Fourier Transform Infrared Spectroscopy of Chemical Systems

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Infrared spectroscopy is recognized as one of our most powerful physical tools for investigating chemical systems. The introduction of interferometric techniques lead to the development of Fourier transform infrared spectroscopy (FT-IR). The impact of these improved instruments has been dramatic and has penetrated nearly every aspect of chemical research from analyzing the environment on other planets to cells in the blood stream. It behooves chemists, therefore, to develop some insight into the nature of this evolving technique in order to appreciate the large amount of activity in this area. This Account describes the technique of FT-IF and selected chemical applications.

The heart of an FT-IR spectrometer is a Michelson interferometer, diagrammed in Figure 1.[1,2] One arm of the interferometer contains a stationary, plane mirror; the other arm contains a movable mirror. When the light beam enters the interferometer, it is split into two equal beams by the beam splitter. These two beams travel down their respective arms of the interferometer and are reflected back to the beam splitter and to the detector. The two reunited beams will interfere constructively or destructively, depending on the relationship between their path difference (x) and the wavelengths of the light. When the movable mirror and the stationary mirror are positioned the same distance from the beam splitter in their respective arms of the interferometer ($x = 0$), the paths of the light beams are identical. Under these conditions all wavelengths of the radiation striking the beam splitter after reflection add coherently to produce a maximum flux at the detector and generate what is known as the "center" burst. As the movable mirror is displaced from this point, the path length in that arm of the interferometer is changed. This difference in path length causes each wavelength of source radiation to destructively interfere with itself at the beam splitter. The resulting flux at the detector, which is the sum of the fluxes for each of the individual wavelengths, rapidly decrease with mirror displacement. By sampling the flux at the detector, one obtains an interferogram, as shown in Figure 2.

Each point in the interferogram, $I(n)$, is given by eq 1, where $b(\nu_k)$ is the intensity of each wavelength and

$$I(x) = \sum_{k=1}^{j} B(\nu_k) \cos (2\pi\nu_k x) \qquad (1)$$

ν_k is the wave number (expressed in cm^{-1}) for each wavelength. Each data point on the interferogram is the sum of individual frequencies. Hence, the frequency

domain data of interest to the spectroscopist must be recovered from the interferogram. In the general case, eq 1 can be written in integral form:

$$I(x) = \int_{-\infty}^{+\infty} B(\nu_k) \cos (2\pi\nu_k x) \, d\nu \qquad (2)$$

This equation is one-half of a cosine Fourier transform pair.

$$B(\nu_k) = \int_{-\infty}^{+\infty} I(x) \cos(2\pi x\nu) \, dx \qquad (3)$$

Equations 2 and 3 define the relationship between the interferogram and the spectrum. The transform from the interferogram to the spectrum is carried out on the dedicated minicomputer on the instrument.

Fourier Transform interferometric techniques have three principal advantages. The Fellget or multiplex advantage arises from the fact that the FT-IR spectrometer examines the entire spectrum in the same period of time required for a dispersive instrument to examine a single spectral element. Theoretically, an FT-IR can acquire the spectrum from 0 to 4000 cm^{-1} with 1-cm^{-1} resolution 4000 times faster than a dispersive instrument with the same signal-to-noise ratio. Or from another point of view, for the same measurement time a factor of approximately 63 increase in signal-to-noise can be achieved on the FT-IR instrument.

Jacquinot's or the throughput advantage arises from the loss of energy in the dispersive system due to the gratings and slits. These losses do not occur in an FT-IR which does not contain these elements. The throughput for an FT-IR instrument is limited by the size of the mirrors. This higher throughput is particularly important in the infrared region where the signals are extremely weak since the infrared sources are weak.

The Conne or frequency accuracy advantage comes from the fact that the frequencies of an FT-IR instrument are internally calibrated by a laser whereas conventional IR instruments exhibit drifts when changes in allignment occur. This latter advantage is particularly useful for coaddition of spectra to signal average since the frequency accuracy is an absolute requirement in this case. Another advantage of FT-IR resides in the speed of scanning a spectrum. For a dispersive instrument routine scans take several minutes, whereas an interferometer can be scanned many times per minute.[3]

The limitation of any spectroscopic device ultimately is determined by the signal-to-noise ratio of the spectra

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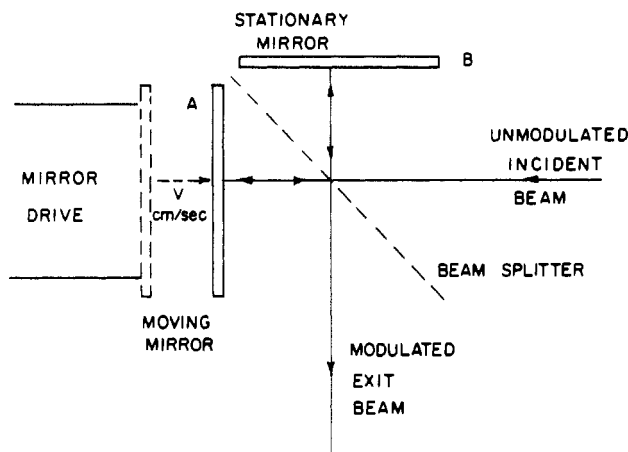
(1) R. J. Bell, "Introductory Fourier Transform Spectroscopy", Academic Press, New York, 1972.
(2) P. R. Griffiths, "Chemical Infrared Fourier Transform Spectroscopy", Wiley-Interscience, New York, 1975.
(3) P. R. Griffiths, H. J. Sloane, and R. W. Hannah, *Appl. Spectrosc.*, 31, 485 (1977).

