High-resolution Gas Chromatography/Matrix Isolation Infrared Spectrometry

ARTICLE *in* ANALYTICAL CHEMISTRY · JULY 1985

Impact Factor: 5.64 · DOI: 10.1021/ac00285a024

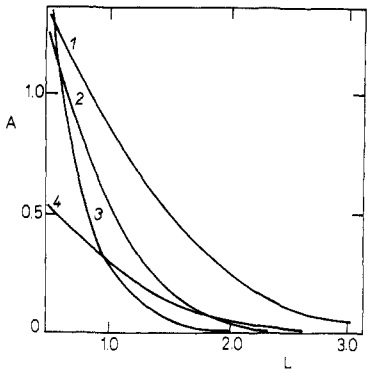

Figure 5. Dependence of the equivalent of accessible polymeric mass A [m/g × 10^{-12}] on the effective size of molecules L [nm] entering the porous system of a swollen polymer: 1, 0/2, 0/5; 3, 30Ste/7; 4, 50Bu/20.

nation of relative differences inside a certain series of gel polymers. Values of the structure parameters of these materials hold only for the medium in which they have been measured, but one is justified in assuming that the relative participation of variously dense fractions of polymeric mass remains preserved also with changing degree of swelling or even after functionalization which does not perturb the polymeric skeleton itself. Presenting structure parameters in terms of the Ogston model which uses the concentration of polymer chains instead of pore diameter is for this purpose very suitable and is, in our opinion, its greates advantage in comparison with the model of cylindrical pores. Thus, e.g., texture data obtained as described in this study have been successfully used in a quantitative description of the dependence of catalytic activity of strongly acidic ion exchangers prepared on the basis of copolymers of styrene and divinylbenzene (*14*).

Registry No. (Divinylbenzene)·(styrene) (copolymer), 9003-70-7.

LITERATURE CITED

(1) Jeřábek K. *Anal. Chem.* **1985**, *57*, 1595-1597.
(2) Freeman D. H.; Polnescu, I. C. *Anal. Chem.* **1977**, *49*, 1183.
(3) Halász I.; Vogtel P.; Groh R. *Z. Phys. Chem. (Munich)* **1978**, *112*, 235.
(4) Giddings J. C.; Kucera E.; Russel Ch. P.; Myers M. N. *J. Phys. Chem.* **1968**, *72*, 4397.
(5) Boublík T. *Collect. Czech. Chem. Commun.* **1972**, *37*, 2499.
(6) Porath J. *Pure Appl. Chem.* **1963**, *6*, 233.
(7) Waldmann-Meyer H. M. "Chromatography of Synthetic and Biological Polymers", Vol. I; Ellis Horwood, Ltd.: Cichester, 1978; p 289.
(8) Laurent J. C.; Killander J. *J. Chromatogr.* **1964**, *14*, 317.
(9) Ogston A. G. *Trans. Faraday Soc.* **1958**, *54*, 1754.
(10) Freeman D. H.; Schram S. B. *Anal. Chem.* **1981**, *53*, 1235.
(11) Heitz W.; Korn W. *Angew. Makromol. Chem.* **1967**, *1*, 150.
(12) Jeřábek K. *Collect. Czech. Chem. Commun.* **1977**, *42*, 805.
(13) Jeřábek K. *Collect. Czech. Chem. Commun.* **1981**, *46*, 1577.
(14) Jeřábek K.; Setínek K. "8th International Congress on Catalysis, Berlin (West), Proceedings"; Verlag Chemie: Weinheim, Deerfield Beach, Basel 1984; Vol. IV, pp 781-789.

RECEIVED for review November 2, 1984. Resubmitted and accepted February 25, 1985.

High-Resolution Gas Chromatography/Matrix Isolation Infrared Spectrometry

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An apparatus is described that allows the collection, within a matrix of condensed inert gas, of the effluent compounds from a high-resolution gas chromatograph. Each collected compound is contained within an area typically 0.3 mm in diameter yielding a concomitant high level of infrared spectral absorbance per nanogram of sample. Tests demonstrate the level of infrared sensitivity, the achievement of matrix isolation of the sample molecules, and the maintenance of compound separation achieved by the gas chromatograph. Use of the apparatus is demonstrated for PAH, PCB, dioxin, and aliphatic hydrocarbon compounds.

