FT approach. These have been named after the original workers who cited them. The Felgett advantage results from the simultaneous measurement of all wavelengths of the IR spectrum. This is also known as the time advantage since it takes the FTIR the same time to produce a complete spectrum as it takes a dispersive instrument to scan one resolution element. Therefore, the FTIR is $N$ times faster (where $N$ is the number of resolution elements in the spectrum) than a dispersive IR or, by signal averaging over the same measurement time, the FTIR is the $\sqrt{N}$ more sensitive (due to the noise reduction). A second advantage is the Jacquinot or throughput advantage. This results from the lack of slits and dispersive optics in the FTIR, allowing more energy to reach the detector which results in higher sensitivity. Conne's advantage is the highly accurate wavelength calibration of the FTIR. This is the result of the use of a helium-neon laser to calibrate the wavelength of each scan. This allows accurate spectral manipulation such as subtraction.
techniques.

The modulation of the mirror produces two advantages. First, the mirrors chop each IR frequency at a different frequency (proportional to the mirror velocity). This eliminates stray light from an interferometer experiment. Also, the modulation frequency of the mirrors allow the use of detectors which are more sensitive than the detectors used in dispersive IR.

Finally, an additional advantage of FTIR spectrometers is the built-in computer which is necessary to perform the Fourier Transforms to produce frequency domain data from the time domain data collected. Since this type of computing power is built in, it has been put to additional good use through spectral manipulation and display routines to name a few. This has been such an advantage that many dispersive systems now have dedicated computers built-in. It should be noted that all of these advantages do not come cheaply, but the price of FTIR's have dropped dramatically for routine instruments and their performance continues to improve.

The earliest example of the use of interference spectrometry as a chromatographic detector (gas) was in 1967 (44). Even in this primitive form, its great potential was recognized. Since that time, commercial instruments of high performance have provided the chromatographer with a unique new detector. The first published LC-FTIR experiment appeared in 1975 (45). Size exclusion separations were employed in much of the early work and continues to be the most popular type of chromatography when using FTIR detection. The coupling of other chromatographic modes
will be left for subsequent chapters. Vidrine presents (42) a review of HPLC-FTIR with emphasis on the flow cell method. The chromatographic and spectroscopic requirements are stressed. Specific SEC applications to the analysis of several polymer samples as well as the detection of 525 ng of injected paraffin oil (11.2 mm cell pathlength) are presented. Non-chromatographically resolved components are also spectroscopically resolved via subtraction methods. The latter review is a compilation of two previously published (46,47) papers also dealing with SEC-FTIR. While other workers are most assuredly using this technique in industry, little experimentation has found its way into the open literature.

As a logical extension of the work with conventional IR coupled with size exclusion separation, the results of similar experiments using FTIR detection are presented.

3.2 Experimental Section

Samples

SRC samples derived from Indiana No. 5 feed stock were obtained from a Southern Services Inc. pilot plant (Wilsonville, AL) funded by Electric Power Research Institute and operated by Catalytic, Inc. A chloroform soluble fraction was generated by stirring a chloroform-SRC suspension (40:1 w/w) for 3 hours after which the soluble material was removed by filtration. This was followed by a preliminary separation into rough chemical classes by a modification of the sequential elution selected solvent chromatography (SESC) procedure developed by Farcasiu et al. (4) in which the SRC material is sequentially eluted from silica gel by use of various mobile-phase compositions. Of the nine fractions
generated (5), SESC Nos. 5, 7, and 9 (designated basic nitrogen heterocycles, polyphenols, and increasing oxygen-nitrogen content and basicity, respectively) were chosen because of their suspected high heteroatom content for analysis by LC-FTIR. Appropriate amounts of each fraction were weighed and dissolved in chloroform in an attempt to prepare 1% solutions. Limited solubility of the samples in chloroform only permitted the examination of the resulting saturated solutions. The solubility of the separated material differs considerably from the solubility of the total SRC. We have observed that lower SESC fractions have greater solubility in chloroform; whereas, the higher fractions are increasingly less soluble. This is most probably due to the much higher heteroatom content of the later SESC fractions which greatly increases the sample's polarity.

**Chromatography**

Size exclusion chromatography employing a Waters 6000A reciprocating piston pump and a Waters 100-A µ-Styragel column with ~14,000 plates/m was performed on all samples. A flow rate of 0.5 mL/min with chloroform as the mobile phase and a Nicolet flow cell (KBr, 1 mm pathlength, ~20 µL volume) designed for use with the Nicolet FTIR system were employed. A differential refractometer (LDC Model 1107) was used as an auxiliary detector during the separation.

A Nicolet 7199 FTIR spectrometer was employed as an LC detector. The system used a KBr beam splitter and TGS (triglycine sulfate) detector with a useable range of 5000-400 cm\(^{-1}\). The more sensitive MCT (HgCdTe) detector system would have been desirable but was not
available. A total of 4096 data points were transformed and a Happ-
Genzel apodization function was applied giving a resultant resolution of
~4 cm\(^{-1}\) for stored spectra. Each stored spectrum (representing ~28 s
elution time or ~0.25 mL of elution volume) was the result of averaging
32 individual spectra. Up to five IR window regions with 16 cm\(^{-1}\)
resolution could be selected over which the total integrated absorbance
can be plotted vs. time to produce a pseudo-real-time chromatogram.
Concurrently, individual averaged spectra are filed whenever an operator
pre-determined threshold value is exceeded in any IR window region. The
software employed in this study was that developed by the manufacturer
and provided with the instrument.

3.3 Detailed Analysis

Fourier transform infrared spectrometry (FTIR) has been employed as
a liquid chromatographic detector in the size exclusion separation of
selected CHCl\(_3\)-soluble solvent refined coal (SRC) fractions. Indiana
No. 5 feed coal served as the source of SRC. Crude separation of the
sample into nine fractions (SESC No. 1-9) was achieved via separation on
a silica gel column utilizing a constant elution volume (twice the bed
volume) for each eluting solvent. SESC No. 5, 7 and 9 dissolved in
CHCl\(_3\) provided the specific samples for SEC-FTIR. Scheme III (Figure
11) outlines the history of these samples.

Prior to size separation, FTIR spectra were measured on
approximately 1% (w/w) CHCl\(_3\) solutions of SESC fractions 5, 7, and 9.
The 4000-3200 cm\(^{-1}\) region (Figure 12) is similar for all samples. Bands
at ~3690 and 3600 cm\(^{-1}\) dominate this part of the spectrum and are
Figure 11. Preliminary Fractionation Scheme to Generate Chloroform Soluble SESC Fractions of Indiana No. 5 SRC.
Figure 12. IR Spectra of Whole SESC Fractions 5, 7 and 9 Dissolved in CHCl₃.
assignable to an O-H stretching vibrational mode. The lower wavenumber value is no doubt due to nonbonded phenolic and/or alcoholic functionality. The band at 3690 cm⁻¹ is tentatively assigned to traces of moisture in the sample. Broad bonded O-H absorbances which dominate the spectra of KBr pellets and cast films (21) are of little consequence here since our spectra are of dilute solutions. Weak absorbance in the 3600-3400 cm⁻¹ region is observed which may be due to bonded OH and/or N-H. While solvent interference obscures the aromatic C-H region, there appears to be a progression to less aliphatic C-H in the higher SESC fractions. Estimates of this type are difficult at this stage since the solubility of each fraction in CHCl₃ is different. Each FTIR spectrum exhibits a sharp absorbance around 1600 cm⁻¹ attributable to aromatic ring vibrations. Weaker unresolved absorbances at slightly higher wavenumber are apparent in most fractions and are likely due to low concentrations of a variety of carbonylic material. This becomes most pronounced in fraction No. 9 where now the major peak in the spectrum appears at ~1800 cm⁻¹. The 1200-900 cm⁻¹ window demonstrates the most dramatic spectral changes in going to higher SESC fractions. Aliphatic ethers, aryl-alkyl mixed ethers, and alcoholic functionality exhibit absorbance in this region. Considering the complexity of each fraction, the assignment of individual bands to specific moieties is at best conjecture. Detailed interpretation of these and other spectral bands has been deferred to smaller cuts of further size-separated SESC fractions. This method will be shown to provided considerably more detail concerning the speciation of each SESC fraction.
Real-time size-exclusion chromatograms employing "aliphatic C-H" (C-H_{α}ph) detection, "aromatic ring" (C_{aro}) vibration detection, and "alkyl ethereal" (C-O-C) detection for SESC fractions 5, 7 and 9 are reproduced in Figure 13. "Alkyl ethers" are most pronounced in fraction No. 5 and occur in predominantly large size molecules. The distribution of C-O-C is similar in fraction No. 5 and 7 whereas fraction No. 9 contains practically no large size molecules possessing C-O-C moieties. The same is true of C-H_{α}ph with fraction No. 9 having few species of any size with C-H_{α}ph. The C_{aro} chromatograms show an interesting pattern with major elution in the K_{D}=0-0.5 region followed by a significant group of materials near the totally permeated region. Again the amount of detectable material in fraction No. 9 is less. The relatively low solubility of fraction No. 9 in CHCl_{3} probably accounts for this observation rather than the absence of organic functionality. While this type of data provides an overall picture of the distribution of specific functionalities with respect to (a) individual samples and (b) molecular size for a specific sample, an examination of file spectra gives a more complete description of the eluting species.

Each file spectrum represented approximately 0.25 mL elution volume and was the result of averaging 32 individual scans. As Figure 13 attests major chromatographic peak maxima from the SESC No. 5 separation coincide with files 28, 44, and 54 for the IR windows monitored. An examination of each file confirms the observation that no new information pertaining to functionality is supplied beyond what is revealed in the files noted above. Figure 14 illustrates the C-H_{α}ph,
Figure 13. Selected Window Regions Monitored Via FTIR During the Chromatographic Separation of SESC 5, 7, and 9.
Figure 14. Portions of Selected Spectra Collected During the Separation of SESC No. 5 Showing Various Functionality Absorbance Regions.
Caro, and C-O-C regions for these files. The larger size fraction (file 28) contains appreciable methyl (2965 cm\(^{-1}\)) and methylene (2930 cm\(^{-1}\)) along with aromatic (1588 cm\(^{-1}\)) moieties which either may contain heteroatoms or have extended conjugation. The assignment of the band at 1588 cm\(^{-1}\) is based on its relatively high peak intensity. Dominant bands at 1153 and 1108 cm\(^{-1}\) are assigned to ether functionalities. Aryl ethers are obscured by CHCl\(_3\) absorption; however, aliphatic, acyclic, and alkyl aryl ethers give rise to IR activity in the 1100-1000 cm\(^{-1}\) region. The lack of IR bands above 3200 cm\(^{-1}\) suggests that there is no contribution from large size alcoholic or phenolic materials which would otherwise also absorb in this region. Less intense bands at 1168 and 1015 cm\(^{-1}\) may therefore be attributed to smaller concentrations of alkyl containing ethers and/or the symmetric C-O-C stretching vibration for aryl ethers, respectively. In going to intermediate size molecules (files 43 and 44), etheral bands have reappeared but it has somewhat different character. The band at 1108 cm\(^{-1}\) (file 43) assigned to aliphatic ethers is extremely weak; whereas, the C-H\(_{\text{alph}}\) is surprisingly intense. This could be due to the presence of high molecular weight aliphatic or alkyl aryl ethers. The 1108 cm\(^{-1}\) band intensifies and broadens on going to file 44 but the C-H\(_{\text{alph}}\) has essentially disappeared. This suggests a variety of mixed ethers with minimal aliphatic content. Bands at 1042 and 1033 cm\(^{-1}\) may again arise from aryl ethers (symmetric stretch). This assignment is supported by IR activity around 1600 cm\(^{-1}\). The intense 1168 cm\(^{-1}\) peak possibly arises from an alicyclic ether such as benzofuran. The doublet at 1606 and
1588 cm\(^{-1}\) may alternatively be due to pyridinic material. This would be consistent with the designation of SESC No. 5 material as basic nitrogen heterocycles. The only other significant absorbances in the regions that one is able to monitor elute in the near totally permeated region (file 54). Intense absorption is noted at 1606 cm\(^{-1}\) with simultaneous onset of IR activity at 3600 cm\(^{-1}\) (Figure 15). With the evidence presented in Figure 15 coupled with the absence of ethereal and alcoholic bands in the C-O-C and C-H\(_{\text{alp}}\) region, we believe the eluting species to be phenols with traces of moisture present (3691 cm\(^{-1}\)).

File spectra 153 and 154 obtained during the size exclusion separation, Figure 16, of SESC No. 7 reveal carboxylic (1771 cm\(^{-1}\)) material eluting with the larger size molecules. Its concentration is low since it is not readily observed in the static spectrum of SESC No. 7 and it only appears in a few file spectra. The high frequency band precludes simple aldehydes, ketones, amides, and esters. Aroyl halides absorb in the region of interest but they should not survive the SESC chromatographic separation. Anhydrides also appear around 1770 cm\(^{-1}\) but they have a symmetric and asymmetric IR active modes. The band at 1771 cm\(^{-1}\) is a singlet. Nonbonded acids would appear to be candidates. Even more likely would be cyclic carbonyl materials (i.e., ketones, lactones, and lactams) of relatively small ring size. It should be noted that no carboxylic or potential carboxylic (e.g., THF) material has been employed in either the SESC or SEC procedures at this point. Little additional absorbance is detected in the IR windows that can be monitored. Very weak, poorly resolved C-H\(_{\text{alp}}\) appears in files 153
Figure 15. OH/NH Region of File Spectrum No. 54 Collected During SESC Fraction 5 Separation and Corresponding to Small Size Material.
Figure 16. Portions of Selected Spectra Collected During the Separation of SESC Fraction 7 Showing Various Functionality Absorbance Regions.
and 154. (Most of the C-H\textsubscript{Aph} is seen in files 140-150.) The aromatic carbon region (C\textsubscript{aro}) exhibits little absorbance as does the O-H, N-H stretching region. Bands in the ether region which are unique only to files 153-156 are 1159 and 1038 cm\textsuperscript{-1} (Figure 17). The assignment of one or both to C-O-C stretching modes in an \alpha-lactone is tempting. Phenyl acetate appears to be an excellent prototype which exhibits absorbance at 1770 cm\textsuperscript{-1} and around 1100 cm\textsuperscript{-1}.

Material eluting immediately after (files 155-159) the carboxylic species shows the familiar doublet around 1600 cm\textsuperscript{-1} observed in the SEC of SESC No. 5. Again no O-H, N-H activity and weak C-H\textsubscript{Aph} absorbances were found. The appearance of the doublet, however, is accompanied by a considerable number of new peaks in the C-O-C region (i.e., 1151, 1067, 1032, and 993 cm\textsuperscript{-1}), Figure 17. Mixed aryl alkyl ethers are known to absorb in the 1075-1020 cm\textsuperscript{-1} region. SESC No. 7 has been previously designated polyphenols (4). No files except 166-168 show O-H\textsubscript{str} absorbances. This feature, as was the case with SESC No. 5, is accompanied by the appearance of a 1607 cm\textsuperscript{-1} peak suggestive of phenols. Larger size phenols appear to be absent. However, evidence from an independent model study has shown a non-size-exclusion mechanism for phenols (44). The original designation of SESC No. 7 as polyphenols was based on limited model studies with low molecular weight polyphenolic material (e.g., 2,3-dihydroxy-naphthalene)(6). Several of the phenolic vibrational modes are obscured by CHCl\textsubscript{3} absorbances (e.g., C-O\textsubscript{str}). Furthermore, the possibility of intramolecular association may cause the 0-H\textsubscript{str} region to be weak and broadened. Alternatively, the eluting
Figure 17. C-O-C Region of Selected Spectra Collected During the Separation of SESC No. 7.
material may be so broadly distributed that many species are undetected.