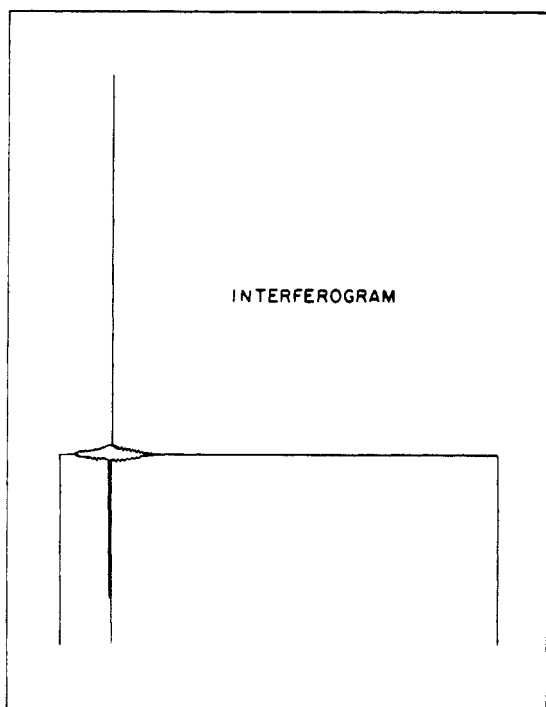Figure 1. The Michelson interferometer.

Figure 2. An interferogram.

obtained. For FT-IR, signal-to-noise ratios of 60 000:1 have been recorded.[4] However, in FT-IR, practical problems arise in the determination of the 100% line, in the interferometer stability, and in the data digitization process, so these especially high S/N ratios are not generally available. A maximum S/N of 500 can be obtained from a single scan.

Application of FT-IR to Detection of Trace Gaseous Constituents

One of the most obvious applications of FT-IR is the improved analysis of trace constituents in air samples. Most small molecules can be detected at concentrations down to $\simeq 100$ ppb in 5-L samples.[5] Complex mixtures can be handled and trace components quantitated in the presence of large amounts of interfering substances. In fact, with proper programming and calibration, one can carry out these analysis completely automatically and the samples can be handled at a rate of one every
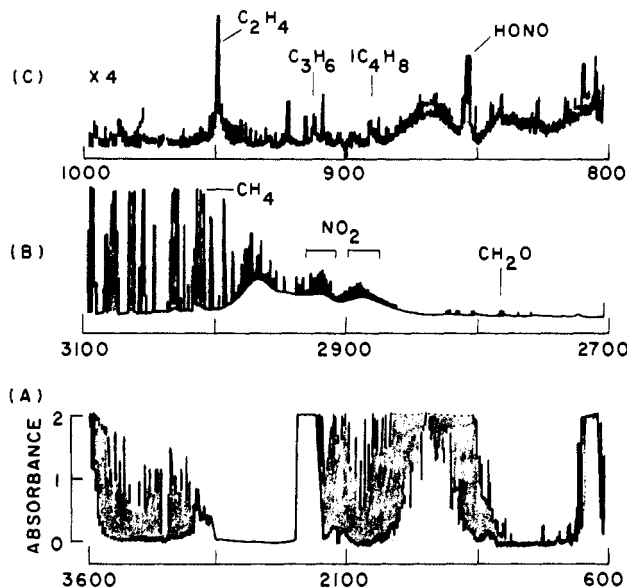
(4) C. Foskett and T. Hirschfeld, *Appl. Spectrosc.*, **31**, 239 (1977).
(5) P. D. Maker, H. Niki, C. M. Savage, and L. P. Breitenbach, *ACS Symp. Ser.*, No. 94, 170 (1979).


Figure 3. Absorbance spectrum of typical cold–hot cycle, constant volume sampled auto exhaust, at various scale expansions with prominent bands identified (taken from Figure 6 of ref 5).