The technique of matrix isolation has been extensively used to study the infrared spectrometric properties of molecules (*1-4*). In this technique the molecules to be studied are cocondensed with an excess of inert gas, such as argon. Trapped in the solid argon matrix, at temperatures between 10 and 15 K, the individual molecules do not interact with each other and interact only slightly with the inert argon atoms surrounding them. This lack of intermolecular interaction, coupled with the absence of molecular rotation, leads to infrared absorption spectra having narrow lines (frequently <0.5 cm^{-1} fwhm for small molecules). Such spectra have proven to be valuable in detailed spectrometric studies of a large variety of molecules, free radicals, and ions (*5-7*). The adaptation of this technique to analytical chemistry applications could, in principle, be very fruitful; but, until now, this technique has not been very sensitive and the deposition of chemical compounds in the matrix, followed by the measurement of infrared absorption spectra, has been an involved and lengthy process. As a result, analytical applications of matrix isolation infrared spectrometry (*8*) have been of limited utility in the past.

Gas chromatography, by contrast, has become a universally practiced analytical technique applicable to a vast number of volatile compounds. Efforts at Argonne National Laboratory (ANL) have been directed toward combining gas chromatography and matrix isolation infrared spectrometry to develop a high-sensitivity technique for rapidly measuring

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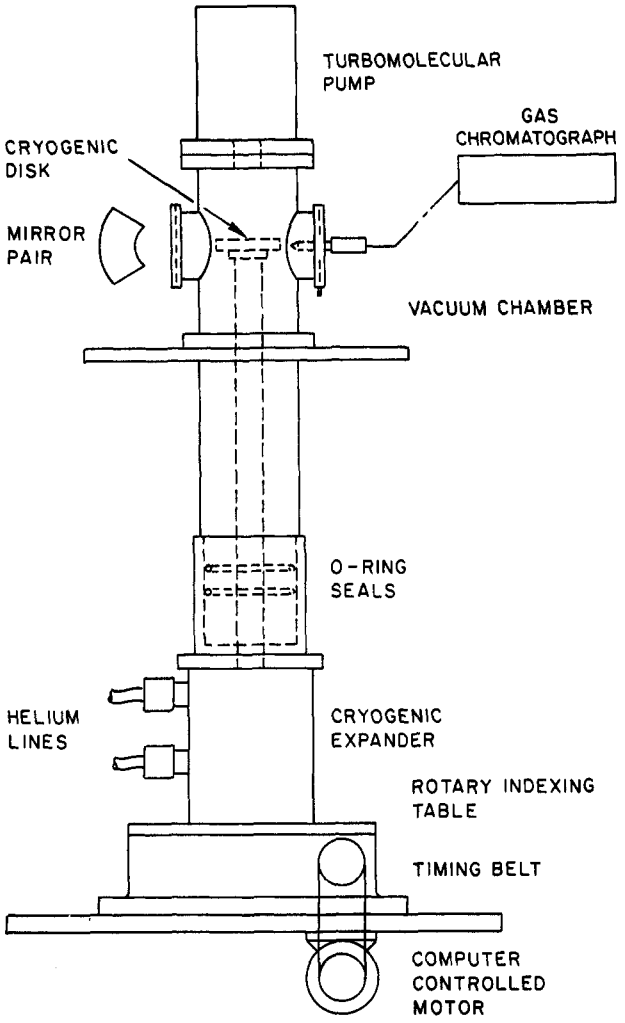

Figure 1. Side view of the cryogenic apparatus.

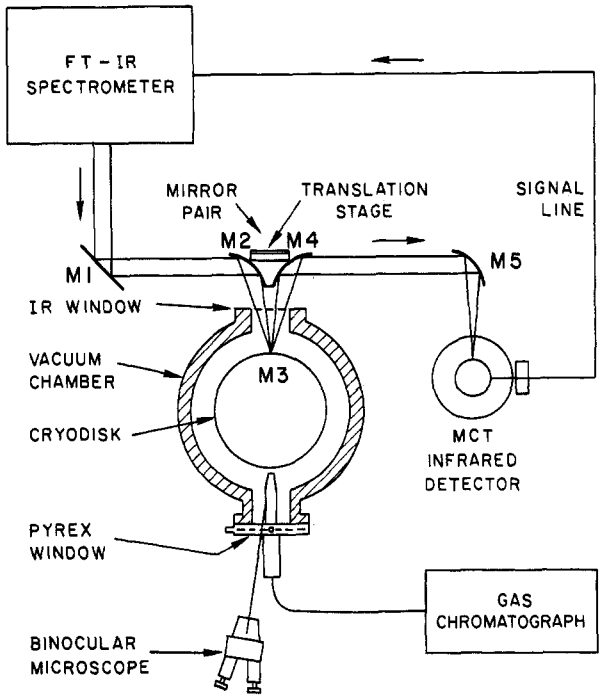

Figure 2. Relative arrangement of components in a horizontal plane at the elevation of the cryodisk.

the infrared absorption spectra of the components of complex mixtures.