Although we have been concerned with CHCl₃ soluble SRC, an appreciable amount of material still appears in SESC No. 9; even though very few files exhibit IR activity. One reason for this no doubt is due to the fact that all of SESC No. 9 did not redissolve in CHCl₃ for injection onto the SEC column. This is no doubt due to co-solvent effects present in the whole SRC material which are absent in the separated fractions. The 1770 cm⁻¹ band observed in select SESC No. 7 file spectra is much more prominent in the file and static spectra of SESC No. 9. A chromatogram employing 1780-1760 cm⁻¹ detection is shown in Figure 18. The carbonylic material again elutes in a very narrow range of Kₐ values corresponding to "C₂₀ alkane-size" molecules. This high frequency carbonyl peak, as previously suggested, probably originates from a cyclic carbonyl. Several other IR bands track the 1700 cm⁻¹ band. Relatively sharp bands at 2922, 1464, and 1380 cm⁻¹ can be assigned to the asymmetric methyl C-H stretching band and the asymmetric and symmetric methyl bending vibrational modes, respectively. For files 116-118 the C-O-C region is also uniquely active with bands at 1178, 1044, and 993 cm⁻¹, Figure 19. The assignment of these bands to cyclic esters and/or mixed ethers seems appropriate as discussed in SESC No. 7. An examination of files 130-133 reveals phenolic material again of apparent small size, Figure 20. Surprisingly little C₇₀ vibrations are observed in any of the files before the elution of phenols.
Figure 18. Carbonyl Window Region Monitored Via FTIR During the Separation of SESC No. 9.
Figure 19. C-O-C Region of Selected Spectra Collected During the Separation of SESC No. 9.
Figure 20. Portions of Selected Spectra Collected During the Separation of SESC No. 9 Showing Various Functionality Absorance Regions.
Several points should be made regarding this investigation. Relative to more conventional detectors, FTIR coupled with chromatography yields a much clearer picture of the composition of a typical synfuel. Highly colored material elutes continuously from the column; yet, all files are not rich in IR bands. This observation could mean that considerable material which exhibits infrared absorption in several of the non-transparent regions obscured by CHCl₃ is eluting from the column, or an insignificant part of the material which possesses extraordinarily high molar absorptivities is eluting. Clearly, quantitative elution data are required in this regard. The observation of the intermediate size carbonylic material which appears to be concentrated in SESC No. 9 (yet which also appears in SESC No. 7) is a novel finding which must be extended to other synfuels. Significant "spill-over" from one SESC fraction to another apparently occurs and the chemical class designation of specific SESC fractions should be broadly interpreted.

3.4 Conclusions

The feasibility of SEC-FTIR has been demonstrated for monitoring differences in chemical functionality as a function of molecular size. As was expected, SRC material shows a large diversity of functionality and molecular sizes. SEC provides a means for grossly separating SRC components with FTIR providing specific functionality information. This technique appears to provide a means for routine monitoring of SRC products with regard to evaluating the effects of changes in the processing parameters.
As a method for identifying individual components in complex mixtures, the separation by size (SEC), even after preliminary separation by chemical class (SESC), is insufficient. Also, for FTIR to be more universally used as an HPLC detector, it needs to be employed with some of the more frequently encountered means of HPLC such as normal and reversed phase chromatography. For this reason, we abandoned SEC separations in favor of normal phase chromatography, although the SEC-FTIR technique remains a powerful tool for the analysis of simple mixtures, polymers and copolymers. SEC-FTIR has been fundamental in laying the ground work for the use of FTIR detection with other chromatographic systems.
CHAPTER 4

NORMAL PHASE HPLC-FTIR EMPLOYING ANALYTICAL SCALE COLUMNS

4.1 HPLC-FTIR Methods

Flow cell methods have been the most popular means for interfacing SEC and FTIR. This is due both to the large sample capacity of SEC and its tolerance of any solvent which will dissolve the sample of interest. Since solvents with large IR transparent regions can be employed, solvent absorptions are not an important consideration. Also, the sensitivity of the FTIR is more than sufficient for the quantities of sample normally separated by SEC. However, the use of flow cell methods with normal and reversed phase chromatographic separations is not nearly as simple.

Several problems are encountered when trying to interface the FTIR to these much more widely used chromatographic modes. Water based solvent systems employed in most reversed phase separations are totally incompatible with FTIR detection. The highly absorbing nature of water presents the biggest obstacle, while the solubility of common (NaCl, KBr, KCl) IR support materials is less critical since insoluble substitutes can be found such as AgCl, BaF₂ and CaF₂. However, their transmission cut off and refractive indices are not as favorable. Normal phase HPLC presents almost as formidable a problem. One of the biggest advantages of HPLC is the ability to gain different selectivity for a particular separation by changing the mobile phase employed. The
highly absorbing (IR) nature of most of the common HPLC solvents remains the key obstacle to successful interfacing of HPLC-FTIR. Also, application of the popular technique of mobile phase gradients to accomplish a particular separation is a problem in that the solvent compensation method normally employed (simple ratioing) is inadequate to handle a continuous change in mobile phase. The solutions to these problems have not been simple and are still not entirely satisfactorily resolved.

The methods to cope with these problems and their associated restrictions have basically taken two approaches. One is based upon an elaborate system to concentrate the chromatographic eluent while removing the solvent and collecting fractions for subsequent analysis by reflectance spectroscopy. The second is based upon standard transmission spectroscopy with a suitable flow cell of various path-lengths and is similar to most conventional HPLC detectors based upon optical spectroscopy. Both approaches have distinct advantages and disadvantages which will be examined further.

4.1.1 Solvent Removal Methods

There have been several groups working on a satisfactory means of automatic fraction collection and solvent removal. Basically, it is the logical extension of the classic method of fraction collection followed by spectroscopic measurements of the fractions. In its most developed state, the entire process is almost totally automated. The first generation system to employ this method for HPLC-FTIR (49) appeared in 1977. A better description of this system was provided in a subsequent
publication (48). The effluent from the chromatograph was sprayed into one of four heated light pipes which evaporated the chromatographic solvent (and any volatile sample) and deposited the sample on the walls of the lightpipe. The reflection-absorbance spectra were taken as the lightpipe was moved into the IR beam. The lightpipe was then cleaned and returned to its original position. The remaining three lightpipes were operated identically, each at one of the three remaining sampling stages. Several problems are encountered with this analysis method, with the principal problem cited by the authors being insensitivity. The authors (48) next developed an improved version of their solvent elimination and concentration system which has been more successful.

This refined method employed a different form of reflection spectroscopy as the method for sampling the evaporated sample. By employing diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy many of the original problems cited were overcome. The principal of DRIFT spectroscopy has been described (50) and several applications (51) reported. Reflectance techniques in general have undergone a renaissance with the advent of FTIR spectrometers and their intrinsic high optical throughput. DRIFT spectroscopy employs an ellipsoidal mirror to collect the reflected sample radiation. In the HPLC interface, the effluent is concentrated through heating and dripped into small cups filled with powered KCl. As a peak was monitored via an auxiliary UV (254 nm) detector, the cups would advance on a carousel device to collect the concentrated effluent. The cups were then evaporated further with a stream of air. The cup is then passed to the
spectrometer and a DRIFT spectrum is collected. Sub-microgram detection limits for several dyes were reported (48), as well as its use with a gradient separation. An expanded study of a computer controlled interface has been described (52) which has dealt with optimizing the interface.

Preliminary use of this system with a reversed phase separation was reported (53) by addition of an extraction system to remove most of the water. An improved and expanded interface was subsequently (54) reported by the same research group for reversed phase separations. Another group has reported (55) a similar method for reversed phase separations. DRIFT spectroscopy was employed with the sample deposited on a hard crusted KCl surface and water eliminated by reaction with dimethoxy propane (acid catalyzed) to form volatile methanol and acetone. These are removed by evaporation prior to taking the DRIFT spectrum.

Although the solvent removal methods have been shown to be successfully used with both gradient and reversed phase separations, several problems still exist and have precluded there widespread use. Even in its automated state, the method remains an off-line technique in that sample manipulation is required prior to data collection. Also, the method remains mechanically complex and has been accepted generally no better than similar techniques employed in some HPLC mass spectrometer interfaces based on solvent removal. Any volatile components of mixtures also can not be analyzed by this method. The improved sensitivity is partially due to the increased number of averaged spectra (400 in ref. 48) which results in long spectral
collection times for complex chromatographic runs. Also, in complex mixtures, many peaks are not totally separated and the collection of fractions results in loss of chromatographic resolution, especially considering the practical limitations on the number of previously prepared sites for sample collection. Finally, the ability to remove many polar solvents used in chromatography either as polar modifiers (normal phase) or as a large portion of the mobile phase (reversed phase) from polar type compounds which can strongly hydrogen bond is still debatable with these solvent removal systems. However, this is not to say that they might not be satisfactory for many analyses.

4.1.2 Flow Cell Methods

While conceptually it is the easiest means for the direct interfacing of HPLC and FTIR, the application of the flow cell technique has not gained wide acceptance outside of SEC applications. This is due in part to the restrictions it places on the separation due to its inability to handle reversed phase and gradient separations. However, with normal phase separations, much progress has been made in the last few years with developing the technique more fully. Much of this work is described in this thesis.

The number of workers publishing in this area, as in all areas of HPLC-FTIR, remains small. Griffiths' group (56) did some early work with flow cell HPLC-FTIR with relatively poor results. This employed a dual beam approach with a reference cell containing the solvent. The advantages of this approach (57) to the more conventional single beam analysis is questionable due to the need for exactly matching both the
cells and double beam optics. While this approach may have an advantage for GC-FTIR (58), in most HPLC-FTIR applications one is throughput limited and so the interferogram does not overload the 15 bit A/D. This is the principal reason for the double beam technique which reduces the dynamic range of the interferogram for easier digitization. Therefore, this double beam technique does not aid the HPLC-FTIR experiment. By employing a well designed single beam FTIR system, the same cell and optics can be used to measure the background, resulting in a perfect match. Sample to background ratioing is also preferred to the more common subtraction method since fewer artifacts are produced. Perhaps for these reasons, less than adequate results were obtained. Also, the choice of the chromatographic solvent system (n-hexane) was less than ideal and Freon 113 would have performed equally as well chromatographically and would have been a superior spectroscopic solvent. Another group of workers (59) employed HPLC-FTIR (flow cell), GC-FTIR and GC-MS in a complimentary fashion. However, these workers also performed spectral subtraction, which again caused problems with the spectra produced.

Most of the work with flow cell FTIR coupled to normal phase chromatography is several years old. New developments in FTIR instrumentation have improved its ability to perform the flow cell FTIR experiment. Although it is currently restricted to normal phase separations, this appears to be the mode of choice for SRC products due to solubility problems with reversed phase solvent systems. Gradient elution would be desirable for such complex mixtures, and it should not
be written off as impossible for flow cell FTIR until it is investigated further, although it presents a challenging problem. Reversed phase separations may prove feasible through the use of non-aqueous solvents systems (38,39). Flow cell HPLC-FTIR's biggest advantages are its simplicity of interfacing and continuous monitoring of the chromatographic effluent. By monitoring continuously, complex mixtures partially resolved through chromatography can be analyzed since spectra are produced at several points along each chromatographic peak. Also, any volatile components, which may also be present, can be simultaneously monitored as well as the non-volatiles. Dilute solution spectra have the added advantage of having sharper absorbance bands than the spectra of solids (i.e., DRIFT) and subtraction techniques for mixtures are easier to apply. The remainder of this chapter and the following chapters deal with our efforts to more fully develop the potential of flow cell HPLC-FTIR and its application to SRC products. Our solutions to some of the restrictions with this technique will be discussed as well as future plans for continued improvements.

4.2 Experimental

A Varian model 5060 ternary solvent liquid chromatograph (Walnut Creek, CA) was employed equipped with a Rheodyne model 7125 injector (50 μl loop) and standard fixed wavelength (254 nm) UV detector. Solvents were Burdick and Jackson Laboratories Inc. (Muskegon, MI) preservative-free chloroform and Aldrich (Milwaukee, WI) 99.6+ atom % deuterated chloroform. Two Whatman Partisil-10 Polar Amino Cyano (PAC) bonded phase columns (25 cm x 4.6 cm I.D.; 10 μm particles) were employed.
One was equilibrated in chloroform and was used for methods development with the UV detector, while the other was equilibrated in deuterochloroform and used with FTIR detection.

A Nicolet 6000 FTIR (Madison, WI) with a broad range (5000-400 cm\(^{-1}\)) cooled MCT-B detector was employed as a LC detector. A directly coupled flow cell obtained from Nicolet Instrument Corporation was employed. This cell which had a 3 mm circular aperture and a fixed 1 mm pathlength had an effective cell volume including transfer tubing of 23 \(\mu\)L. FTIR file spectra were obtained by coadding eight "on-the-fly" spectra taken at 4 cm\(^{-1}\) resolution and ratioing these versus 32 coadded background files. This amounted to storage of a file spectrum every 6.2 seconds during an LC run.

Two process recycle solvents (boiling range: 400-800°F) originating at the SRC-I pilot plant (approximately 1979 in Wilsonville, AL were obtained from Mobil Research and Development Corporation, Central Research Division, Princeton, NJ (66). One process solvent (92-26-019) had been previously hydrotreated by the Conoco Co. and contained 9.6% hydrogen. The other process solvent (92-03-035) was not hydrotreated and contained 8.15% hydrogen. Each solvent was separated into several fractions (64,65) of varying polarity as outlined in Scheme IV. Twenty grams of the process solvent was mixed with 40 mL of heptane and loaded onto a 500 g Silica Gel 60 (E. Merck) column which also was in heptane. The column dimensions were 3.5 inches I.D. and 2 feet in length. By using the solvents indicated in Scheme IV (Figure 21), the process solvent is sequentially eluted with two liters of each solvent at
Figure 21. Preparative Scale Separation of Coal Derived Process Solvents.
a flow rate of 30-40 ml/min. Collected fractions are rotary evaporated to remove the mobile phase and dried at 40°C and 1 torr for approximately 10 hours. The second fraction from this preliminary separation, termed "intermediate polar", was subjected to further analysis by LC-FTIR.

4.3 Process Solvent Analysis
4.3.1 Background

Process solvents play a key role in the conversion chemistry leading from coal to coal-derived products. The effects of various heteroatom containing components in the process solvent on liquefaction behavior is only just beginning to be understood and explored. Certain model nitrogen-containing compounds have recently been shown to greatly enhance conversion to pyridine and toluene soluble products (24,60,61, 63). The overall detailed composition of process solvents, however, is poorly known and this has hampered an understanding of the complex chemistries which occur during coal liquefaction. Clearly, more specific analytical techniques for process solvent characterization are needed.

Liquid chromatography (LC) is an obvious means to separate the non-volatile components in process solvents. Early attempts at characterizing the whole process solvent by normal phase LC were frustrated by the wide polarity range of components and the low concentration level of the heteroatom containing components which were of particular interest. Gradient elution reversed phase (RP) chromatography, (63,27) which can handle wide polarity ranges, has been investigated as a partial solution to this problem. However, RP
chromatography does not separate by chemical class and conventional LC detectors (UV, RI) give little more than retention time data which are of limited use in highly complex mixture analysis. Furthermore, solubility of process solvents is a problem with the H_2O based solvent systems normally employed in RP chromatography. Employment of information-specific detectors (infrared and ^1H nuclear magnetic resonance) is hampered by the fact that the solvents used in RP chromatography are for the most part incompatible with these spectroscopic modes.

One solution in our laboratory to both of the above process solvent analytical problems (wide polarity range-low heteroatom content) has been a preliminary preparative LC separation on silica into several fractions prior to analytical scale LC. This separation (see Figure 21) allows concentration of the more polar components (~20% of the total) into several fractions. The polarity range of the components contained in each fraction is also limited which simplifies subsequent analysis under isocratic normal phase LC conditions.

From earlier work, (62) it was originally thought that the "intermediate polar" (IP) fraction would be rich in such oxygen containing functionalities as ethers and carbonyls. With this in mind, 10 model compounds containing the above functionalities were chosen and a separation was developed using CHCl_3 on a PAC column. These compounds are tabulated along with their retention times in Table II. It can be seen that a variety of ethers, ketones, esters and lactones elute under these conditions over a fairly broad range. Figure 22 shows the
**TABLE II**

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<th>Name</th>
<th>Structure Formula</th>
<th>$R_T$, min</th>
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<td>2. dibenzothiophene</td>
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<td>6. anthraquinone</td>
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<td>10. hydrocoumarin</td>
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</tbody>
</table>

*Flow rate = 1 mL/min.*
SEPARATION OF INTERMEDIATE POLAR MODELS

PARTISIL - 10 PAC
FLOW RATE: 1 ML/MIN
MOBILE PHASE: CHCl₃
DETECTION: UV (254 nm)

TIME IN MINUTES

Figure 22. Chromatogram of the Separation of IP Models on Partisil-10 PAC with UV (254nm) Detection; CHCl₃ Elution; 1 ml/min.
separation achieved using UV detection (254 nm). Ethers initially elute followed by ketones. Cyclic esters elute last preceded by linear esters. The totally aliphatic t-butyl ketone and cyclohexyl acetate have no UV absorption. Figure 23 represents the IR reconstructed chromatogram, based on the Gram-Schmidt (67) algorithm, of these same models employing the IR flow cell and CHCl₃ elution. The slight difference here in resolution is due to the superior dryness of the CHCl₃ relative to CH₃Cl which causes the PAC column to be more activated. It is apparent that a better separation is obtained with the dryer solvent. Identification of each chromatographic peak was made by comparing file spectra obtained during the separation with the solution spectrum of each model compound. A sample spectrum collected during the separation (maximum of the third peak) is shown in Figure 24. It is easily identified as di-t-butyl-ketone.