Table I
Computer Report of the Quantitative Analysis of the Spectrum of Figure 3

compound	amount	error[a]	% THC
H_2O	1.51	0.1%	0.000
CO_2	1.35	0.1%	0.000
CO	781.29	10.0 ppm	0.000
$HC(c)$	112.00	8.0 ppm C^d	0.693
NO	15.49	0.3 ppm	0.000
NO_2	47.25	0.3 ppm	0.000
N_2O	0.81	0.1 ppm	0.000
$HONO$	4.90	0.1 ppm	0.000
HCN	-0.12	0.2 ppm	0.000
NH_3	0.03	0.1 ppm	0.000
SO_2	0.19	0.2 ppm	0.000
CH_4	11.44	0.1 ppm C	0.071
C_2H_2	3.06	0.2 ppm C	0.019
C_2H_4	13.42	0.5 ppm C	0.083
C_2H_6	1.36	0.2 ppm C	0.008
C_3H_6	10.76	1.0 ppm C	0.067
IC_4H_8	6.08	1.0 ppm C	0.038
CH_2O	2.27	0.1 ppm C	0.014
$HCOOH$	1.00	0.1 ppm C	0.006
CH_3OH	0.28	0.1 ppm C	0.002

total HC = 161.66 ppm C = 53.89 ppm C_3
total NO_x = 67.65 ppm

[a] Estimated error of measurement. [b] Percent of total hydrocarbon. [c] Heavy hydrocarbon, representing the area under the unresolved CH stretching band, normalizing to raw fuel (indolene clear). [d] ppm C = ppm ÷ molecular carbon number; taken as 8 for HC.

10 min. An interesting example of such analysis is that of a typical auto exhaust.[5] Figure 3 shows the complete absorbance spectrum (A) which is dominated by water and CO_2 absorptions, but one can see the CO band and absorption due to hydrocarbons. With scale expansion (spectrum B), absorption due to CH_4, NO_2, formaldehyde, and other heavy hydrocarbons are clearly seen. With further scale expansion (spectrum C), ethylene, propylene, isobutylene, and nitrous acid become visible. The Ford researchers have developed a completely automatic software program to analyse spectra of this type. Table I gives the computer printout of such an analysis for the spectra given in Figure 3 using a 40-m path cell length. Analysis of this
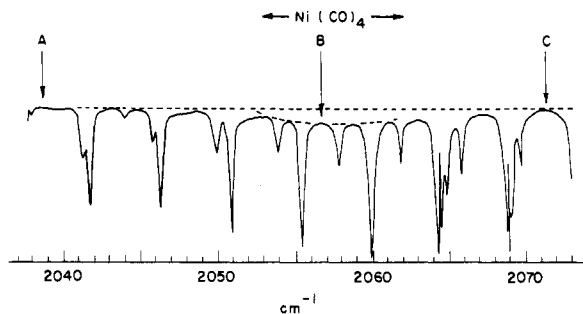
Figure 4. FT-IR absorption spectrum of 75 ppb of Ni(CO)₄ in 750 ppm of CO (path length 42 m, resolution 0.065 cm⁻¹) (taken from Figure 2 of ref 7).

Table II
Calculated Detection Limits with 1-km FT-IR System

compound	measurement frequency, cm⁻¹	detection limit for present system, ppb
NH₃	931	2
	967.5	2
HCHO	2779 ⎱	
	2781.5 ⎰	4
HCOOH	1105.3	4
HNO₃	896	4
HONO	853 (cis)	4
N₂O₅	740	2
	1248	2
O₃	1055	10
PAN	793	4
	1162	3
CH₄	2970	23

kind can be developed for a variety of different applications, including furnace atmosphere analyses, stack emission checks, and health-related ambient atmosphere measurements.[5]

One of the more demanding problems is the monitoring of ambient air for toxic species. An example is nickel carbonyl which is an intermediate in the synthesis of acrylate monomers. Evidence exists that it is a source of cancer in rats, and hence a limit of 1 ppb has been set by OSHA for ambient plant air.[6] This is the lowest environmental air standard established to date. When FT-IR with 0.1-cm⁻¹ resolution and a path length of 42 m is used, the spectra of nickel carbonyl in carbon monoxide at 100 000 times concentration was obtained in the region of 2058 cm⁻¹.[7] The spectrum is shown in Figure 4. The antisymmetric C=O mode of Ni(CO)₄ is a broad band centered at 2058 cm⁻¹. The sharp bands are the individual vibrational–rotational lines of CO. A calibration curve was established by using concentrations as low as 1.1 ppb. The practical limits of detection are about 0.2–0.3 ppb, and the precision is about ±0.2 ppb and the accuracy ±0.5 ppb at the 1-ppb level in actually monitoring the environmental air of a process control room. Using FT-IR instrumentation it is possible to automatically and continuously monitor nickel carbonyl concentrations in the sub-1-ppb range.[7]

In order to benefit from the increased sensitivity of FT-IR for gas analysis, multiple reflection cells have been constructed with path lengths in excess of 1 km.[8]
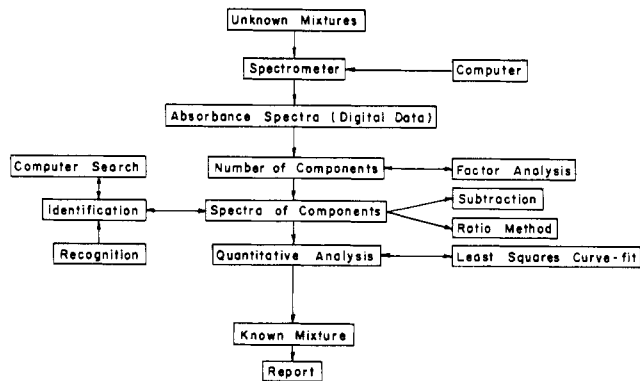

Figure 5. Logic diagram for the analysis of mixtures.