The combined use of gas chromatography and matrix isolation infrared spectrometry (GC/MI-IR) was first demonstrated in this laboratory in 1979 (*9*, *10*). The apparatus constructed for this demonstration allowed one to collect effluent compounds from a packed column gas chromatograph in discrete matrices on a movable cryogenic surface that was capable of holding up to 32 individually deposited compounds. Several limitations were inherent in that early instrument. First, the large quantity of gas passing through the packed column of the gas chromatograph made it necessary to use a molecular jet separator as a preconcentration step in the collection process. Second, the apparatus did not provide for precise positioning of the deposited matrix or for close observation of the matrix as it was being deposited. Third, the apparatus required the operator to advance the collector before the collection of each compound. Fourth, and most importantly, the area of the cryogenic surface over which each eluting compound was collected was relatively large ($\sim$3 mm^2) and, as a result, the infrared absorbance was low.

A more advanced GC/MI-IR instrument has now been constructed and tested at ANL (*11*). The design and performance of this instrument are discussed here. The new GC/MI-IR system has proven to be a very sensitive and precise tool for the analysis of complex mixtures.

EXPERIMENTAL SECTION

Apparatus. Figure 1 shows a side view of the vacuum chamber and cryogenic components of the GC/MI-IR instrument. The vacuum chamber is constructed of welded stainless steel tubing and flanges and includes four ports. A 330 L/s Balzers turbomolecular pump is mounted on the top port (100 mm i.d.). The turbomolecular pump is backed by a direct drive mechanical pump. The right-hand port, as shown in Figure 1, serves for the introduction of a heated capillary tube carrying the effluent from a gas chromatograph. The capillary tube passes through a copper block and a metal nozzle that are sealed into a Pyrex window. The end of the capillary tube protrudes slightly from the nozzle. The window permits observation of the nozzle and capillary tip so that the position of the latter with respect to the rotating cryogenic disk or "cryodisk" can be accurately determined. A set of six adjustment screws allows the window, with the attached nozzle, to be positioned along three axes. A small mirror (not shown) within the vacuum chamber just above the end of the capillary tube aids in observing the spacing between the nozzle and the collection surface of the cryodisk. The infrared beam from the spectrometer passes through a window mounted in the opposite port of the vacuum chamber.

The cryogenic expander of an Air Products Displex 202 closed cycle helium refrigerator is mounted, upward, through the bottom port of the vacuum chamber. The refrigerator is capable of cooling the cryodisk to about 11 K in 1 h and does not require the use of liquid helium. A stainless steel extension tube, attached to the bottom port of the vacuum chamber, is employed to seal the base of the cryostat to the vacuum chamber. A double O-ring seal between the base of the cryostat and the stainless steel tube allows the entire cryostat and attached cryodisk to rotate independently of the vacuum chamber. Connectors on the cryostat base provide for attachment of flexible tubes leading to the helium compressor. The base of the cryostat is fixed at the center of a machinist's rotary indexing table so that the axis of the cryostat is coincident with the table rotation axis. The indexing table is driven by a computer-controlled stepper motor through a timing belt arrangement. Not shown in Figure 1 is the radiant heat shield within the vacuum chamber that partially encloses the cryodisk and is attached to the first refrigeration stage of the cryostat. The problem of refrigerator vibration described in the first paper on GC/MI-IR (*9*) has been eliminated by direct attachment of the sample collector to the refrigerator cold stage, adjustment of the ballast volume of the cryogenic expander, and use of a higher FT-IR scan velocity.

Figure 2 shows a cross-sectional top view of the vacuum chamber at the elevation of the cryodisk. The sample inlet port and the infrared beam port mentioned earlier are shown on opposite sides, facing the curved surface of the cryodisk. A binocular microscope of long focal length provides a 10× view through the