After this preliminary work, the two IP fractions generated from the hydrogenated (92-026-019) (HPS) and non-hydrogenated (92-03-035) (PS) process solvents were chromatographed first using CHCl₃ dried over molecular sieves on a PAC column with UV (254 nm) detection. Figures 25 and 26 shows the chromatograms obtained for PS and HPS. As can be seen, the UV absorbing components elute almost identically over the same time range (3-8 minutes) as the models. This result, without a more specific detector, might lead one to believe that these fractions are truly rich in aromatic ethers and carbonyls. In addition, the compositional differences between PS and HPS are not apparent from this experiment. The UV mode of detection also provides no information regarding
Figure 23. Chromatogram of the Separation of IP Models. Conditions as in Figure 22.
Figure 24. Sample on-the-fly Generated IR Spectrum from IP Model Separation. File Spectrum Number 111 and Identified as Anisole.
Figure 25. Chromatogram of the Separation of the IP Fraction of PS: UV (254 nm); CHCl$_3$ Elution, 1 ml/min.
Figure 26. Chromatogram of the Separation of the IP Fraction of HPS: UV (254nm); CHCl₃ Elution, 1 ml/min.
non-aromatic containing moieties. Clearly a more detailed picture of each fraction is needed.

Prior to separation, both IP fractions were subjected to "static" solution IR analysis (Figure 27). Noticeable differences are observed in the two spectra. First, the ratio of the CH aliphatic (3000-2800 cm⁻¹) to CH aromatic (3100-3000 cm⁻¹) absorbance is drastically increased upon hydrogenation as would be expected. Secondly, the carbonyl stretching region around 1700 cm⁻¹ shows very weak absorbance indicating little of this functionality is present in these fractions. Thirdly, ether related absorbance bands appear between 1250 cm⁻¹ and 1100 cm⁻¹. Since ethers have IR absorbance bands which greatly depend on substitution, the type of ether is debatable. The most distinctive bands, which are most prevalent in the IP fraction of HPS but which are also present in the IP fraction of PS, occur between 3600-3400 cm⁻¹. These absorbances are very indicative of alcohols/phenols and amines. This analysis of the IR spectra of the IP fractions of PS and HPS indicates that our initial choice of models based totally on known chromatographic elution behavior with UV detection was incorrect. Although small amounts of carbonyl compounds and ethers are present in each IP fraction, major constituents containing -OH and -NH functionalities appear.

4.4. HPLC-FTIR of Intermediate Polar Fraction

4.4.1 Preliminary Analysis

The suggested presence of -OH and -NH functionalities in both IP
Figure 27. Infrared Spectra of the Whole IP Fraction of (A) HPS and (B) PS: in CDCl₃, 0.2 mm pathlength cell.
fractions was puzzling since we had previously found that simple alcohols-phenols-amines do not elute off of a PAC column (UV detection) with CHCl₃ as the mobile phase. FTIR has been employed as a mode of detection with CDCl₃ elution to better understand the species being separated. The employment of CDCl₃ in place of CHCl₃ enabled the C-H stretching region to be monitored. Preliminary analysis of "on-the-fly" IR spectra generated during the CDCl₃ separation of the IP fraction of HPS and PS indicated that indeed a wide range of -OH and -NH materials elute, but their identities were still a question. With the realization that certain -NH and -OH compounds in each IP fraction were being chromatographed, we searched further for acceptable models. The -OH/-NH compounds which were discovered to elute with 100% CHCl₃/CDCl₃ on a PAC column are tabulated in Tables III and IV along with their retention times. Figure 28 is a representative chromatogram of the eluting hindered phenols. A wide range of alcohols and phenols were chromatographed but only compounds having very hindered -OH groups eluted. With the exception of diphenylamine and N-phenyl-2-naphthylamine, both aromatic substituted secondary amines, only "indole-like" heterocyclic secondary amines were found to elute. Interestingly, no aromatic primary amines (e.g. aniline) were found to be chromatographable under our conditions. None are present in the IP fraction of either HPS or PS since primary amines exhibit both symmetric and asymmetric (\(\sim 3450\) and \(\sim 3380\) cm\(^{-1}\)) absorbance bands which do not appear in the "static" IR spectra or in any file spectrum of our samples. This preliminary analysis narrows down the possible compound classes which can be present in the IP fraction.
<table>
<thead>
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<th>Compound</th>
<th>Structural Formula</th>
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</tr>
<tr>
<td>3,5-di-tert-butylcatechol</td>
<td><img src="image" alt="Structure" /></td>
<td>not eluted</td>
</tr>
<tr>
<td>4,6-di-tert-butylresorcinol</td>
<td><img src="image" alt="Structure" /></td>
<td>not eluted</td>
</tr>
</tbody>
</table>

$^a$ Flow rate = 1 mL/min.
### TABLE IV

**Elution Behavior of Selected Amines**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>( R_T ) min</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N )-phenyl-2-naphthylamine</td>
<td><img src="image1" alt="Structure" /></td>
<td>3.95</td>
</tr>
<tr>
<td>Diphenylamine</td>
<td><img src="image2" alt="Structure" /></td>
<td>4.25</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydrocarbazole</td>
<td><img src="image3" alt="Structure" /></td>
<td>5.05</td>
</tr>
<tr>
<td>2,3-diphenylindole</td>
<td><img src="image4" alt="Structure" /></td>
<td>5.20</td>
</tr>
<tr>
<td>2,3-dimethylindole</td>
<td><img src="image5" alt="Structure" /></td>
<td>5.90</td>
</tr>
<tr>
<td>5-ethylindole</td>
<td><img src="image6" alt="Structure" /></td>
<td>6.42</td>
</tr>
<tr>
<td>7-methylindole</td>
<td><img src="image7" alt="Structure" /></td>
<td>6.60</td>
</tr>
<tr>
<td>2-methylindole</td>
<td><img src="image8" alt="Structure" /></td>
<td>7.35</td>
</tr>
<tr>
<td>( \alpha )-phenylindole</td>
<td><img src="image9" alt="Structure" /></td>
<td>7.45</td>
</tr>
<tr>
<td>Indole</td>
<td><img src="image10" alt="Structure" /></td>
<td>7.80</td>
</tr>
<tr>
<td>Pyrrole</td>
<td><img src="image11" alt="Structure" /></td>
<td>7.85</td>
</tr>
<tr>
<td>Carbazole</td>
<td><img src="image12" alt="Structure" /></td>
<td>8.00</td>
</tr>
</tbody>
</table>
Figure 28. Chromatogram of the Separation of selected Model Hindered Phenols with UV (254nm) Detection; CHCl₃ Elution, 0.4 ml/min.
and will aid greatly in the analysis of the individual file spectra.

4.4.2 Hydrotreated Process Solvent

Figure 29 represents the Gram-Schmidt reconstructed chromatogram of the IP fraction of HPS. Note the similarity of it with the UV trace in Figure 24. The comparison is a fairly good one with the exception of the early eluting peak which is not present in the UV trace (retention time ~3 minutes, file spectrum #312). This no doubt is due to the non-UV absorbing nature of this component. File spectrum #312 is shown in Figure 30. As is evidenced by the lack of aromatic C-H absorbance bands at 3150-3000 cm\(^{-1}\) and phenyl ring absorbance bands ~1600 cm\(^{-1}\), this component is not aromatic and hence non-UV absorbing. Analysis of the spectrum shows that it is a siloxane-like material with associated characteristic absorbance bands. A spectrum of polydimethyl siloxane appears to match our unknown very well. This material is believed to be a contaminant from the silicone rubber septums employed in the storage of the sample. Different septums are currently used which do not seem to produce this problem.

What appears to be small amounts of mixed aromatic and aliphatic ethers elute between files #312-331. These are in low concentration and are not retained sufficiently to be resolved chromatographically. The next significant eluting species appear in file spectrum #331 (Figure 31) which shows four prominent bands between 3600-3400 cm\(^{-1}\) which are indicative of -OH and -NH functionality. These bands are more prevalent in HPS as compared to PS. The weak absorbance band at ~3420 cm\(^{-1}\) is assignable to a secondary amine such as diphenyl amine (Figure 32) which
Figure 29. Chromatogram of the Separation of the IP Fraction of HPS: Gram-Schmidt Reconstruction, FTIR Detection; CDCl₃ Elution, 1 ml/min.
Figure 32. Reference Spectrum of Diphenylamine Dissolved in CDCl$_3$, 0.2 mm Pathlength cell.
elutes in this region of the chromatogram (see Table IV). Non-
heterocyclic secondary aromatic amines, however, are a minor component
class which elute only in files #330-340 in the IP fraction of HPS.

The absorbance band at ~3475 cm⁻¹ in file spectrum #351 (Figure 33)
is due to ring -NH such as is found in indole (Figure 34). This class
of compound elutes over almost the entire chromatographic region (Table
III) depending upon substitution and is a major constituent of the IP
fraction eluting from files #330-396. These cyclic aromatic secondary
amines are not sufficiently resolved chromatographically (from the -OH
components in the fraction and each other) to identify any specific
compounds.

The final two IR bands (3601 and 3536 cm⁻¹) represent -OH function-
alities. Chromatographically, only highly hindered phenols (Table III)
elute under the given conditions. Figure 35 shows a spectrum of
4-methyl-2-t-butyl phenol. The -OH absorbance band exhibited at 3600
cm⁻¹ is similar to the band seen in files #338-396 and its chromatog-
ographic retention time (Table III) is consistent with the components
suspected in this fraction. Unhindered phenols do not elute. Alcohols
and hindered phenols have a tendency to have slightly higher energy O-H
stretching absorbance bands. 2,6-Di-t-butylphenol has an absorbance
band at ~3640 cm⁻¹, while benzyl alcohol (Figure 36) exhibits an
absorbance band at 3609 cm⁻¹. These absorbance bands are similar to
those briefly observed in the earlier files such as file #334. Later in
the separation this absorbance band shifts to ~3600 cm⁻¹ which is more
indicative of phenols. To date, no model alcohols have been found to
Figure 33. Infrared Spectrum of File 351 From the Separation in Figure 29.
Figure 34. Reference IR Spectrum of Indole Dissolved in CDCl₃, 0.2 mm pathlength Cell.
Figure 35. Reference 4-methyl-2-t-butyl Phenol Infrared Spectrum Dissolved in CDCl₃, 0.2 mm Pathlength Cell.
elute from the PAC column. Hindered "benzyl alcohol-like" compounds could be present but the 3600 cm\(^{-1}\) band is most likely a hindered phenol.

The absorbance band around 3536 cm\(^{-1}\) may originate from polyphenolic material. For example, bands at \(~3545\) cm\(^{-1}\) and 3600 cm\(^{-1}\) are observed in the CDCl\(_3\) solution FTIR spectrum of catechol (Figure 37). These two bands are probably due to the symmetric and asymmetric stretching vibrational modes of the two adjacent -OH groups. Again, catechol itself is too strongly retained to chromatographically elute under our conditions, but a hindered catechol might elute similarly to a hindered phenol. This combination of bands (3545 cm\(^{-1}\) and 3600 cm\(^{-1}\)) occurs in files #328-392 with the relative intensities of the two varying. This could be due to overlap of a "phenol-type" absorbance band (at 3600 cm\(^{-1}\) only) with a "catechol-like" absorbance band since the 3600 cm\(^{-1}\) band is almost always more intense than the 3545 cm\(^{-1}\) band. Figures 33 and 38 show two file spectra (#351 and 371) taken during the chromatographic run which illustrates this comparison. Alternately, this band at 3536 cm\(^{-1}\) could be due to a compound similar to 9-hydroxy fluorene which exhibits its hydroxyl stretch at 3587 cm\(^{-1}\) (Figure 39).

Generally, all files show very strong aliphatic absorbance bands (3000-2800 cm\(^{-1}\)) as one would expect for a hydro-treated sample. Better fractional separation is needed to identify individual components. This is more easily accomplished in the IP fraction of PS which appears to contain fewer -OH/-NH components (\textit{vide infra}).
Figure 37. Reference Catechol Infrared Spectrum Dissolved in CDCl₃, 0.2 mm Pathlength Cell.
Figure 39. Reference 9-Hydroxyfluorene infrared spectrum dissolved in CDCl₃, 0.2 mm pathlength cell.
4.4.3 Non-Hydrogenated Process Solvent

Figure 40 shows the Gram-Schmidt reconstructed chromatogram for the separation of the IP fraction of PS. Several distinctions can be made between the hydrotreated and non-hydrotreated fraction. There is a notable absence of the siloxane component in the non-hydrogenated sample indicating that the IP fraction of PS is much less corrosive to the septums used in storage. The separation, as can be seen in the reconstructed chromatogram, is nevertheless dominated by an early eluting peak. Examination of the file spectrum (#212) taken at the maximum of the first peak reveals a significant concentration of ethers (Figure 41). The eluting components are highly aromatic in nature with a much lower level of aliphatic -CH absorbance than was seen in the hydrotreated sample. The band at 1230 cm\(^{-1}\) is characteristic of aromatic ethers, while the smaller bands at \(\sim 1000\) cm\(^{-1}\) could be due to aliphatic ethers. The complicated overtone pattern between 1800 and 2000 cm\(^{-1}\) is characteristic of several types of substituted benzenes.

File spectrum #222 (Figure 42) shows the 4 distinctive bands above 3300 cm\(^{-1}\) due to -OH and -NH as was seen in the hydrotreated fraction. Note also the reversal from file #212 to a considerably higher -CH aliphatic/-CH aromatic absorbance ratio, although not as high as for the IP fraction of HPS. The assignments of the -OH and -NH bands are believed to be the same as in the HPS sample, except that there is much less (weaker absorbance) phenol-amine material than was found in the hydrotreated sample.
Figure 40. Chromatogram of the Separation of the IP Fraction of PS: Gram-Schmidt Reconstruction, FTIR Detection; CDCl$_3$ Elution, 1 ml/min.
Figure 41. Infrared Spectrum of File 212 From the Separation in Figure 40.
Figure 42. Infrared Spectrum of File 222 from the Separation in Figure 40.
The IP fraction of PS appears to have more well-defined chromatographic peaks due to a fewer number of components than exist in the IP fraction of HPS. A well-defined chromatographic peak (Figure 43) is reflected in file spectrum #235. Here we see that the \(-\text{NH}\) band at \(-3420\) cm\(^{-1}\) has disappeared as well as the \(-\text{OH}\) band at \(3540\) cm\(^{-1}\) that was present through most of the chromatography of the hydrotreated fraction. In addition, the \(3600\) cm\(^{-1}\) band due to phenols is very weak. The major "indole-like" band at \(-3575\) cm\(^{-1}\) dominates the spectrum at this point. Additionally, a carbonyl band at \(1675\) cm\(^{-1}\) is apparent in files #227-242, which is probably an aromatic ketone (such as benzophenone). This band was not in the IP fraction of HPS to as great an extent.

By file spectrum #247 (Figure 44), the third major chromatographic peak, only the band at \(-3475\) cm\(^{-1}\) is present in the \(-\text{OH}/-\text{NH}\) region. It is most probably a highly aliphatic substituted (note the 3000-2800 cm\(^{-1}\) absorbance) indole. Finally, file spectrum #257, which is the maximum of the last chromatographic peak, is due to the elution of a single component, Figure 45. This spectrum can readily be identified as carbazole. See Figure 46 which is a spectrum of sublimed carbazole dissolved in CDCl\(_3\) taken in our laboratory.

In conclusion, the effect of hydrotreating a sample can be seen not only in the increased \(\text{-CH aliphatic/\text{-CH aromatic IR absorbance ratio but also in the smaller amounts of ethers present. It appears that ether material may be readily converted to hydroxyl containing components upon hydrotreating with the result that highly hindered phenols are produced. Hindered dihydroxy components may also be produced in hydrotreating,
Figure 43. Infrared Spectrum of File 235 From the Separation in Figure 40.
Figure 44. Infrared Spectrum of File 247 From the Separation in Figure 40.
Figure 45. Infrared Spectrum of File 257 From the Separation in Figure 40.
Figure 46. Reference Carbazole Infrared Spectrum Dissolved in CDCl₃, 0.2 mm Pathlength Cell.
possibly from ether substituted phenols. Additional mono phenols may also be produced by hydrotreating but they should appear in later fractions of our preparative-scale separation. Future analysis of the more polar fractions of the two process solvents with FTIR detection may answer these questions. The presence of very small amounts of carbonyl containing compounds may be due to sample oxidation which is a problem with coal derived products. If this is the case, then this method would also lend itself to monitoring the integrity of the sample vs. time. We have reanalyzed this fraction after one year and have found no evidence of sample change.