When such a cell is used, detection limits of 2–4 ppb were established for many species in smog, including HCHO, HCOOH, HNO₃, HONO, NH₃, and PAN and other peroxyacyl nitrates.[8] In Table II are given the calculated detection limits using the 1-km cell FT-IR system.

Data Processing Techniques for Quantitative Analysis of Liquid and Solid Mixtures

Quantitative analysis of mixtures by infrared spectroscopy can be considerably improved by using modern interferometric techniques. The improvement arises for two different reasons. First, the signal-to-noise ratios for the Fourier transform spectra are considerably enhanced; second, the digitized nature of the spectra allow the introduction of computerized data processing techniques. New data processing methods are utilized for the quantitative analysis of mixtures. The logic pattern to be followed in developing the spectral methods is shown in Figure 5. The FT-IR spectrum of the mixture is obtained and recorded as a digitized absorbance spectrum. The first question is the number of spectroscopically distinguishable components in the mixture. A method termed factor analysis answers this question.[9] The next logical step is the determination of the spectra of each of the components. If only two components are present, absorbance subtraction is simplest while the absorbance ratio method is more suitable for more complex mixtures.[10,11] An analysis of the spectra of the components yields identification and structural information. A quantitative analysis of the mixture requires an evaluation of the spectra of the mixture in terms of the spectra of the components.[12] Classically, only a single analytic frequency is used for quantitative measurement, but with digitized spectra and a computer, the entire frequency spectrum of each component can be fit to the mixture by curve-fitting techniques using various methods including least-squares refinements which can yield an indication of the precision and the goodness of fit.[13] In this fashion, quantitative analysis of mixtures can be carried out.

An absorbance ratio method has been applied to mixtures of simple organic molecules and also to poly-

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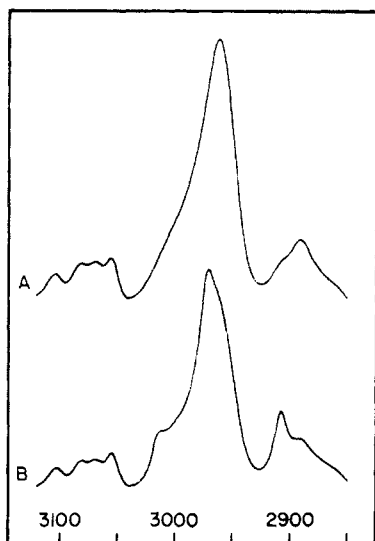
Figure 6. PET C-H stretching region: (A) unannealed film; (B) film annealed at 160 °C for 2 h (taken from ref 14).

mers[10,11] to determine the component spectra without separation or isolation. The method can also be applied to determine the relative concentrations of the components without external calibration.[12]

Once the component spectra of a mixture have been determined and properly scaled, perhaps via the ratio method, other mixtures composed of identical components can be analyzed by using the method of least-squares curve-fitting. A program to do so has been developed in our laboratory.[13] The fitting equation employed is shown in eq 4. N is the number of the

$$\sum_{i=1}^{N} R_{i,k} = \sum_{j=1}^{M} (\sum_{i=1}^{N} W_i R_{i,j} R_{i,k}) X_j \qquad (4)$$

$$S_i = \sum_{j=1}^{M} X_j R_{i,j} \qquad (5)$$

spectral elements (data points) in each spectrum and M the number of basis spectra used in the fitting procedure. $R_{i,k}$ represents the absorbance data for the ith spectral element of the kth basis spectrum. S_i is the data for the spectral range of the mixture spectrum. W_i is a weighting factor equal to $1/X_i$ (statistical weighting). S_i is the number which when multiplied by the appropriate basis spectrum and summed for all components best fits the experimental mixture spectrum. When the calibrated component spectra are used, it is from the computer-determined X_j values that volume fractions of the components in the mixture can be obtained.[14]

The above techniques have been used to study the crystalline and amorphous phases of poly(ethylene terephthalate) (PET).[15] Figure 6 illustrates the effect of annealing an amorphous PET film in the carbon–hydrogen stretching region. The spectra shown include the amorphous spectrum and the spectrum of the same sample after annealing at 160 °C for 2 h. A shoulder band at 2909 cm^{-1} becomes much more pronounced, as does a band at 3015 cm^{-1}. Increases in the amount of the trans conformer were suggested due to either
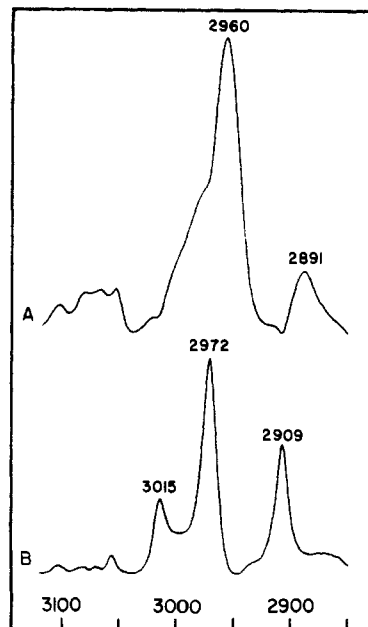

Figure 7. PET: (A) amorphous gauche C–H stretch: (B) crystalline and amorphous trans C–H stretch.

changes in the amorphous state and/or crystallization.

Factor analysis was used on a set of eight spectra taken of annealed PET that covers the range of amorphous to annealed at 200 °C for 3 h. This led to the assignment of two components, the trans and gauche rotational isomers of the PET repeat. Since crystallization under the annealing conditions used was observed, the trans component must be present in both the amorphous and crystalline domains.

With the number of components determined, the ratio method was applied to the binary mixture of conformational isomers in PET. We sought information regarding the spectra of the two components as well as a measure of the amounts of each component in the samples investigated. For the C–H stretching region, an absorbance ratio plot of an annealed film divided by an unannealed amorphous film was obtained. The ratio coefficients R_{p_1} and R_{p_2} are determined to be 2.170 and 0.626, respectively. When this information in used and the ratio technique is applied, the concentration of the trans isomer for the unannealed sample was found to be 47.4%, the remainder being gauche. The film annealed for 2 h at 160 °C was found to be 75.8% trans with 24.2% gauche. Figure 7 details the deconvoluted and internally scaled pure gauche and trans spectra. The trans spectrum shows characteristic bands at 2909, 2972, and 3015 cm^{-1} as well as C–H ring stretching modes at higher frequencies. These glycol trans and gauche conformational spectra were isolated and their volume fraction was determined.

An obvious application of these improved quantitative results is in the area of quality control. An application has been reported[16] which use the least-squares refinement method for the quality control of high-strength composite materials where control of the concentration of the initial reactants and the resin is essential to reproducible manufacture of fiber-reinforced laminates. The results obtained for the initial

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uncured components are excellent when the spectra of the pure components are used to refine the mixing spectra. However, when the epoxy matrix was cured, the results, using the spectra of the initial ractants, were unsatisfactory due to the change in the spectra arising from the reaction products.[16] When the difference spectra, that is, the spectra obtained when the spectra after curing was subtracted from the spectra of the initial reactants, was used as one of the components, good agreement was found as well as a quantitative measurement of the degree of cure.[17]

FT-IR Absorbance Subtraction Spectroscopy

Since the first reported FT-IR digitally subtracted spectra, that of 1,4-*trans*-poly(chloroprene),[18] and the subsequent demonstration of its utility to a wide variety of spectroscopic problems,[19] this area has grown at an enormous rate as spectroscopists recognized the ease of using digital subtraction for isolation of spectra and the determination of small spectroscopic differences in similar samples. Commercial instruments now have an autosubtract command. Unfortunately, almost as many artifacts have been generated by using subtraction techniques as facts due to its careless or inappropriate application. Since the subtraction technique allows computer-scale expansion of the difference spectra, the resultant high sensitivity to real spectroscopic differences also applies to the detection of sampling and photometric artifacts as well. A number of papers have been written outlining areas where problems can arise[20,21] and these papers should be consulted to assure that proper consideration is given to these factors. The most common problem arises from attempts to do digital absorbance subtraction when the absorbances of the samples are too high. In these cases, all of the photometric problems occur along with the numerous sampling problems. Spectra should never undergo digital subtraction when the absorbances are greater than 2, and in most cases an upper limit of 1.0 should be observed. However, absorbance subtraction is a highly useful method, and most of the applications cited later involve this technique.