4.4.4 Conclusions on Normal Phase HPLC-FTIR

Several additional points need to be made. The experimental work presented up to now show the feasibility of the flow cell technique when coupled to normal phase chromatography. Its applicability can be extended to either more or less polar materials by judiciously choosing both the solvent system and column packing material. The polarity range of bonded phases currently available to the chromatographer for normal phase separations allow much flexibility. Although solvents are somewhat generally restricted by this technique to such good IR solvents as Freon 113, carbon tetrachloride and chloroform (to name a few), almost any solvent can be added as a modifier to these base solvents to aide in effecting the desired separation. Additionally, new and more selective bonded phases could be developed for particular separations. Even when a peak is not resolved chromatographically, the spectroscopic data acquired versus time can isolate and identify several of the
various two components. While some selectivity is lost chromato-
graphically, more is acquired spectroscopically. Even though the
technique at present is limited to isocratic elution, preliminary
fractionation provides a means for reducing the polarity range of the
sample, while concentrating the individual species. This lessens the
need to employ gradients. It should be noted that few samples are more
demanding than coal-derived products. The acquisition of the infrared
spectrum of an unknown component as it elutes can aide tremendously in
identifying it. This is particularly powerful when appropriate
reference spectra are available. The capability of automatic comparison
of an unknown with stored reference spectra is currently available on
our system with only a suitable dilute solution phase data base
preventing its use (68). Such a data base has been started in our
laboratory for model SRC materials, but it will take several years to
become an effective tool. The extensive current data bases available of
gas and solid phase spectra are not suitable for identification of
dilute solutions. In general, it seems this technique has much promise
for many samples which can be separated by normal phase chromatography.

While individual components were not identified, in general, for
the process solvents studied this was not due to the detection mode.
The FTIR provided detailed structural information on all eluting
components. Only the complexity of the sample, which resulted in the
col-elution of multiple components, coupled with insufficient reference
spectra, prevented component identification. Also, although deuter-
chloroform is an excellent spectroscopic solvent, its cost, though not
prohibitive, is expensive. These problems lead us naturally into the area of microbore HPLC columns as a way to reduce our consumption of expensive deuterated solvent as well as increasing our separating power. It was also hoped that even more exotic and costly solvents (such as CD$_3$CN) might prove feasible.
CHAPTER 5

MICROBORE HPLC-FTIR

5.1 Background on Microbore Columns

Microbore HPLC columns have recently been the focus of much attention in the field of HPLC. They are one example of the trend toward microcolumns in HPLC. Novotny (69) presents a good review of both the theoretical and practical aspects of this new emerging HPLC technology. Microcolumns encompass a wide range of column types. This discussion, however, will be limited to microbore columns, which have generally, to date, found the most success.

The microbore column as it exists currently, is a logical extension of current HPLC technology. While a conventional analytical scale column normally has a 4.6 mm or greater internal diameter (i.d.), the microbore column is generally defined as having an internal diameter between 0.5 mm-1.0 mm, with 1.0 mm i.d. being the most common. It is packed with the same particle size and types of stationary phases as conventional HPLC columns. This offers several advantages and disadvantages to the chromatographer. Perhaps the first example of microbore columns was demonstrated by Horvath et al. (70) in 1969. They employed ion-exchange columns of 1 mm i.d. x 150 cm to separate nucleic acids. The associated dead-volume problems for the narrow peaks eluted from these columns coupled with the success of larger bore (4.6 mm) HPLC columns and packing technologies left the microbore column unable to
compete until chromatographic hardware improvements could be made. It was not until a report by Scott and Kucera (71) in 1976 that microbore columns began to reappear. There resurgence was due to a desire to develop much larger efficiencies in HPLC than achievable with the normal 20-30 cm long analytical columns. In this regard, microbore columns have been the only currently practical method of achieving extremely high numbers of theoretical plates in HPLC.

A good general overview of microbore columns including the advantages and disadvantages of the technique was presented by Ryall and Kessler (72). When the technological difficulties with packing these small bore columns as well as re-designing the chromatographic system to reduce total system dead volume to appropriate levels were accomplished, several unique advantages were discovered. Kirkland et al. (73) have considered chromatographic dead volume; while, both Knox (74) and Guiochon (75,77) have discussed the theoretical aspects and performance of microbore systems. In general, the advantages can be summarized as follows.

By reducing column geometry from the usual analytical scale (4.6 mm i.d.) to microbore scale (1 mm i.d.), the flow rate necessary to produce the same linear velocity in the column is reduced approximately 20-fold, resulting in a 20-fold decrease in solvent consumption. This reduces both solvent cost and disposal problems. Secondly, the concentration of the eluted peak is increased by a factor of twenty due to the decreased peak volume. This can mean an increase in detector signal by a factor of twenty for the same mass injected. However, due to design
considerations which are necessary to reduce the detector cell dead volume, (i.e. shorter pathlength) this is not necessarily achieved in practice. Nevertheless, this can be an important consideration in sample limited separations. Perhaps the biggest advantage of the microbore column is its ability to produce extremely high numbers of theoretical plates, thus providing a means (similar to capillary GC) to separate highly complex mixtures. While microbore columns produce no more theoretical plates/meter as compared to analytical scale columns when both are packed with the same particle sizes to their theoretical limits, they do possess the ability to be successfully coupled end-to-end, with nearly additive total numbers of theoretical plates. While analytical columns can be coupled in a somewhat similar manner, they soon reach a limiting total plate count (76) which has been attributed to either poor heat dissipation which affects the mass transfer process or inhomogeneous permeability across the column which produces a large multipath contribution to peak broadening. This says nothing for the vast amounts of solvent which would be consumed on an analytical column of such lengths.

Work over the last seven years has brought microbore columns to the forefront of HPLC and commerical equipment has begun to appear which will open up the field to more workers. Since Scott's and Kucera's original paper in 1976 (71) which dealt with a 10 m x 1 mm i.d. silica gel exclusion column with a total of 250,000 theoretical plates and which could separate substituted benzenes differing by only two carbons (i.e. ethyl from butyl from hexyl etc.), much additional work by these
group and others has appeared. The same authors report (76) a modification to conventional HPLC equipment for use with microbore columns as well as data on separations via normal phase columns of over 500,000 theoretical plates. Additional papers by these authors have dealt with the application of microbore columns to gradient separations of biological samples (78) and to high speed chromatographic separations (79) with associated chromatographic hardware. Scott (80) has published packing procedures for microbore columns and compares the reduced plate heights for 20, 10 and 5 μm particles as well as their sample capacity for microbore columns. The 20 μm packed columns gave the theoretical minimum reduced plate height of two particle diameters; while, the 5 and 10 μm particles gave correspondingly lower plate heights although they did not reach the theoretical limit. This showed that the packing method used was not ideal for these small particles. A follow-up paper described the theoretical basis for microbore columns (81) and the effects of extra-column band broadening were described.

Kucera (82) has presented work with 10 μm particle packed microbore columns demonstrating the theoretical minimum plate height of 20 μm for a 25 cm x 1 mm column. Sample capacity was also treated both experimentally and theoretically. Other factors affecting microbore separations were also presented. In a later paper (83) the extension to recycle chromatography was presented for microbore columns. A final paper (84) by the preceeding authors, deals with reversed phase microbore columns. Data is presented showing the concatenation of multiple reversed phase columns with no loss of efficiency in the coupling stage
(50 cm x 1 mm i.d.); while, conventional columns produced a 60% loss of column efficiency at each coupling step. The baseline separation of benzene and hexadeuterobenzene was shown on a 4.5 m x 1 mm column.

Concurrent to Scott and Kucera's work, Ishii's group in Japan was doing similar work. They employed 1.0 mm and 0.5 mm i.d. PTFE (teflon) tubing instead of stainless steel columns. These generally have been short (15-25 cm) columns without any column coupling involved. Column preparation and system miniturization (85) was described. Its extention to gel permeation chromatography (86) has been presented as well as the development (87) of a micro pre-column method for sample pretreatment. The latter was employed (88) in the analysis of corticosteroids in serum. A microscale cation exchange system for the separation of alkali metals (89) was also described. Finally, the use of fused silica tubing (0.25 mm x 10 cm) packed with 5 μm particle was also demonstrated (90).

Currently, interest in microbore HPLC is very high. Several groups have begun work in this area. Harwick et al. have discussed the designed of gradient elution microbore systems (94) as well as methods for efficient packing and coupling of small particle microbore columns (95). Trosper et al. have compared microbore and Fast (short analytical columns) HPLC (96). New commercial chromatographic equipment designed for microbore use continues to be introduced (97).

With this success by other workers and the benefits of lower solvent consumption (CDCl₃) and higher resolving power, the extension of this technique to FTIR detection seemed natural. As can be seen, microbore columns have been around for several years; yet, few instances
of their coupling with FTIR exist. These will be briefly examined.

5.2 Other Studies Involving Microbore HPLC-FTIR

Several Japanese workers have been involved in microbore HPLC-FTIR. Jinno et al. (91) interfaced a 0.5 mm x 15 cm teflon tube silica column in the same way as they had done previously (34,35) with dispersive IR solvent removal methods coupled via a KBr crystal plate and transmission spectroscopy. A simple three component mixture was partially separated and only a reconstructed chromatogram presented. Subsequently (92), this was expanded to size exclusion chromatography with the same technique and only reconstructed chromatograms were again presented. Teramae et al. (93) have described the interfacing of a 0.5 mm x 19 cm teflon SEC column via the flow cell method. Partial IR spectra (2000-1400 cm⁻¹) versus time were presented. Detection limits of 2 µg for diethylphthalate were obtained. Finally, Griffiths et al. (54) have described preliminary work with their DRIFT interface and microbore columns. Collection problems with the small effluent volume and decreased column capacity were cited with the microbore-DRIFT interface. Since this technique is predicated on collecting all or most of the effluent, the increased concentration of the effluent with the microbore column is not helpful. This coupled with the small peak volumes which the DRIFT technique cannot handle well (i.e. cannot take fine enough cuts), makes exploitation of the microbore column's advantages difficult. However, the microbore technique should aid tremendously the flow cell approach, both in sensitivity and resolution of complex mixtures.
5.3 Benefits of Microbore Columns Coupled with Flow Cell FTIR

For successful coupling of HPLC and FTIR via flow cells, care needs to be exercised in developing the chromatographic separation so that the chromatographic solvent system is compatible with regard to spectroscopic properties (i.e. IR transparency). As can be seen in Figure 47, many solvents which exhibit a wide polarity and solvent strength (98) range also exhibit good IR transparency. As has been noted previously, many modifiers to these "base" solvent systems can be added in small (normally <5%) amounts to effect the chromatographic separation without loss of additional spectral information. These allow reasonable flexibility, coupled with the various bonded silica columns available, for normal phase chromatography. Deuterated solvents are particularly good IR solvents but suffer from moderate (CDCl3) to extremely high (CD3CN) cost. Lower solvent consumption would ease the cost burden of the former and improve the feasibility of using the latter. Microbore columns offer this possibility.

While one criticism of the flow cell technique is the loss of parts of the IR spectrum generated due to total solvent absorbance, the remaining information is still substantial and, depending on the separation and reference data base, provides the analyst with either structural information (functional groups) or positive compound identification. While decreasing the pathlength of the flow cell produces additional transparent regions, this is only accomplished at the expense of sensitivity. Since the FTIR is already substantially less sensitive than a standard UV detector, this is not desirable.
Figure 47. Transparent Regions in the Mid-IR for a Variety of Solvents Using a 0.2 mm Cell Pathlength.
Since the effluent concentration is increased (~20-fold) for a microbore column over a conventional analytical column, shorter pathlengths can be employed with microbore columns while still improving absolute sensitivity (as amount injected). Alternately, higher sensitivity can be obtained with equivalent spectroscopic information at the longer pathlengths. The latter assumes such a flow cell can be produced with sufficient low dead volume for microbore work.

Another problem encountered with complex mixtures such as coal-derived process solvents, is the difficulty in resolving all of the components for spectral identification. Again, coupled microbore columns offer an extremely large number (~750,000 (76)) of theoretical plates which can be of tremendous value in the analysis of such complex mixtures and for the first time offers the possibility for individual compound separation and identification for these mixtures by HPLC. In addition, the criticism has been leveled, with some merit, that the solvent restrictions of flow cell FTIR remove the greatest advantage of HPLC, that being its great selectivity through the use of diverse solvent systems, many of which are not IR compatible. However, this is most important when the column's efficiency is limited. The large number of theoretical plates generated by microbore columns reduces the need for such a high degree of selectivity (as in capillary GC) and also shortens the time for methods development. It should be pointed out that such large numbers of theoretical plates are only possible at the expense of very long (>10 hours) analysis times. Smaller diameter packings (3 μm) may improve this somewhat when they can be successfully
packed. This is preferable from a sensitivity standpoint, since less dilution will occur during the chromatographic separation if shorter columns can be employed.

For these reasons, work with microbore columns was begun. This chapter describes work with microbore columns coupled with FTIR detection for the identification of polar material in complex mixtures. Comparisons are made between coupled microbore columns and similar work done with an analytical scale column containing the same bonded phase packing. Separations performed on the two systems will be compared. Minimum injected detectable quantities for various organic functionalities will be measured using suitable vibrational absorption modes.

5.4 Experimental

Analytical scale separations were performed on a Varian model 5060 ternary solvent liquid chromatograph (Walnut Creek, CA) which was equipped with a Rheodyne model 7125 injector, a 10 µL loop and a standard 254 nm UV detector. A Whatman Partisil-10 Polar Amino Cyano (PAC) bonded phase column (25 cm x 4.6 mm i.d.; 10 µm particles) which had been equilibrated in CDCl₃ was employed for separations.

Microbore separations were performed on a modular chromatographic system. A Waters 6000A pump was used without modification except 1/16 inch tubing was used for connecting the solvent reservoir to the pump solvent inlet. To pump at the required reduced flow rates (10-100 µL/min) an auxiliary controller was used. This connected directly to the pump's stepper motor via the standard connection provided on the 6000A for external pump control. The controller was an 8085 (Intel
Corp.) based microprocessor (99) assembled in-house and appropriate software was written so as to produce a variable frequency square wave (0-5 volts peak to peak) which was fed into the pump motor. The resulting flow rate was linearly related to frequency (10 Hz to 1 KHz). The pump operated well down to about 15 μL/min, below which the resulting back pressure from the column was insufficient for proper functioning of the check valves. Use of a restrictor valve to increase the back pressure and placed between the pump and injector should allow lower flow rates. The microprocessor also monitors the pump pressure sensor signal to turn off the system in case of over pressurization for safety purposes. It is hoped that in the future the microprocessor can be used to allow flow programing during a separation. Application to gradient elution control can also be envisioned. Remaining chromatographic components include a Rheodyne model 7413 injector with 0.5, 1.0 and 5.0 μL internal loops; two 50 cm x 1 mm i.d. coupled microbore columns packed with 10 μm Partisil-10 PAC bonded silica by CM Laboratories (Nutley, NJ) which connected directly into the injector; and a LDC model 1205 UV detector with a 3 μL (3 mm pathlength) preparative flow cell.

A Nicolet 6000C FTIR equipped with a model 7010 B Mercury-Cadmium-Telluride (MCT-B) detector was used to monitor the eluting components from the chromatographic system. A flow cell, based upon the Nicolet cell holder previously used was modified for microbore separations. Figure 48 outlines its simple design. Low volume stainless steel connecting tubing (~3 cm x 0.009 inch i.d.) was employed to connect the
Figure 48. Diagram of Modified Flow Cell with Minimized Cell Volume (3.2 μL) for Microbore LC-FTIR.
flow cell to the column. To help minimize dead volume in this first generation cell, an Analabs 0.2 mm KBr cell was employed. Although this reduced sensitivity compared to the 1.0 mm pathlength cell previously used, larger regions of solvent transparency were also produced (Figure 49). Ultimately, unlike UV detection, the pathlength will be restricted by solvent absorptions and not volume, since pathlengths much greater than 1.0 mm result in very limited solvent transparency. The overall dead volume of this cell was 3.2 μL. The effect of this rather large dead volume (for microbore applications) will be discussed presently. All extra column dead volume other than that already noted was eliminated with both delivery systems except for a small (3 cm) piece of 0.009 inch i.d. capillary stainless steel tubing used to connect the analytical column to its injector. The same detector cell was used for both analytical and microbore separations with FTIR detection. Although the analytical separations would not suffer significantly from an increase in pathlength to 1.0 mm, it is important to compare the two systems at the same pathlength since ultimately with better designed flow cells, the restrictions in pathlength will not be due to volume considerations but will be inherently limited by solvent transparency regardless of the type of chromatographic column employed. The standard Nicolet FTIR software package was used to collect 4 cm⁻¹ resolution spectra with a time resolution between spectra of approximately 9.5 seconds (e.g. 12 sample scans ratioed to 24 background scans per spectrum at a mirror velocity of approximately 0.59 cm/sec). Flow rates of 20 μL/min and 0.4 mL/min which were calculated to produce
Figure 49. Infrared Spectra of CHCl₃ and CDCl₃ Showing IR Transparency vs. Pathlength.
the same column linear velocity were employed for the microbore and analytical separations respectively.

The elution solvent was Aldrich (Milwaukee, WI) 99.6+ atom % deuterated chloroform. Model indoles and phenols were also obtained from Aldrich. The process recycle solvent fraction analyzed here is the same as in the previous chapter.

5.5 Normal Phase HPLC-FTIR Employing Microbore Columns

One of the major reasons to switch to microbore scale separations with FTIR detection lies in the drastically reduced solvent consumption. This allows us to economically substitute CDC13 for CHCl3 in our separations since total solvent consumption is on the order of 1 mL/ separation. The benefit of CDC13 over CHCl3 can be seen in Figure 49 where the IR spectrum of each solvent is shown. The dark bands represent IR transparent regions where spectral information can be obtained at the indicated flowcell pathlengths. Blank regions represent total IR absorbance. It can be seen that CDC13 enhances the transparent regions especially around 3000 cm⁻¹ where C-H stretching absorbances occur. At 0.2 mm for CDC13, there is essentially no loss of information above 950 cm⁻¹.

Although a 1 mm flow cell pathlength is preferred for sensitivity reasons a 0.2 mm pathlength was employed here in order to help minimize the system dead volume which is especially critical for early eluting peaks. This decision led to a cell volume with associated tubing of 3.2 μL. An H vs μ plot was constructed from data obtained with the 1 meter coupled microbore columns and the 3 μL volume UV detector (Figure 50).
Figure 50. $H$ vs. $\mu$ Plot for Indole ($K'=0.72$) and Diphenylamine ($K'=0.18$) (1 m x 1 mm PAC column with 3 $\mu$L UV Detector) Showing Increased Efficiency at Larger $K'$; 500 ng; 1.0 $\mu$L Injected.
Diphenylamine \((k' = 0.18)\) and indole \((k' = 0.72)\) were chosen as test compounds. Flow rates between 15 and 100 \(\mu\text{L/min}\) were employed. Reproducible flow rates below 15 \(\mu\text{L/min}\) were not possible with our pumping system, probably due to insufficient backpressure for the proper operation of the check values. Several points can be drawn from this study. Our efficiency does not approach the theoretical limit of \(2d_p\) \((20 \ \mu\text{m})\) but is closer to \(3d_p\). This was to be expected given the relatively large dead volume of our detector cell. Secondly, the efficiency improves with increased \(k'\) indicating cell volume is less critical with longer retained components where the peak volume is already relatively large. In other words, reasonably efficient separations can be expected with the present system, especially if components with high \(k'\) values are to be separated.

Our interest in nitrogen containing components in synthetic fuel samples and knowledge obtained from the analytical scale HPLC-FTIR led us to develop a separation for a series of weakly basic cyclic and non-cyclic secondary aromatic amines. Figure 51 shows the separation with UV detection of a series of these models specifically chosen to test the performance of FTIR as an on-line microbore HPLC detector. Chromatographic conditions are described in the Figure caption. Figure 51A represents the injection of approximately 300 hundred nanograms of each component while Figure 51B is the injection of about three micrograms of each component. The slight resolution between components 6 and 7 in 51A is lost upon increasing the sample load as evidenced in 51B. This is an important point since several micrograms of material
Figure 51. Separation of Model Amines. 1 m x 1 mm PAC Column; 20 μL/min; 1 μL Injected; 3 μL UV Detector. (A) 300 Nanograms/component; (B) 3 Micrograms/component.
are needed per injection for adequate FTIR detection of these compounds with our present system. A more quantitative look at sample capacity will be presented in the next chapter. It should also be noted that the final peak has a k'-1. A better separation could have been achieved by using a weaker solvent system (such as adding CCl₄), however, this would have resulted in broader peaks and subsequently higher detection limits.

The same models were chromatographed with FTIR detection. This separation is shown in Figure 52 which is a Gram-Schmidt reconstructed chromatogram which is based on changes in interferograms (69). Approximately 3 μg of the first two components and 6 μg each of the remaining components were injected with chromatographic conditions noted in the Figure caption. Comparison between Figures 51B and 52 show as good if not better resolution with FTIR detection. Peak labels in Figure 52 refer to individually stored IR spectra as a function of retention time. Each data point represents an unique IR spectrum. The real utility of FTIR detection, however, is not in the reconstructed chromatogram, but in the individual IR spectra generated. Figure 53A represents the spectrum taken at the peak maximum of the last eluting component. Figure 53B is a reference spectrum of indole dissolved in CDCl₃ for comparison. The "unknown" can easily be identified as indole from the reference spectrum even though it is the last component to elute. A more complex example of eluent identification is illustrated by the peak preceeding the indole peak (labeled 301 in the reconstructed chromatogram and 8 in the UV chromatogram) which from Figure 51 can be
Figure 52. Gram-Schmidt Based Infrared Reconstruction of Model Amines. Conditions as in Figure 51 except 5 μL injected. Weights are noted in text.
Figure 53. (A) Sample on-the-fly FTIR Spectrum From the Final Peak in Figure 51 (12 Coadded Scans/Spectrum). (B) Reference FTIR Spectrum of Indole.
seen to consist of 3 compounds (determined from separated individual injections). The FTIR can spectroscopically resolve and identify these compounds; whereas, they seem to co-elute with UV detection (and GSR). Two methods were employed to determine the composition of this peak. First, the IR spectra taken from the front, heart and back of the chromatographic peak were compared with a small library (~50 compounds) of dilute solution reference spectra collected in our laboratory via spectral search software (100) provided by the manufacturer. The reference library contained all of the unknowns as well as many other indoles, amines, phenols, esters and amides. Although this data base is limited, an idea as to the resolving power of the FTIR as a chromatographic detector can be gained. The reference spectrum is compared to the unknown and the computer calculates the difference between the two. The quality of the match is based upon the sum of the square of the absolute difference between the two spectra and this value is termed the metric. The reference spectra with the smallest metric (normally the

\[ M_{SQ} = \sum_{i=1}^{N} |X_i - Y_i|^2 \]

top 5) are returned as possible "hits". This particular algorithm emphasises large differences between spectra as opposed to subtle differences. After the best hit was returned, visual comparisons between the unknown and reference spectra were made. The front (299), center (302) and back (307) spectra are presented with the corresponding best hit reference match in Figures 54-56. As can be seen, the correct
Figure 54. Comparison of IR File Spectrum 299 from the Separation in Figure 51 with the Reference Library's Closest Match, 2-phenyl Indole.
Figure 55. Comparison of IR File Spectrum 302 from the Separation in Figure 51 with the Reference Library's Closest Match, Carbazole.
2-METHYL INDOLE

FILE 307

Figure 56. Comparison of IR File Spectrum 307 from the Separation in Figure 51 with the Reference Library's Closest Match, 2-methyl Indole.
reference spectra were chosen by the computer. Visually, Figures 54 and 55 are good matches while Figure 56 has some spill over from the preceding eluting compounds and is visually a somewhat poorer match. Its metric, however, was still almost one third that of the second best hit. The limited number of reference spectra makes the use of spectral matching risky for true unknowns, but the method shows its potential given a larger data base. All of the remaining peaks were easily identifiable using the same method.

The same process solvent fraction which had been analyzed with FTIR detection on an analytical scale column (25 cm x 4.6 mm i.d.) and discussed in the preceding chapter was chromatographed on the microbore column (100 cm x 1 mm i.d.) to test the increased resolving power of the latter. Figure 57A and 57B show UV traces for both separations. Identical linear velocities were employed. Resolution is clearly improved in the microbore separation, however, not as great as would be expected especially for the early eluting components. This is no doubt due to the large cell volume of the UV detector. The situation is slightly worse (Figure 57C) with FTIR detection. In this case, the large cell volume is coupled with the relative insensitivity of the FTIR. For such a complex sample which contains a large number of components, there is a need to inject a higher concentration of material for FTIR detection. Although the chromatographic resolution is poorer than with UV detection, good quality IR spectra are obtained as evidenced in Figure 58 which is a spectrum of the last major species to elute which appears to be an as yet unidentified alkyl substituted
Figure 57. Comparison of Complex Coal Derived Material. (A) 4.6 mm x 25 cm PAC Column; 0.4 ml/min; 10 µL Injected; UV (254 nm) Detection. (B) 1 mm x 100 cm PAC Column; 20 µL/min; 1 µL Injected; UV (254 nm) Detection. (C) 1 mm x 100 cm PAC Column; 20 µL/min; 5 µL Injected; FTIR Detection with 0.2 mm Flow Cell.
Sample FTIR Spectrum From the Separation in Figure 57c Collected Just After the Maximum of the Final Major Chromatographic Peak.
carbazole. Additional analysis will not be presented since this sample has been discussed previously (Chapter 4). Although we have not yet realized all of the resolving power that the microbore can provide, our overall separation is improved when compared to the separation reported in Chapter 4 obtained via analytical scale LC-FTIR with a 1 mm pathlength cell.

This brings us finally to the determination and comparison of detection limits for LC-FTIR. For this work we chose 2,6-di-t-butyl phenol (k' - 0.1) as a test compound for quantitatively comparing the sensitivity of both the microbore and analytical scale LC-FTIR systems. For this determination various amounts of the test compound were injected onto both columns with their elution monitored with FTIR detection. At the completion of each separation, the three most absorbing spectra were coadded to produce an averaged spectrum. Figure 59 represents the spectra obtained with the microbore system for the indicated injected amounts. For each of the four major absorption bands absolute absorbance was plotted vs. μg injected producing a least squares fit with R² greater than 0.99 for both the analytical and microbore separations. A representative plot (Figure 60) is shown for the 2962 cm⁻¹ assymetrical -CH stretch. Peak to peak noise levels were measured at ±50 cm⁻¹ around the absorbance maximum. Injected minimum detectable quantity (IMDQ) defined as 3x the noise level is tabulated in Table V for each of the four major absorption bands appearing in the IR of 2,6-di-t-butyl phenol. Also listed is the ratio of analytical to microbore IMDQ.
Figure 60. Plot of 2962 cm⁻¹ (Assymmetric -CH Stretch) Absorbance Band of 2,6-di-t-butyl phenol Peak Height vs. weight of material injected.
TABLE V

INJECTED MINIMUM DETECTABLE QUANTITY\(^a\) VS. ABSORBANCE BAND

<table>
<thead>
<tr>
<th>IR band</th>
<th>Assignment</th>
<th>Noise Level (^b)</th>
<th>Analytical (^c) (µg)</th>
<th>Microbore (^c) (µg)</th>
<th>Analytical/Microbore</th>
</tr>
</thead>
<tbody>
<tr>
<td>3640 cm(^{-1})</td>
<td>-OH stretch</td>
<td>0.0007</td>
<td>12.87</td>
<td>1.67</td>
<td>7.71</td>
</tr>
<tr>
<td>2960 cm(^{-1})</td>
<td>Asymmetric -CH(_3) stretch</td>
<td>0.0008</td>
<td>10.52</td>
<td>1.32</td>
<td>7.90</td>
</tr>
<tr>
<td>1426 cm(^{-1})</td>
<td>Asymmetric -CH(_3) bend</td>
<td>0.0004</td>
<td>6.04</td>
<td>0.63</td>
<td>9.59</td>
</tr>
<tr>
<td>1232 cm(^{-1})</td>
<td>-C-O- stretch</td>
<td>0.0002</td>
<td>6.38</td>
<td>0.76</td>
<td>8.39</td>
</tr>
</tbody>
</table>

\(^a\)IMDQ = 3x noise level

\(^b\)Peak to peak noise level in absorbance units from ±50 cm\(^{-1}\) around band maximum

\(^c\)3 sets of 12 sample scans were ratioed against 24 background scans and coadded
Theoretically, the detection limit for the microbore separation should be approximately 20 times that of the analytical scale separation (76). The actual value obtained is only 8 or 9 times depending on the absorption band employed. This is due to two facts. First, the microbore column is 4 times the length of the analytical scale column causing its peaks to be correspondingly broader and less intense. Chromatographic theory shows (see next chapter) that for columns of equal efficiency (plates/meter), an increase of a factor of four in column length will cause a sample eluting at the same k' to yield a chromatographic peak half as intense (peak width doubled) as the same compound eluting from the shorter column assuming Gaussian peak shapes. The remaining small difference between theory and experiment can be attributed to the cell volume which is large by microbore standards. This would cause significant band broadening, especially for a component with k'=0.1, whereas, a negligible contribution to band broadening by the cell volume would be expected in the analytical scale separation. The use of a 1 meter microbore column was employed to try to offset somewhat the large cell volume. It was, however, impractical to employ the same length analytical column.
CHAPTER 6

DETECTION LIMITS FOR MICROBORE HPLC-FTIR

6.1 Background

The methods for interfacing FTIR with HPLC and the associated problems involved have been described in the previous chapters. While the flow-cell technique has been shown to be easy to implement, it still is not widely used as an analytical method. Chromatographic-spectroscopic solvent restrictions (currently limited to isocratic elution with IR transparent solvents) are part of the problem; while, an equally important obstacle is the relative insensitivity of FTIR when compared to conventional HPLC detectors such as U.V. and fluorescence. Several authors have reported on-line LC-FTIR applications and detection limits for a few compounds have been presented (56,93,100), but no report has looked at a wide range of chemical classes with varying molar absorptivities. Also, no good criteria have been established to determine detection limits as a function of chromatographic parameters (i.e. column length, k', column i.d. or amount injected) for FTIR detection. Since the unique advantage of FTIR as a chromatographic detector lies in the real-time IR spectra that can be generated, detection limits should reflect the acquisition of useful structural information from the generated spectra and not just component elution time via reconstructions which emulate single wavelength chromatograms.
This chapter will deal with the detectability of several compound classes with flow-cell HPLC-FTIR. Several chromatographic parameters will be examined to determine their effect on detectability. A method to determine the injected minimum detectable quantity (IMDQ) for HPLC-FTIR will be shown using microbore (1 mm i.d.) columns which is extendable to any HPLC-FTIR system. Such data will provide criteria for determining when HPLC-FTIR can be used successfully. Finally, several factors will be discussed for improving HPLC-FTIR detectability.

6.2 Experimental

The system employed for microbore HPLC-FTIR was more extensively described in the previous chapter. It consisted basically of a Waters 6000A pump modified for reduced flow rates (10-100 µL/min), a Rheodyne model 7413 injector (1 µL loop used); a 50 cm x 1 mm i.d. microbore column packed with 10 µm Partisil-10 PAC bonded silica (N=25,000 plates/meter at 20 µL/min). The flow cell employed had a dead volume of 3.2 µL and a pathlength of 0.2 mm. A Nicolet 6000C FTIR equipped with both a model 7010 B Mercury-Cadmium Telluride (MCT-B) detector and 7010 A Mercury-Cadmium Telluride (MCT-A) detector was employed. On-the-fly spectra from 4000-400 cm⁻¹ were obtained with the MCT-B detector, while the MCT-A was evaluated as to noise characteristics only. A flow rate of 20 µL/min was employed with 99.6+ atom % deuterated chloroform obtained from Aldrich Chemical Co. (Milwaukee, WI) as the elution solvent. Standard Nicolet software was employed to collect 4 cm⁻¹ resolution spectra. Time resolution was 4.8 seconds/stored spectrum (i.e. 6 sample scans ratioed to 24 background scans at an approximate
mirror velocity of 0.59 cm/sec). Additional spectra were coadded post-run. Phenols, amines and indoles were obtained commercially from Aldrich, Fisher, J. T. Baker and Eastman. Some amines and phenols were distilled or recrystallized prior to use. p-Fluorobenzoyl ester and amide derivatives of the corresponding phenols and amines were prepared in-house by a previously reported technique (101). This derivatization was carried out in chloroform with a slight excess of p-fluorobenzoyl chloride and the appropriate amine/phenol. Pyridine was also used as a catalyst. The reaction was refluxed for one hour, after which the reaction mixture was washed with a dilute sodium bicarbonate solution to remove the p-fluorobenzoic acid by-product and dried with sodium sulfate. The product was then isolated by rotary evaporation.