FT-IR Emission Spectroscopy

In principle, the emission spectra yields the same information as the absorption spectra but has the advantage of allowing the study of those systems not amenable to transmission spectra, i.e., remote species,[22] surface species,[23] and those samples which are sufficiently corrosive to be difficult or impossible to handle by the usual IR sampling techniques.[24] Measurements have been made of planet surfaces, gypsum ponds, jet engines, oil refineries, flames, power plant emissions, and flares.[22] Additionally, the spectra obtained from IR chemiluminescence have been obtained with FT-IR
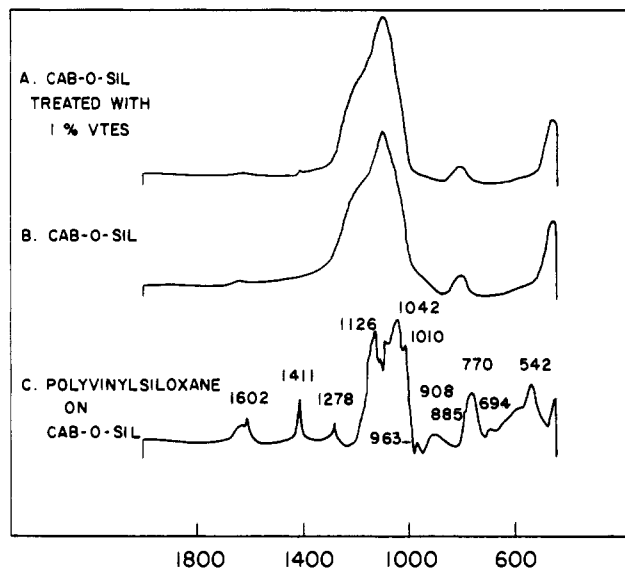
Figure 8. FT-IR spectra of high surface area fumed silica (Cab-O-Sil, 390 m²/g): (A) Cab-O-Sil treated with 1% by weight of vinyltriethoxysilane (VETS) and dried at room temperature; (b) Cab-O-Sil mixed with dilute acid and dried in the same manner as A; (c) difference spectrum (A minus B) of poly(vinylsiloxane) on Cab-O-Sil.

in the emission mode.[25] Emission spectra of surface species have also been obtained.[23]

Measurements have been made of the gaseous fluorides from a phosphate fertilizer plant gypsum pond.[22] The HF concentration can be determined for the path-averaged path length of 860 m and was found to be 45 ppb. When the 1031.5-cm⁻¹ band of SiF₄ was used, the concentration of SiF₄ was found to be 0.5 ppb.

Although the high acoustical noise limited the operation of the interferometer, the emission spectra of jet engines operated at idle, military, and afterburner power have been obtained. The amount of acetylene in the jet engine exhaust at the idle (10 m path) was found to be 1.6 ppm; for ethylene, 3.2 ppm; and for carbon monoxide, 28 ppm.[22]

FT-IR Applications in Surface Science

The importance of the study of surface structure on the molecular level has long been recognized, but advanced spectroscopic techniques have only appeared in the last decade. It is feasible to analyze, using FT-IR within a reasonable period of time, the spectrum of the monomolecular solid/gas interface by examining the solid, the solid in contact with a gas, and the gas all at the same pressure and temperature. When the appropriate absorbance subtraction is done, the spectrum of the interface should be obtained. One particular advantage of this method is that the same solid sample (not a dummy solid) is used and that the measurements are carried out under appropriate experimental conditions of temperature and pressure. An in situ IR cell can be constructed which fits in the FT–IR spectrometer and the measurements can be made at different temperatures and pressures systematically on the catalyst without repositioning or touching the system. Measurements can be made with coaddition of the spectra to increase the signal-to-noise ratio. The

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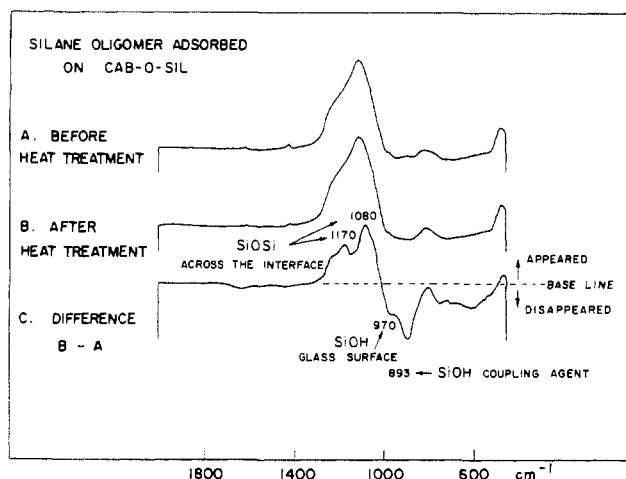
Figure 9. FT-IR spectrum of Cab-O-Sil treated with a silane oligomer: (a) Cab-O-sil mixed with precipitated poly(vinyl-siloxane)/methanol solution with subsequent drying; (b) sample A heat treated at 150 °C for 30 min; (c) difference spectrum showing the change before and after heat treatment.

spectrum of the product resulting from the interaction of CO with Ni/SiO$_2$ at 75 °C was obtained by subtracting the spectra of the initial gas phase at 75 °C and the catalyst at 20 °C.[26]

Another example of surface studies concerns coupling agents on glass surfaces. These coupling agents are used to enhance the adhesion between the fiber and the polymer matrix in the fabrication of glass-reinforced composites. These systems have received a considerable amount of study in order to establish the basis of the reinforcement mechanism. A high surface area fumed silica (390 m^2/g) was modified using a 1% by weight vinyltriethoxysilane aqueous solution.[27] In Figure 8, the spectrum is shown of the modified silica and the spectrum of the control. The absorption of the coupling agent is barely observable as a small peak at 1411 cm^{-1}. When spectral subtraction is used, the difference spectrum reveals the spectrum of the coupling agent, including the changes arising from the silica surface. The difference spectrum shows poly(vinylsiloxane), indicating that the coupling agent has polymerized on the surface.

An important question is whether the coupling agent has chemically bonded to the glass surface or whether it is only physically absorbed. Figure 9 shows the spectra of silica with a coupling agent before and after heat treatment at 150 °C for 30 min. Since the amount of coupling agent does not change before and after the heat treatment, no obvious differences are seen between the original spectrum and the final. However, the difference spectrum clearly demonstrates the condensation reaction of the SiOH groups of the silica surface (970 cm^{-1}) and the coupling agent (893 cm^{-1}) as illustrated by the negative absorbances of these bands. The positive absorbances at 1170 and 1080 cm^{-1} frequencies arise from Si–O–Si bonds and have been interpreted as associated with the interfacial bond formed between the coupling agent and the silica surface.[27] For this example, the amount of material on the surface is at the monolayer coverage and the quality of the difference

spectrum indicates the sensitivity of the FT-IR spectrometer.

FT-IR Applications to Polymers

Fourier transform infrared spectroscopy is having a major impact on the study of polymer systems. The advantages of FT-IR arise primarily from the higher signal-to-noise ratio obtainable from the higher energy throughput of the system and the capability of signal averaging of the spectra to achieve a further increase in the signal-to-noise ratio. Additionally, the full benefit of the higher signal-to-noise ratio can be realized through the use of the data-processing potential arising from the availability of the minicomputer.

The experimental difficulties arising from the study of polymer spectra result from the fact that the systems are all complex mixtures of components and structures. Therefore the observed bands are broad and weak for polymers. The structure elucidation is considerably improved by using absorbance subtraction to remove interfering absorbances and absorbance magnification to enhance the observed signals. Curve fitting techniques allow the observation of very small differences in absorbance and the removal of spectral features which do not contribute to the problem at hand. Several reviews have been written on the application of FT-IR to polymers.[19,28,29]

FT-IR is a potential tool for the investigation of the mutual compatibility of the various components, including additives. The very small spectral effects which are introduced as a result of the interactions required for the polymers to be compatible can be detected by using FT-IR. As a result, FT-IR has been used to investigate the interactions of additives such as antioxidants[30] as well as for blends of different polymers in a search for the structural basis of compatibility.[31,32]

FT-IR Application to Biological Systems

Infrared spectroscopy has been limited in its application to biological systems for two reasons: (1) water, omnipresent in biological systems, is an intense infrared absorber and masks or overlaps most bands arising from the compounds of interest; (2) the concentrations of the biological species are usually low further complicating the spectroscopic problems. Nonetheless, the spectroscopic results yield detailed information at the molecular level concerning the internal groups in a native macromolecular structure including the local environment of the group and the manner of its changes with biological interactions. Therefore, the motivation has been high to apply FT-IR to biological systems, and the results have been rewarding. Of course, the first requirement is the subtraction of the spectrum of water in order to examine the vibrational features. This is accomplished by using thin calcium fluoride or barium fluoride windows with pathlengths of 2.5–15 μm in order to keep the absorbance of one or less in the regions of

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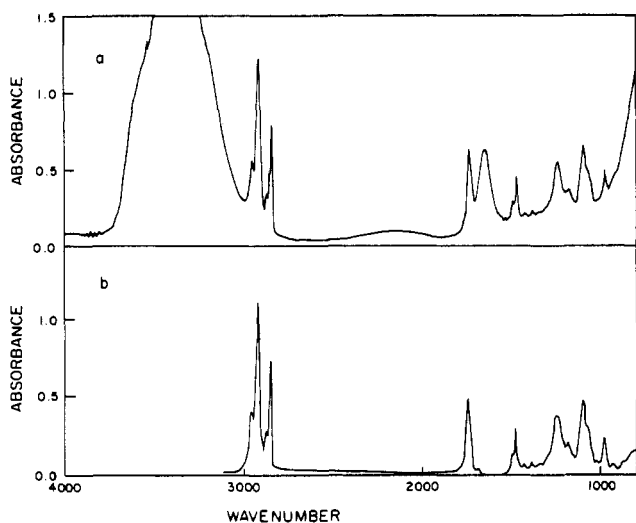(29) M. M. Coleman and P. C. Painter, *Rev. Macromol. Sci.*, C16, 197 (1977).
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Figure 10. FT-IR absorbance spectra of phospholipid multilayers at 33 °C in the presence of excess water (a) and after water subtraction (b), using a water spectrum recorded under identical conditions. Reprinted with permission from ref 33. Copyright 1979, Elsevier/North-Holland.