6.3 Detectability as a Function of Chromatographic Parameters

In the previous chapter we reported a method for experimentally determining FTIR detection limits for 2,6-di-t-butyl phenol employing both microbore and analytical scale HPLC. By injecting known weights of the test compound, a calibration curve was generated by plotting a particular IR absorbance band intensity vs. injected sample weight. Extrapolation of the plot by a linear least squares fit to the weight required to produce a signal which was 3x the detector peak to peak noise level (±50 cm⁻¹ around the absorbance band maximum) yielded a detection limit. This procedure was performed for several of the major absorbance bands in 2,6-di-t-butyl phenol with the results termed the injected minimum detectable quantity (IMDQ). These data provided a reasonable means for determining the IMDQ of a particular compound for
a given chromatographic separation. However, a change in chromato-
graphic conditions will result in a change in IMDQ. The three major
chromatographic factors affecting detectability are column length (L),
plate count (N) and capacity factor (k'). All these factors affect band
broadening in the chromatography column which produces a dilution of the
injected compound in the flow cell thereby causing a decrease in signal
for concentration detectors such as FTIR. For most analytical scale
separations, column length is generally 25-30 cm. Microbore columns, on
the other hand, ranging from 25 cm to 14 meters have been employed
(82,76) in an effort to increase the number of theoretical plates.
Plate counts which affect the concentration of eluting components vary
depending on particle diameter, packing proficiency and flow rate. Once
column hardware has been chosen these two parameters (N and L) are
normally held constant. At this point, the actual chromatographic
separation becomes important. This is reflected in the capacity factor
(k') which determines elution time. Larger k' values cause longer
retention times and a more dilute sample presented to the detector.
Chromatographic theory allows us to treat each of these three parameters
quantitatively.

6.3.1 Column Length and Plate Count

Coupled microbore columns have been shown (84) to produce an
additive effect in total number of plates (N). Thus, two coupled
microbore columns produce the sum of the individual plates of each of
the two columns. This property allows columns to be constructed which
have a large number of theoretical plates (>500,000 (76)). This is
accomplished, however, at the expense of band broadening. The effect of column length on Gaussian peak width can be determined from chromatographic theory by starting with the fundamental equation for calculating theoretical plates. Assuming microbore columns of length L and 2L, we can write equations (1 and 2) for calculating the number of theoretical plates employing the same compound separated under the same conditions on the two columns where \( t_r \) = retention time, \( N \) = number of theoretical plates and \( t_w \) = peak width at peak base.

\[
\text{Column Length=}\, L: \quad 16(t_r/t_w)^2 = N \quad \text{(1)}
\]

\[
\text{Column Length=}\, 2L: \quad 16(2t_r/t_w')^2 = 2N \quad \text{(2)}
\]

The column of length 2L will elute the compound with a width \( t_w' \) at double the retention time \( (2t_r) \). This of course assumes that the two coupled microbore columns are identically packed and therefore will have a plate count twice that \( (2N) \) of the shorter column. The ratio of equation 1 to equation 2 yields equation 3 which relates the broadness of the peak \( (t_w') \) eluted from the longer column \( (2L) \) to the broadness of the peak \( (t_w) \) eluted from the shorter column \( (L) \).

\[
t_w'/t_w = \sqrt{2} \quad t_w' = \sqrt{2} \, t_w \quad \text{(3)}
\]

This can be related to peak height and detectability by the equation for a Gaussian function of unit normalized area \( (102) \)

\[
f(x) = \frac{1}{\beta \sqrt{2\pi}} e^{-\frac{(x-\alpha)^2}{2\beta^2}} \quad \text{(4)}
\]
where \( \alpha = \text{mean or retention time}, \beta = \sigma \) (standard deviation) and \( t_w \) is defined as \( 4\sigma \). At the chromatographic peak maximum, where the most intense IR spectra are produced, \( x = \alpha \) and equation 4 reduces to

\[
f(x) = \text{peak height}(P_h) = \frac{1}{\beta\sqrt{2\pi}}
\]  

(5)

Upon substituting for \( \beta \) in terms of \( t_w \) \( (t_w = 4\sigma) \), equation 5 can be written as

\[
P_h = \frac{4}{t_w\sqrt{2\pi}}
\]  

(6)

For a column of length \( 2L \) and using equation 3 to substitute for \( t_w \) gives

\[
P_h' = \frac{4}{t_w'\sqrt{2\pi}} = \frac{4}{2\sqrt{\pi}t_w} = \frac{2}{t_w\sqrt{\pi}}
\]  

(7)

Again taking the ratio of equation 7 to equation 6 yields

\[
P_h'/P_h = \frac{1}{\sqrt{2}} \quad P_h' = \frac{P_h}{\sqrt{2}}
\]  

(8)

which is the reciprocal of the increase in peak width on going to twice the column length. Thus, the peak height (and detectability) decreases by the square root of the increase in column length. This means that a compound separated on a microbore column of 100 cm would have a peak height one-half that of the same compound separated on a microbore column of 25 cm. At the same time the separation would benefit from 4 times the number of theoretical plates.
By using a similar treatment and substituting the appropriate factors into equation 1, it can be shown that for a column of given length an increase in the number of theoretical plates by a factor of \( n \) yields an increase in the peak height equivalent to the \( \sqrt{n} \). Both treatments assume IR spectra are taken at the peak maxima, which is a reasonable assumption since spectra are collected continuously across the peak. Non-weighted averaging of IR spectra across the chromatographic peak will, however, cause some deviation from the above.

6.3.2 Capacity Factor

For a column of given length and efficiency, the remaining factor affecting peak height is the length of time the compound remains in the column, expressed as the capacity factor, \( K' \)

\[
K' = \frac{(t_r - t_0)}{t_r}
\]  

(9)

where \( t_r \)=retention time of the compound of interest and \( t_0 \)=retention time for a non-retained compound. Kucera (82) gives an equation for the peak volume (width) of Gaussian shape peaks as

\[
V_p = \frac{\pi D L \varepsilon (1 + K')}{\sqrt{N}} = 4\sigma = t_w
\]  

(10)

where \( V_p \)=volume of the peak \((4\sigma)\), \( D \)=inner column diameter, \( \varepsilon \)=porosity of the bed, \( L \)=column length and \( N \)=plate count. This allows us to compare, with the same column parameters, changes in peak widths for various \( K' \) values which is inversely equivalent to peak height. For peaks \( P_1 \) and
from equation 10

\[ \frac{V_{p1}}{V_{p2}} = \frac{t_{w1}}{t_{w2}} = \frac{(1+K'_1)}{(1+K'_2)} \]  \hspace{1cm} (11)

For example, the peak height for a compound eluting at \( K' = 1 \) compared to \( K' = 3 \) should be

\[ \frac{t_{w1}}{t_{w3}} = \frac{P_{h3}}{P_{h1}} = \frac{(1+1)}{(1+3)} = 0.5 \]  \hspace{1cm} (12)

6.3.3 Limiting Sample Mass

The limiting sample mass \( (M_s) \) has been defined as the mass of sample that can be placed on a column without seriously impairing column efficiencies. Typically this is set at a loss in efficiency of 10% of the total column theoretical plates. For 20 \( \mu m \) particles, Kucera reports (82) values of 20 \( \mu g/\)component \( (K' = 2; \ 1 \ mm \times 1 \ m \ column) \) for the limiting sample mass. However, this will be affected by several factors. Kucera gives an equation (82) for \( M_s \) as:

\[ M_s = \frac{\pi \cdot D \cdot L \cdot \varepsilon \cdot (1+K')}{2\sqrt{N}} \]  \hspace{1cm} (13)

This is similar to equation 10 presented for peak volume. Here \( X_m \) is the maximum solute concentration allowable in the mobile phase. For a column of \( L \) and \( 2L \), increase in loading would be:

\[ \text{Column } L \quad M_s = X_m \frac{\pi \cdot D \cdot L \cdot \varepsilon \cdot (1+K')}{2\sqrt{N}} \]  \hspace{1cm} (14)

\[ \text{Column } 2L \quad M_s = X_m \frac{\pi \cdot D \cdot 2L \cdot \varepsilon \cdot (1+K')}{2\sqrt{2N}} \]  \hspace{1cm} (15)
\[ \frac{M_s^2}{\frac{1}{M_s}} = \frac{2}{\sqrt{2}} = \sqrt{2} \]  

(16)

or

\[ M_s^2 = 1.41 \frac{1}{M_s} \]  

(17)

However, as was shown by equation 3, the peak width (and height) is now \( \sqrt{2} \) times broader, having a net cancelling effect on any signal enhancements by additional sample loading. This does not take in any signal averaging improvements which could be realized due to the broader peak width (i.e. more scans for same percentage of the peak). The same trade off would be realized at higher \( K' \)'s (from equation 11). The benefits remaining from longer columns would be increased number of theoretical plates and higher tolerance of chromatographic dead volumes. Analysis time, however would be correspondingly longer.

6.3.4 Analysis Time

To realize several hundred thousand theoretical plates with coupled microbore columns would require analysis times on the order of 10 hours or more. A means of shortening this analysis time while still maintaining the same number of theoretical plates, is the use of smaller size packings such as 5 \( \mu \)m (or given the ability to pack efficiently, 3 \( \mu \)m) particles. The time savings would be a factor of two at the expense of double the operating pressure of the chromatographic equipment. The time savings would come from the shorter length required, since well packed 5 \( \mu \)m particles produce twice as many plates/meter as 10 \( \mu \)m.
particles. The increase in pressure is the tradeoff for the higher number of plates/meter. Since very long coupled microbore columns will reach the pressure limit imposed by the chromatographic equipment (6000-7000 psi), it is this upper limit in pressure which dictates the maximum number of total theoretical plates obtainable. Smaller particle size packings reach this limit in a shorter length of column than would large particles. Still, analysis time would be shortened. As discussed in the previous sections, there are both positive and negative consequences of employing smaller particles.

First, as was discussed in 6.3.1, increasing the number of plates per unit length would decrease the peak volume by the $\sqrt{n}$, $n$ being the factor by which the number of plates/meter were increased. This also would increase the peak height by the same factor. This would simultaneously improve detectability while increasing the effect of system dead volume. While the detectability would improve, the column capacity, as discussed in the preceding section, would be lowered.

6.4 Injected Minimum Detectable Quantity

6.4.1 Experimental IMDQ

Having developed equations which relate the relative effect of $N$, $L$ and $K'$ on chromatographic peak height and therefore detectability, there remains the need to experimentally determine the injected minimum detectable quantity (IMDQ) for a given set of conditions in order to make use of these equations for other sets of chromatographic conditions. Using a 50 cm PAC microbore column with a plate count of
-25,000 plates/meter at a flow rate of 20 µL/min, IMDQ values were determined for the following hindered phenols: 2,6-di-t-butyl phenol (K' = 0.06), 2,4-di-t-butyl phenol (K' = 0.72) and 2-t-butyl-4-methyl phenol (K' = 1.01). The absorbance band chosen for calculating IMDQ was the characteristic -OH stretching vibration at ~3600 cm⁻¹. The noise level for this IR band was determined by taking the average peak to peak noise ±50 cm⁻¹ around the absorption maximum for 8 sets of background spectra. Each of the 8 sets consisted of 3 individual coadded spectra of six scans each. In other words 18 scans were coadded for the noise determination with a time resolution of about 14 seconds.

Similarly, 3 sets of 6 scans each were coadded across the chromatographic peak (post-run) to obtain a high S/N composite spectrum for each of the three component phenols. This procedure was followed for a series of chromatographic runs of varying injected amounts. The total absorbance of the appropriate band (3600 cm⁻¹) was plotted vs µg of compound injected and a linear least squares line fitted. Extrapolating to 3X the noise level in absorbance units produced experimentally IMDQ values which are tabulated in Table VI. By using 2,6-di-t-butyl phenol as the reference IMDQ and correcting for molar absorptivity and molecular weight differences, the predicted IMDQ values as a function of K' for the two other phenols (from equation 11) can be determined (Table VI; see Appendix I for sample calculation). The calculated IMDQ values agree very well with the experimentally determined values, with the differences possibly arising from small variations in experimental K' values and coadding spectra across the
### Table VI

EXPERIMENTAL AND CALCULATED IMDQ

<table>
<thead>
<tr>
<th>Compound</th>
<th>K'</th>
<th>IMDQ&lt;sub&gt;exp&lt;/sub&gt;</th>
<th>ε</th>
<th>IMDQ&lt;sub&gt;calcd.&lt;/sub&gt;</th>
<th>Dilution Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,6-di-t-butyl phenol</td>
<td>0.06</td>
<td>1.06</td>
<td>19.96</td>
<td>--</td>
<td>6.71</td>
</tr>
<tr>
<td>2,4-di-t-butyl phenol</td>
<td>0.72</td>
<td>2.37</td>
<td>14.20</td>
<td>2.42</td>
<td>10.89</td>
</tr>
<tr>
<td>2-t-butyl-4-methyl phenol</td>
<td>1.01</td>
<td>2.24</td>
<td>14.48</td>
<td>2.20</td>
<td>12.72</td>
</tr>
</tbody>
</table>

<sup>a</sup>3X Noise = 0.00216 ABS

<sup>b</sup>Injected Minimum Detectable Quantity (μg) (0.2 mm pathlength)

abs.-liter/mm-mole
peak maximum. The decrease in IMDQ for 2-t-butyl-4-methyl phenol (K' = 1.01) over the earlier eluting (K' = 0.72) 2,4-di-t-butyl phenol should be noted and shows how the increased molar absorptivity and lower molecular weight of the former can offset the increased dilution as a result of its higher K'.

6.4.2 IMDQ determined from molar absorptivity for a given K'

Although the above treatment lets us determine the effect of changing K' on detectability, it is useful to determine detectability for a standard K' value for comparison of compounds with different molar absorptivities. We have chosen a K' = 1.0 for this purpose which represents a reasonably retained component. By knowing the molar absorptivity of a particular compound at a specific frequency along with the associated detector noise level, we can calculate the minimum weight of material which needs to be present in the flow cell for detection. Comparison of this value to the IMDQ value determined experimentally at its particular K' value allows us to determine the chromatographic dilution factor for that compound as it passes through the chromatographic column. To determine this dilution factor we employ Beer's law

\[ A = \varepsilon b C \]  

(18)

where A = sample absorbance, \( \varepsilon \) = molar absorptivity, b = pathlength and C = sample concentration. Plugging in the values tabulated in Table VII produces the concentration necessary in the cell to produce a signal for the -OH band that is 3x noise (i.e. A = 0.00216 absorbance). Knowing the molecular weight (206.33 amu) and the illuminated cell volume (1.41 \( \mu l \))
allows us to calculate the minimum weight of 2,6-di-t-butyl phenol needed in the flow cell (0.157 μg). Dividing the experimental IMDQ (Table VI) by this weight determines the chromatographic dilution factor at K' = 0.06 (6.71). From equation 11 we can extrapolate this factor to any desired K'. Using the dilution factor obtained for 2,6-di-t-butyl phenol (6.71 at K' = 0.06) and correcting it to a K' = 1, we obtain a dilution factor of 12.66. For additional compounds we now need in addition to the above, the compound's molecular weight and molar absorptivity (determined experimentally), as well as the detector noise at the given wavelength and spectroscopic parameters (also determined experimentally). With this we can again employ Beer's law to determine the minimum amount of sample needed to produce a signal 3X the noise. When multiplied by the previously determined chromatographic dilution factor for the given K' (in this case K' = 1.0) this will produce an IMDQ value without the need of performing any separations after the initial system characterization (see Appendix II for sample calculation). Tables VII-VIII list IMDQ values determined in this way for a variety of chemical classes for a K' = 1. Two bands were chosen to report IMDQ values, one band was characteristic of the compound class and a second band produced the lowest IMDQ value. For positive compound identification, normally an amount of sample between these two IMDQ values would be needed.

6.4.3 Improving IMDQ

Several ways are available to improve IMDQ for a given microbore LC-FTIR separation. Current employment of a 0.2 mm pathlength cell is
Table VII

IMDO \(^a\) for Selected Amines

<table>
<thead>
<tr>
<th>Compound</th>
<th>(\text{Abs. Max.} )</th>
<th>Assignment</th>
<th>Noise</th>
<th>(\epsilon)</th>
<th>IMDO ((\mu g))</th>
</tr>
</thead>
<tbody>
<tr>
<td>aniline</td>
<td>3454 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>1.74</td>
<td>12.31</td>
</tr>
<tr>
<td></td>
<td>1619 (\text{cm}^{-1})</td>
<td>(\text{NH}_2) deformation</td>
<td>.00029</td>
<td>19.26</td>
<td>0.38</td>
</tr>
<tr>
<td>1,2,3,4-THQ</td>
<td>3438 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00082</td>
<td>3.15</td>
<td>9.49</td>
</tr>
<tr>
<td></td>
<td>1499 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>20.31</td>
<td>0.56</td>
</tr>
<tr>
<td>N-phenyl-naphthylamine</td>
<td>3413 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00085</td>
<td>4.33</td>
<td>11.78</td>
</tr>
<tr>
<td></td>
<td>1498 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>51.08</td>
<td>0.36</td>
</tr>
<tr>
<td>indole</td>
<td>3482 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>26.07</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>1455 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>12.44</td>
<td>0.80</td>
</tr>
<tr>
<td>2-methyl indole</td>
<td>3474 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>23.50</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>1455 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>18.09</td>
<td>0.62</td>
</tr>
<tr>
<td>7-methyl indole</td>
<td>3474 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>22.93</td>
<td>1.32</td>
</tr>
<tr>
<td></td>
<td>1339 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00045</td>
<td>14.74</td>
<td>1.10</td>
</tr>
<tr>
<td>5-ethyl indole</td>
<td>3484 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>25.17</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td>2966 (\text{cm}^{-1})</td>
<td>Sym. methyl stretch</td>
<td>.00084</td>
<td>12.23</td>
<td>2.69</td>
</tr>
<tr>
<td>2,3-dimethyl indole</td>
<td>3475 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>23.33</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>1464 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>23.82</td>
<td>0.52</td>
</tr>
<tr>
<td>carbazole</td>
<td>3475 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>22.96</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>1238 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00033</td>
<td>36.35</td>
<td>0.42</td>
</tr>
<tr>
<td>1,2,3,4-tetrahydrocarbazole</td>
<td>3474 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>23.19</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>1467 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>19.88</td>
<td>0.73</td>
</tr>
<tr>
<td>(\alpha)-phenyl indole</td>
<td>3474 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>16.47</td>
<td>2.70</td>
</tr>
<tr>
<td></td>
<td>1298 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00029</td>
<td>24.04</td>
<td>0.64</td>
</tr>
<tr>
<td>2,3-diphenyl indole</td>
<td>3464 (\text{cm}^{-1})</td>
<td>(\text{N-H stretch})</td>
<td>.00084</td>
<td>22.04</td>
<td>2.81</td>
</tr>
<tr>
<td></td>
<td>1456 (\text{cm}^{-1})</td>
<td>Unknown</td>
<td>.00031</td>
<td>18.03</td>
<td>1.27</td>
</tr>
</tbody>
</table>

\(^a\)Assuming \(k'=1.0\); 18 coadded spectra; MCT-B detector, 3x noise

\(^b\)First listing=characteristic class absorbance band; second listing=absorbance band yielding lowest IMDO unless first listing is lowest.

\(^c\)Peak to Peak \(\pm 50\ \text{cm}^{-1}\) around absorbance maximum in absorbance units

\(^d\)Abs./liter/mm-mole
Table VIII

IMDQ for Selected Phenols

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abs. Max.</th>
<th>Assignment</th>
<th>Noise</th>
<th>ε</th>
<th>IMDQ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenol</td>
<td>3598 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>15.32</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td>1184 cm⁻¹</td>
<td>C-O stretch</td>
<td>0.00033</td>
<td>24.81</td>
<td>0.34</td>
</tr>
<tr>
<td>2-t-butyl-4-methyl phenol</td>
<td>3601 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>14.38</td>
<td>2.38</td>
</tr>
<tr>
<td></td>
<td>1179 cm⁻¹</td>
<td>C-O stretch</td>
<td>0.00033</td>
<td>22.55</td>
<td>0.66</td>
</tr>
<tr>
<td>β-naphthol</td>
<td>3596 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>16.98</td>
<td>1.77</td>
</tr>
<tr>
<td></td>
<td>1173 cm⁻¹</td>
<td>C-O stretch</td>
<td>0.00033</td>
<td>30.16</td>
<td>0.43</td>
</tr>
<tr>
<td>catechol</td>
<td>3601 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>16.83</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>1271 cm⁻¹</td>
<td>Unknown</td>
<td>0.00035</td>
<td>35.85</td>
<td>0.29</td>
</tr>
<tr>
<td>2,5-di-t-butylhydroquinone</td>
<td>3602 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>16.14</td>
<td>2.87</td>
</tr>
<tr>
<td></td>
<td>1157 cm⁻¹</td>
<td>C-O stretch</td>
<td>0.00033</td>
<td>26.91</td>
<td>0.75</td>
</tr>
<tr>
<td>9-hydroxy fluorene</td>
<td>3587 cm⁻¹</td>
<td>O-H stretch</td>
<td>0.00076</td>
<td>9.70</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>1190 cm⁻¹</td>
<td>C-O stretch</td>
<td>0.00033</td>
<td>14.26</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Assuming a k'=1.0; 18 coadded spectra; MCT-B detector, 3x noise

First listing=characteristic class absorbance band; second listing=absorbance band yielding lowest IMDQ unless where first listing is lowest.

Peak to Peak ±50 cm⁻¹ around absorbance maximum in absorbance units

Abs.-liter/mm-mole
due to the present cell design and volume considerations. Solvent transparency is reasonably good up to pathlengths of 1.0 mm for many solvents used in LC-FTIR. At 1.0 mm this could increase the signal for a given compound by a factor of 5 with some increase in detector noise due to throughput losses. Currently, we are working on cells which are of lower volume (~1 μL) yet longer pathlength (0.5 mm) for future improvement both in IMDQ and in the chromatography.

The noise level for a MCT detector is a function of wavelength. It is a combination of both detector response at a particular wavelength and solvent absorbance, both of which contribute to the optical throughput. High optical throughput reduces the corresponding noise level at a particular wavelength. Figure 61 illustrates this point visually. The top part of the figure is the Fourier transformed single beam IR spectrum from 4000-400 cm⁻¹ showing solvent absorbance superimposed on the detector response. This is an absolute measure of the amount of IR radiation reaching the detector. The lower part of the Figure is a plot of the reciprocal of the noise (averaged ±50 cm⁻¹ around data point) as a function of wavelength. It can be seen that lower IR throughput corresponds to higher noise levels and the two can be superimposed. This includes the strong solvent bands as well, indicating a solvent which strongly absorbs at a particular wavelength will increase the relative noise at that wavelength. The lower the noise can be made; the lower the IMDQ will become.

We have examined the use of a restricted wavelength (MCT-A) detector, normally employed with GC-FTIR, which has lower detector
Figure 61. Comparison of Optical Throughput vs. Wavelength to the Reciprocal of the Spectrum Noise vs. Wavelength.
noise. The cutoff on this detector is ~750 cm\(^{-1}\), below which many LC-FTIR compatible solvents are non-transparent, especially with longer pathlengths. The increase in sensitivity for the MCT-A is due to lower noise levels for this detector. Figure 62 shows IR spectra for the same concentration of 9-hydroxy fluorene taken with both the MCT-A and MCT-B detectors. As is expected, the absolute absorbance is independent of the detector. Note also the 750 cm\(^{-1}\) cutoff of the MCT-A detector.

Figure 63 is a direct comparison of the noise levels of the two detectors as a function of wavelength for carbon tetrachloride ratioed vs. itself. This is quantified in Table IX which compares the absolute noise value for the MCT-A and MCT-B detectors at selected wavelengths. It can be seen that the MCT-A detector should decrease IMDQ by about a factor of two. Additional work with this detector is planned and it is hoped that further optimization might improve this even more by reducing the noise even further. It should be noted also that the aperture employed with the MCT-A was less than 1/2 the size used for the MCT-B (see Table IX footnotes). This has implications for longer pathlength cells where the amount of through-put will be reduced, yet the aperture can be increased to offset this loss somewhat.

IMDQ's may also be decreased by tagging the materials of interest with an intense IR absorbing functional group such as a carbonyl. As can be seen from Table X, by comparing the IMDQ's for the phenols and their corresponding p-fluorobenzoyl ester derivative as well as for the amines and their p-fluorobenzoyl amide derivative, IMDQ's for the p-fluorobenzoyl derivatives have significantly lower IMDQ's. This does
Figure 62. Comparison of MCT-A and MCT-B Detectors for the Same Concentration of 9-hydroxyfluorene.
COMPARISON OF DETECTOR NOISE

SOLVENT CARBON TETRACHLORIDE (1.2MM)
NSS=6
NSB=24

MCT-A

MCT-B

Figure 63. Comparison of MCT-A and MCT-B Detectors for Spectral Noise Characteristics.
Table IX

DETECTOR NOISE $^{a,d}$ COMPARISON

<table>
<thead>
<tr>
<th>Wavelength</th>
<th>MCT-A $^{c}$</th>
<th>MCT-B $^{b}$</th>
<th>B/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>3600 cm$^{-1}$</td>
<td>0.0003548</td>
<td>0.0009989</td>
<td>2.82</td>
</tr>
<tr>
<td>3475 cm$^{-1}$</td>
<td>0.0004532</td>
<td>0.001138</td>
<td>2.51</td>
</tr>
<tr>
<td>2960 cm$^{-1}$</td>
<td>0.0005756</td>
<td>0.001189</td>
<td>2.07</td>
</tr>
<tr>
<td>1735 cm$^{-1}$</td>
<td>0.0001996</td>
<td>0.0003433</td>
<td>1.72</td>
</tr>
<tr>
<td>1630 cm$^{-1}$</td>
<td>0.0001939</td>
<td>0.0003880</td>
<td>2.00</td>
</tr>
<tr>
<td>1265 cm$^{-1}$</td>
<td>0.0001936</td>
<td>0.0003892</td>
<td>2.01</td>
</tr>
</tbody>
</table>

$^{a}$ Average of 25 sets of 12 coadded scans ratioed vs 24 scans of background

$^{b}$ Aperture = 0.250"

$^{c}$ Aperture = 0.091"

$^{d}$ ±50 cm$^{-1}$ peak to peak noise
Table X

IMDO for Ester and Amide Derivatives of Phenols and Amines

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Abs. Max.</th>
<th>Assignment</th>
<th>Noise</th>
<th>IMDQ</th>
<th>IMDQ†</th>
</tr>
</thead>
<tbody>
<tr>
<td>phenol</td>
<td>1737 cm⁻¹</td>
<td>C=O Stretch</td>
<td>.000028</td>
<td>58.09</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>1197 cm⁻¹</td>
<td>C-O-C Stretch</td>
<td>.000033</td>
<td>76.71</td>
<td>0.25</td>
</tr>
<tr>
<td>2-t-butyl-4-methyl phenol</td>
<td>1735 cm⁻¹</td>
<td>C=O Stretch</td>
<td>.000028</td>
<td>55.77</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>1265 cm⁻¹</td>
<td>C-O-C Stretch</td>
<td>.000035</td>
<td>87.72</td>
<td>0.31</td>
</tr>
<tr>
<td>α-naphthol</td>
<td>1737 cm⁻¹</td>
<td>C=O Stretch</td>
<td>.000028</td>
<td>55.01</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>1239 cm⁻¹</td>
<td>C-O-C Stretch</td>
<td>.000035</td>
<td>92.03</td>
<td>0.28</td>
</tr>
<tr>
<td>catechol</td>
<td>1746 cm⁻¹</td>
<td>C=O Stretch</td>
<td>.000028</td>
<td>55.95</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>1238 cm⁻¹</td>
<td>C-O-C Stretch</td>
<td>.000035</td>
<td>78.38</td>
<td>0.28</td>
</tr>
<tr>
<td>benzyl amine</td>
<td>1660 cm⁻¹</td>
<td>Amide I band</td>
<td>.000029</td>
<td>48.37</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>1496 cm⁻¹</td>
<td>Unknown</td>
<td>.000030</td>
<td>74.11</td>
<td>0.25</td>
</tr>
<tr>
<td>1,2,3,4-ThQ</td>
<td>1630 cm⁻¹</td>
<td>C=O Stretch</td>
<td>.000029</td>
<td>54.02</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>1493 cm⁻¹</td>
<td>Unknown</td>
<td>.000030</td>
<td>39.73</td>
<td>0.53</td>
</tr>
<tr>
<td>aniline</td>
<td>1675 cm⁻¹</td>
<td>Amide I band</td>
<td>.000029</td>
<td>42.80</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>1504 cm⁻¹</td>
<td>Unknown</td>
<td>.000030</td>
<td>94.99</td>
<td>0.19</td>
</tr>
</tbody>
</table>

aAssuming a k'=1.0; 18 coadded spectra; MCT-B detector, 3x noise
bFirst listing=characteristic class absorbance band; second listing=absorbance band yielding lowest IMDQ unless where first listing is lowest.

cPeak to Peak ≤50 cm⁻¹ around absorbance maximum in absorbance units
dAbs.-liter/mm-mole

*reported as weight of underivatized parent compound
not take into account the higher molecular weight of the derivatives. If we report the IMDQ's based upon the amount of phenol/amine present prior to derivatization, the IMDQ's as shown in the last column of Table X are obtained and are substantially lower. Not only does the derivatization improve detectability through increased molar absorptivities and lower detector noise (in the region of both maximum and characteristic absorbance), but the resulting derivatives, which are chromatographically stable, are much easier to chromatograph using normal phase chromatography with IR compatible solvents. The derivatives are also not as susceptible to adduction with polar solvents, which can produce broad IR bands, or to chromatographic tailing. This feature is especially attractive since amines are particularly difficult to chromatograph via normal phase chromatography.

6.5 Conclusions

By employing short microbore columns (50 cm) with a 0.2 mm pathlength cell and an MCT-B detector, LC-FTIR is capable of detecting and identifying the injection of sub-microgram quantities of a variety of compound classes. By increasing the pathlength of the flow cell and decreasing its volume to ~1 μL while employing the more sensitive MCT-A detector, good quality spectra should be obtained from several hundred nanograms of injected compounds. Derivatization can aid detectability and improve chromatographic performance for polar compounds such as amines and phenols. This technique should help LC-FTIR find more general utility in the analytical laboratory. Faster acquisition rates can be obtained by going to lower spectral resolution (i.e. 8 cm⁻¹ has
approximately twice the scan rate due to shorter mirror displacement) or by increasing the mirror velocity (up to ~5 times faster). This would allow additional spectral averaging, which would decrease the noise (as the $\sqrt{n}$), without sacrificing any time resolution. This is illustrated in Figure 64 where the effect on the noise level is shown for a Gram Schmidt reconstructed chromatogram of 2,6-di-t-butyl phenol with six scans per spectra vs. 2 scans per spectra. Alternatively, this could provide finer chromatographic detection by increasing the total number of spectra collected without increasing the noise level. The latter would cause a large consumption of disk space which for long chromatographic runs is limited with our current system to 400 4 cm$^{-1}$ or 800 8 cm$^{-1}$ stored spectra. Additional disk hardware with increased storage capacity can alleviate this problem.
Figure 64. Effect of the Number of Scans Averaged per Spectrum on the Spectral noise (Gram-Schmidt Reconstructed Chromatogram).
CHAPTER 7

CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

This thesis has attempted to outline the development of HPLC-FTIR as a viable analytical technique. Much progress has been realized in improving the technique during the course of this research. The current status of the technique with the use of microbore columns capable of delivering both reasonable detection limits and a high number of theoretical plates appear to have placed HPLC-FTIR one large step closer to more general utility as an analytical method. Continued advances in column technology coupled with improvements in flow cell design (longer pathlengths and lower dead volume) should continue this steady progress. It would not be surprising, given the commercial interest currently being shown for microbore columns, to see 5 μm and 3 μm packed columns in the next few years. This would offer extremely high efficiencies (on the order of one hundred thousand plates/meter) in more reasonable analysis time. While capacity would be lessened, detection limits would decrease similarly.

The state of the art in FTIR has also been improved and is expected to continue. The price-performance ratio continues to decline. This has allowed high performance FTIR's to be affordable by many additional labs. These instruments are somewhere between the expensive, high

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resolution (<1 cm⁻¹) instruments and the no frills dummy proof versions which sacrifice flexibility for lower price. Talk has centered around a specialized instrument to perform only the GC-FTIR experiment. The simplicity of the HPLC-FTIR interface makes it a natural application for one of the intermediate cost instruments. This alone might raise the number of users of HPLC-FTIR. The only thing lacking on most of the current moderately priced systems is a computer system capable of handling the large number of tasks along with a large and fast disk storage system. Both of these are becoming increasingly less expensive. The advent of the array-processor will prove to be a great advance for real time data acquisition and manipulation. Continued improvements in all aspects of the FTIR can be expected.

Finally, the application of HPLC-FTIR to extremely complex mixtures such as coal-derived products is perhaps the best test yet of its value as an analytical technique. Certainly, simpler separation problems exist where this technique, with an assist from a good IR data base, could routinely provide important information and sample identification. Complex mixtures, however, stretch any analysis technique to its limits and no one method will be all things to all samples. Certainly, HPLC-FTIR can offer complementary information to that obtained via other hyphenated techniques especially for the analysis of isomers. Its real value lies in the analysis of non-volatile samples which cannot be done by either GC-MS or GC-FTIR. While LC-MS would also provide a great deal of information, the development of a practicle system appears to still require additional work. Also, the instrumentation cost is lower for
LC-FTIR than for LC-MS.

HPLC-FTIR, in its various stages of development, has provided important and often unique information on components in typical coal-derived products. It is now advanced to the point where more specific information can be acquired to aid in the understanding of the complex chemistries involved in coal liquefaction. Hopefully, it will also find a use as an aid in the solution of other types of analyses.