Figure 11. FT-IR difference spectrum of a saline-subtracted protein layer absorbed onto a germanium crystal from a flowing solution of blood plasma. Reprinted with permission from ref 35. Copyright 1979, John Wiley.

interest. It has been found advisable to carry out the solvent subtraction, by using the same cell thickness rather than attempt to use the scaling factors of the computer to change a spectrum obtained at one thickness to another. This arises from the fact that anomalous dispersion is a function of the refractive indices of the cell windows and of the cell contents but is independent of the path length while the absorption is a function of the path length. The peak distortion is thus a function of the path length and may be as high as 20% for very short path lengths. Figure 10 shows the FT-IR spectra of palmitoyl phospholipid multibilayer membranes before and after subtraction of water under identical conditions.[33] The isolation of the membrane spectrum is excellent.

In biological systems, the structural features of the absorbance subtracted spectra are complex and involve changes in peak height, peak position, and band width since the biological changes involve a number of changes in the local environment. Consequently, interpretation is difficult if one attempts to isolate a single variable. The problem is somewhat simplified if one concentrates on a given molecular probe and follows changes in its local interactions. For FT-IR studies of the function of hemoglobin, the sulfhydryl group was selected.[34] Although this group has low absorptivity, this absorptivity is considerably enhanced by hydrogen bonding. Hemoglobin contains cysteine sulfhydryl groups at three different positions. The α-104 cysteine SH vibration absorptions are observed from 2552.6 to 2556.3 cm^{-1} in aqueous solutions of human hemoglobin. The β-112 SH bond absorbs as a low frequency shoulder on the α-104 peak. This frequency of SH vibration reflects strongly H-bonded sulfhydryls surrounded by nonpolar and nonexchanging nearest-neighbor van der Waals contacts that are present at the α,β interface. The β-93 cysteine SH absorption was not observed.

The water content of the hemoglobin samples was measured by the near-IR water absorption band at 1.92
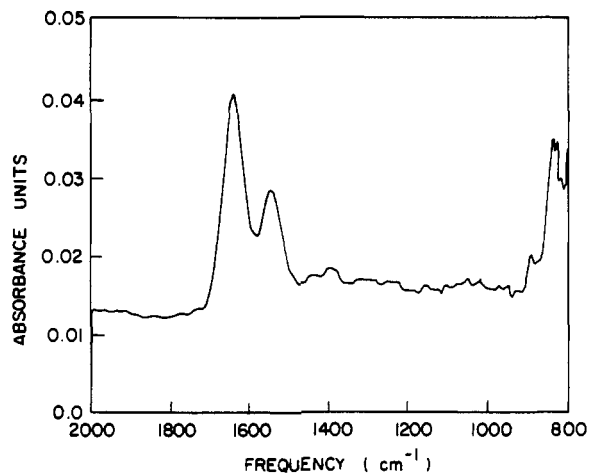
μm, and the FT-IR spectra were corrected for solvent water by subtraction of the appropriate fraction of the water absorbance spectrum. The corrected absorbance spectra due to SH vibrations of the α-104 and β-112 cysteines were curve fit to the sum of two Lorentzian functions. The absorbance subtracted spectrum was obtained between the hemoglobin derivatives and carboxyhemoglobin. In this manner small shifts in absorption band center frequencies are amplified. The center frequency shift is determined from the measured absorbance difference and the difference of computed Lorentzian functions. Measurements of the α-104 cysteine SH absorption band show similar patterns of frequency differences among hemoglobins from humans, pigs, and horses. The SH center frequencies indicate that the strength of hydrogen bonding and the range of differences in H-bond strength with ligation increase proportionately in the series horse < pig < human.

FT-IR has been used to study the problem of blood compatibility of implant materials.[35] The adsorption of blood proteins onto various surfaces in contact with flowing blood plasma or serum has been investigated by using attenuated total reflection (ATR). For these studies, the ATR crystal is incorporated in a sealed cell through which the blood plasma solution is passed. Figure 11 shows a saline-subtracted spectrum of the protein layer absorbed onto a germanium crystal from a flowing solution of blood plasma. Spectral subtraction allows compensation for both saline and the implant materials. The spectral results have yielded two important pieces of information. On a poly(vinyl chloride) (PVC) surface, the blood protein found has a high carbohydrate content and this may be in part responsible for the observation that PVC induces thrombus formation. A stable protein layer is formed from the flowing blood plasma, which is not desorbed on contact with flowing saline. Studies of this type have also been carried out by using whole blood from live laboratory animals under physiological conditions.[36] It is anticipated that these results will yield new insight into the

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molecular events which lead to surface-induced thrombosis.

Concluding Remarks

The inroads that FT-IR spectroscopy has made into the research laboratory are remarkable considering the short time involved and the quality of competing analytical techniques, such as nuclear magnetic resonance spectroscopy. The future also looks bright, particularly in those areas where the rapid scanning rate is important. These areas include using FT-IR as a detector for on-the-fly gas chromatographic and liquid chromatographic separations.

When time-resolved techniques which sample only a portion of the interferogram are used, it is possible to obtain spectra in the microsecond domain. This advance will allow the study of dynamic and kinetic processes. Developments in new hardware will allow a wider variety of samples to be studied, and new software will allow us to obtain more information from the spectra. Ultimately, additional applications of FT-IR will result.