7.2 Thoughts on Future Work

Many experiments can be envisioned for additional work with HPLC-FTIR. These include both fundamental studies and applications. This section will briefly explore some of these experiments which may yet be done.

The microbore separations have suffered somewhat from the relatively large cell volumes which were employed in the first generation flow cells. Design of cavity type flow cells should reduce the volume to acceptable limits while increasing the pathlength for improved detection. It is most probable that a beam condenser will be necessary to reduce (3x) the 3 mm circular spot sufficiently to allow enough radiation to pass through the smaller cell so that additional noise will not be acquired. Alternately, longer coupled columns could and should be explored to produce not only an increase in total plate count but also to reduce the dead volume constraints somewhat. The increased analysis time could be offset somewhat, through the use of 5 \( \mu \)m particle packings. The techniques for packing such columns, as well as 10 \( \mu \)m packings, needs to be developed in-house to make such
applications feasible. Once this is done, the development of new packings with novel bonded phases could be tried. It is possible that these could provide some of the selectivity lost through solvent restrictions. Packings based on a series of hindered phenols is one possibility. As has already been mentioned, the larger number of theoretical plates developed by longer columns would offset somewhat this selectivity loss. Also, the current acquisition of additional disk storage space will make separations, which take several hours, now feasible with FTIR detection. This will also facilitate the development of a dilute solution IR data base which would be very beneficial for the analysis of the large number of IR spectra currently produced. Spectral analysis remains the most time intensive step in the HPLC-FTIR experiment.

Reversed phase separations would be desirable with FTIR detection since a large portion of chromatographic separations are performed by this mode of chromatography. One possible method would be to use a higher surface coverage bonded phase such as Dupont markets. This has allowed limited use of non-aqueous solvent systems such as acetonitrile-methylene chloride for certain types of compounds such as triglycerides. For microbore separations, the possibility of using CD$_3$CN would increase the available transparent solvent regions. This would be useful for the analysis of non-polar components which are more strongly retained on a reversed phase column. Polar components would not be separated well by such a solvent system. Longer microbore columns could aid the separation through increased numbers of theoretical plates since for
such a strong solvent system, low K' values (<2) would be expected.

A more practical method might be to employ water-acetonitrile with microbore separations and remove the water via a post-column reaction. This might be accomplished via a small (5-10 cm) column packed with a dehydrating reagent such as sodium sulfate which could remove most of the water through acquisition of several waters of hydration. A switching valve could be used to isolate the post-column until just prior to the onset of the first chromatographic peak, thereby allowing the post-column to exhibit its maximum lifetime before needing to be replaced. Some loss of polar components might occur on the post-column but weakly and non-polar components should not be effected. An added advantage would be the decrease in volume as the component elutes from the chromatographic column, hopefully offsetting any band broadening due to the post-column. It might even be possible to do gradient elution via such a binary solvent system since hopefully the water content of the mobile phase would be kept constant by the post-column. However, whether practical flow-cell, gradient elution or reversed phase HPLC-FTIR will ever be performed can only be answered by future experimentation.

The use of long microbore columns packed with high efficiency size exclusion material might prove advantageous for use with FTIR detection. The big drawback to earlier use of SEC was its relatively low chromatographic resolution. Several hundred thousand theoretical plates for a size exclusion column might provide quite good separations for very polar components which are hard to separate via normal phase
separations. This would be especially useful for the solid SRC product which exhibits a wide molecular size range. Although the separation would be non-chemical specific, the FTIR detector would provide this information. Also, preliminary polarity separations could be performed to simplify the mixture as is now presently done. The limited total elution volume of size exclusion chromatography (SEC), as compared to normal phase chromatography, would cut down on analysis time. Also, isocratic elution is not a restriction with SEC since the mobile phase plays no part in the separation mechanism.

Finally, another possible area to explore is the coupling of Supercritical Fluid Chromatography (SFC) to FTIR. SFC employs mobile phases which are kept above their critical temperature and pressures, thus producing very dense "fluids". These highly compressed fluids have some very desirable properties. One advantage is the higher solute diffusion coefficients as compared to liquids which decreases analysis time. Also, supercritical fluids possess solvating properties similar to a liquid, which allows for the analysis of non-volatile components. Although it has found its most successful uses with capillary (~2 mm i.d.) columns it has also been employed with standard HPLC columns (4.6 mm i.d.) and should be equally applicable to microbore columns. One of the most common SFC mobile phases is carbon dioxide. This should be a very good solvent for use with FTIR detection. Other potential SCF mobile phases for use with IR detection include many of the Freons (Freon-14, Freon-113, Freon-114) as well as nitrous oxide. All of these should provide reasonably transparent regions in the IR. Also, pressure
programing, analogous to gradient elution in HPLC, is possible with SFC
and this causes solutes to become more soluble and elute faster. The
FTIR would hopefully not be sensitive to this pressure change, although
pressure broadening effects might be seen.
APPENDIX I

Prediction of IMDQ

IMDQ = injected quantity of material necessary to produce an absorbance equal to three times the noise (±50 cm⁻¹) level for a particular IR absorbance band.

For the 3600 cm⁻¹ characteristic -OH absorbance, 3 times the noise (12 scans) = 0.00216 Abs.

For 2,6-di-t-butyl phenol (M.W. = 206.33 amu; K' = 0.06) a 6.630 × 10⁻³ mole/liter solution produced an -OH absorbance = 0.0264 Abs. for a 3 mm x 0.2 mm cell (1.4137 µL). Thus, 9.3728 × 10⁻⁹ mole of 2,6-di-t-butyl phenol was present in the cell.

Given Beer's Law \[ A = εbC \]

\[ ε = \frac{0.0264}{(0.2)(9.3728 \times 10^{-9})} = 1.412 \times 10^7 \text{ Abs/mm-mole} \]

or

\[ ε = \frac{0.0264}{(0.2)(6.630 \times 10^{-3})} = 19.96 \text{ abs-liter/mm-mole} \]

Given 3x noise = 0.00216, we must calculate the concentration of 2,6-di-t-butyl phenol which gives this absorbance

\[ 0.00216 = (0.2)(1.412 \times 10^7)[X] \]

\[ [X]_{\text{min.}} = 7.648 \times 10^{-10} \text{ moles} \]

\[ [X]_{\text{min.}} = 0.1578 \mu \text{g of 2,6-di-t-butyl phenol} \]
This is the minimum detectable quantity in the cell. It is necessary to now determine the chromatographic dilution factor for IMDQ.

Experimentally IMDQ ($K'=0.06$) = 1.06 μg

Therefore, the dilution factor = 1.06/0.1578 = 6.71

Prediction of detection limit for 2,4-di-t-butyl phenol ($K'=0.72$; m.w.=206.33 amu)

From equation 11:

\[
\text{dilution factor } (K'_2) = \frac{(K'_2+1)}{(K'_1+1)} \times K'_1 \text{ (dilution factor)}
\]

\[
\text{dilution factor } (K'_2) = \frac{1.72}{1.06} = 10.69
\]

A $6.59 \times 10^{-3}$ mole/liter solution produces an $-\text{OH}$ absorbance = 0.01872 Abs.

\[
\epsilon = 1.0047 \times 10^7 \text{ Abs/mm-mole}
\]

or \[
\epsilon = 14.20 \text{ Abs-liter/mm-mole}
\]

\[
[X]_{\text{min.}} = 1.07495 \times 10^{-9} \text{ moles}
\]

\[
[X]_{\text{min.}} = 0.2218 \text{ μg}
\]

IMDQ$_{\text{calc.}} = [X]_{\text{min.}} \times \text{dilution factor } (K'_2) = 2.41 \mu g$

The experimental IMDQ = 2.37 μg

A similar treatment was done for 2-t-butyl-4-methyl phenol (m.w. 164.25; $K'=1.01$).
APPENDIX II

IMDQ DETERMINATIONS FOR CONSTANT K' (K' = 1.0)

From equation 11

\[
\text{dilution factor (K' = 1.0)} = \frac{(1+K'_2/1+K'_1)}{K'_1} \times K'_1 \quad \text{(dilution factor)}
\]

\[
\text{dilution factor (K' = 1.0)} = \frac{(2.00/1.06)}{6.71} = 12.66
\]

For a given compound and absorbance band (e.g. say indole and 3482 cm\(^{-1}\) -NH stretch).

Experimental values are:

- M.W. = 117.15 amu
- \(\varepsilon = 26.07\)
- 3x noise = 0.00252 Abs.

Therefore,

\[
[X]_{\text{min.}} = 6.8326 \times 10^{-10} \text{ moles}
\]

\[
[X]_{\text{min.}} = 0.080 \text{ mg}
\]

\[
\text{IMDQ}_{\text{calc.}} = 0.080 \text{ mg} \times 12.66 = 1.03 \text{ mg}
\]
APPENDIX III

Examples of Derivatization Applied to Coal-Derived Products

The derivatization reaction described in Chapter 6 has real potential for the analysis of coal-derived products, both by FTIR and HPLC-FTIR. This appendix deals with preliminary application of the p-fluorobenzoyl chloride derivatizing reagent to the analysis of both simple and complex coal liquefaction products.

In an effort to understand how process conditions affect the products produced during coal liquefaction, a small bench scale reactor system has been employed. Single runs employing different feed coals and model process solvents under various operating parameters have been performed. In the present example, Wyodak #3 coal has been employed with 1,2,3,4-Tetrahydroquinoline (THQ) as the process solvent. The volatile and non-volatile products from this run, designated P-24, have been examined via FTIR spectrometry.

The volatile portion of this run was diluted in CDCl₃ and its IR spectrum is shown in Figure 65. From GC analysis the majority of the product is 1,2,3,4-THQ as expected. However, quinoline and several substituted anilines have also been observed in significant amounts resulting from both dehydrogenation and breakdown of the 1,2,3,4-THQ. Weak absorbances at at ~3400 cm⁻¹ assignable to N-H stretching vibrations are observed which would be expected from the nitrogen rich process solvent. Additional small amounts of hydroxyl components appear to be incorporated into the process solvent from the coal as evidence by

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Figure 65. IR Spectrum of Process Solvent from Run P-24 in CDC13.
the weak -OH stretch at ~3600 cm\(^{-1}\). Figure 66 shows the IR spectrum of the derivatized volatiles. The much greater relative absorbance due to the amide group can be seen. A weak -NH absorbance due to secondary amides is observed as well as a weak -OH absorbance due to unreacted hydroxyl groups. Figure 67 is an expanded IR plot of the amide and ester regions. It can be seen that amide bands (1680-1620 cm\(^{-1}\)) dominate.

The situation changes when the non-volatile portion of the coal-derived product is examined. Figure 68 is the IR spectrum of the non-volatile product. This product was sparingly soluble in CDCl\(_3\). Major absorbances due to hydroxyl groups (3604 cm\(^{-1}\)) and water (3691 cm\(^{-1}\)) are most noticeable. The soluble portion appears also to have a reasonably high aliphatic content from the intensity of the -CH stretching region (~2900 cm\(^{-1}\)). The soluble portion, therefore, appears to consist of predominately aliphatic substituted phenols. The solubility of the non-volatile product is greatly enhanced upon derivatization. Now, essentially the entire sample is soluble in CDCl\(_3\). Figure 69 is the IR spectrum of the derivatized non-volatile product. Little -NH or -OH stretching modes are observed. Again, aliphatic substitution is present due to the -CH stretching absorbance around 2900 cm\(^{-1}\). Figure 70 is an expanded plot of the ester/amide region. Here one can see strong ester absorbances (1736 cm\(^{-1}\)) as well as amide absorbances (1695 cm\(^{-1}\) and 1674 cm\(^{-1}\)). This indicates that while derivatized phenolic material from the coal is present, it appears that some of the nitrogen from the process solvent may have been incorporated in the non-volatile product. This
Figure 66. IR Spectrum of Derivatized Process Solvent from Run P-24 in CDCl₃.
Figure 67. Expanded Plot of Figure 66 Showing Ester/Amide Region.
Figure 68. IR Spectrum of CDCl₃ Soluble Portion of the Non-Volatile Fraction of Run P-24.
Figure 69. IR Spectrum of the Derivatized Non-Volatile Fraction of Run P-24 in CDC<sub>3</sub>. 
Figure 70. Expanded Plot of Figure 69 Showing the Ester/Amide Region.
has implications both in maintaining solvent balance and for recycling
the non-volatile product for further reaction to produce more volatiles.
Most important is the enhanced solubility of the derivatives in
compatible IR solvents. Figures 71 and 72 show the pure derivative
spectra of 1,2,3,4-THQ and aniline while Figures 73 and 74 show spectra
of the derivatives of β-naphthol and 2-t-butyl-4-methy phenol for
reference.

Finally, the derivatization reaction was applied to a complex
recycle solvent (92-03-035) previously described but with a 600-800°F
boiling range. This sample is typical of a real pilot scale product.
After derivatization, a modification of the preparative separation
(Figure 21) performed in Chapter 4 was applied to simplify the product.
Solvents employed were heptane/chloroform/methanol to elute the three
fractions. IR spectra of all samples were run in deuterochloroform.
Figure 75 is the IR spectrum of the heptane fraction. This fraction is
dominated by a single ester absorbance at 1735 cm⁻¹. This appears to be
due to derivatized simple phenolic material. Small amounts of indole
and diphenylamine type compounds are indicated by the expanded -NH
absorbance region.

The chloroform fraction (Figure 76) contains a mixture of esters
and amides. While the exact nature is unknown, the -NH stretching could
be due to either non-basic -NH absorbances (i.e. "indoles" and "diphenyl
amine" compound types) or secondary amides. An underivatized hydroxyl
absorbance (3604 cm⁻¹) is apparent with traces of water (3689 cm⁻¹) also
present. The underivatized hydroxyl groups can either be due to
Figure 71. Reference Spectrum of 1,2,3,4 THQ Derivative.
Figure 72. Reference Spectrum of Aniline Derivative.
Figure 73. Reference Spectrum of β-Napthol Derivative.
Figure 74. Reference Spectrum of 4-methyl-2-t-butyl Phenol Derivative.
Figure 75. IR Spectrum of the Heptane Fraction From the Preparative Separation of Derivatized 92-03-035 Process Solvent in CDCl₃.
Figure 76. IR Spectrum of the Chloroform Fraction From the Preparative Separation of Derivatized 92-03-035 Process Solvent in CDC$_3$. 
hydrolysis during the separation or non-reaction of these groups, possibly due to steric hindrance.

Finally, Figure 77 is the IR spectrum of the methanol fraction. Again, similar assignments can be made with varying amounts of ester and amides. Two -NH stretching bands at 3472 cm\(^{-1}\) and 3435 cm\(^{-1}\) are apparent, most probably due to secondary amides. Water (3690 cm\(^{-1}\)) is again apparent along with unreacted hydroxyl bands. The 3632 cm\(^{-1}\) band is due to a hindered hydroxyl (as was seen in 2,6-di-t-butyl phenol) which may account for its non-reaction. Clearly, HPLC-FTIR on both of these samples would aid analysis greatly.
Figure 77. IR Spectrum of the Methanol Fraction From the Preparative Separation of Derivatized 92-03-035 Process Solvent in CDC$_3$. 
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VITA

Robert Scott Brown was born on June 22, 1956 in Medford, Massachusetts. He grew up in Melrose, Massachusetts where he graduated in June 1974 from Melrose High School.

The following September he began attending Lowell Technological Institute, which in 1975 merged with Lowell State College to form the University of Lowell. In June, 1978, he was awarded the Bachelor of Science degree (Cum Laude) in Chemistry by the University of Lowell.

In September of 1978 he began his graduate studies toward the Doctor of Philosophy degree in the chemistry department of Virginia Polytechnic Institute and State University (Blacksburg, VA) under the direction of Dr. Larry T. Taylor. He has held both a Department of Health Education and Welfare Domestic Mining and Mineral Fuel Conservation Fellowship (1978-1980) and a research assistantship (1980-1983) while at VPI&SU. In April, 1983 he completed the requirements for the Doctor of Philosophy Degree. In May, 1983, he will assume a postdoctoral position at the University of California, Riverside under the direction of Dr. Charles L. Wilkins.

Robert S. Brown

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PUBLICATIONS


"Fate and Nature of Trace Elements During the Fractionation of Solvent Refined Coals", J. W. Hellgeth, R. S. Brown and L. T. Taylor, (Submitted to Fuel)

"Investigation of Microbore Liquid Chromatography with Flow Cell Fourier Transform Infrared Spectrometry Detection", R. S. Brown and L. T. Taylor (Accepted to Analytical Chemistry)

"Detection Limits for Microbore Liquid Chromatography Coupled with Flow Cell Fourier Transform Infrared Spectrometry Detection" (Submitted, Analytical Chemistry).
PRESENTATIONS AT SCIENTIFIC MEETINGS


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