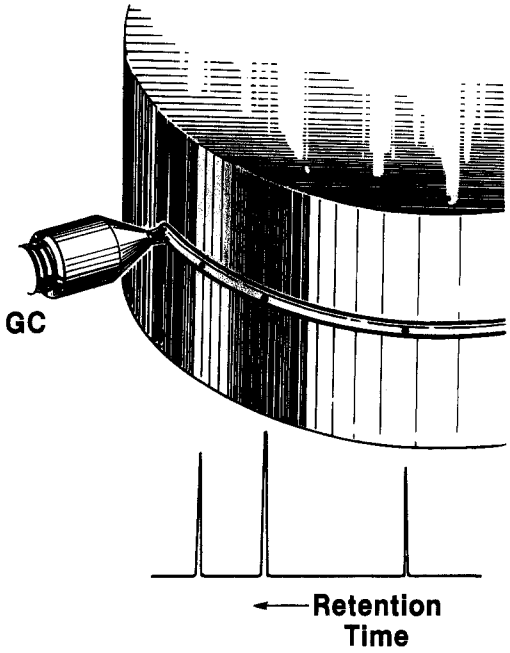

Figure 3. Collection and subsequent infrared examination of gas chromatographically separated compounds in a matrix of solid argon at the cryodisk. The chromatogram in juxtaposition denotes the elution of chromatographically separated compounds.

sample inlet port window, which shows the capillary tip and the disk surface where the matrix is deposited. The cryodisk, 100 mm in diameter and 6 mm thick at the rim, is made of oxygen-free, high-conductivity copper plated with hardened gold. The curved surface of the disk is highly polished to give a uniform mirror finish. An indium gasket beneath the disk provides good thermal contact with the cryostat. A pair of off-axis paraboloid mirrors (M2 and M4) are mounted outside the vacuum chamber. The pair is attached to an X–Y–Z translation stage that allows the mirrors to be positioned precisely. A collimated beam from a Nicolet Model 6000 Fourier transform infrared spectrometer is directed by a plain mirror, M1, onto the first paraboloid mirror, M2. Subsequently it is focused through a thin (~1.5 mm thick) small-diameter window of fused KCl onto mirrored surface M3—the sample collection surface of the cryodisk. In approaching and reflecting from the cryogenic surface, the infrared beam passes twice through material (e.g., the matrix) present on the surface. The reflected beam is redirected and collimated by mirror M4 onto a third parabolic mirror, M5, which focuses the beam onto the mercury cadmium telluride (MCT) infrared detector. A preamplifier at the detector amplifies the resulting electrical signal and transmits it to the data system of the Nicolet 6000 spectrometer for processing. The gas chromatograph indicated in Figure 2 is a Perkin-Elmer Sigma 2000 equipped with a flow splitter that diverts 10% of the effluent to a flame ionization detector (FID), and a Valco three-way valve that directs the remaining effluent to either the heated tranfer line or a waste exhaust.

Figure 3 shows an enlarged view of the region where sample deposition occurs. The fused silica capillary tube, approximately 150 μm (0.006 in.) in diameter, directs the effluent carrier gas stream from the gas chromatograph against the mirrored surface of the cryodisk, M3, in Figure 2. The carrier gas is typically 98% helium, with the balance argon. The helium is noncondensable at the temperature of the mirror surface and is pumped away by the vacuum pump. The argon and sample molecules are condensable and form a solid layer on the cold mirror surface. Slow rotation of the cryodisk keeps the surface in constant motion transverse to the effluent stream. The separated components in the carrier gas stream from the chromatograph are retained, in sequence, in the track of frozen argon on the cold mirror surface.

Several important features of the collection technique are indicated in Figure 3. First, the capillary transfer line, protruding slightly beyond the heated nozzle, is kept hot through its entire length to avoid condensation of the effluent compounds. Second, the collection process occurs in a very small region; the capillary tube is accurately positioned (±25 μm) with respect to the collection surface at a separation that is typically 150 μm. Third, the rate at which the collection surface moves and the width of the effluent stream determine the amount of separation between gas chromatographically separated compounds as they collect on the cold surface. The chromatogram pictured below the cryodisk in Figure 3 illustrates the direct relationship between retention time on the chromatogram and angular rotation of the disk. Under typical matrix deposition conditions, the cryodisk will undergo a complete rotation in 110 min. In order to collect separated components from a chromatographic run of longer duration than 110 min or to collect components from several chromatographic runs, the capillary tube can be repositioned to deposit up to three tracks, side by side, on the surface of the cryodisk.

The measurement of infrared absorption spectra for a large number of matrix isolated compounds collected on the cryodisk is greatly facilitated by automation of the disk motion. The cryodisk is driven by a Compumotor stepper motor that is controlled through an indexer. The indexer is interfaced to the spectrometer computer through an RS-232 port. A Fortran program is run after a chromatographic separation has been completed and the solid argon strip, containing the separated sample components, has been deposited on the cryodisk. The program acepts as input a set of retention times, which are read by the operator from the chromatogram generated by the flame ionization detector. After the retention times of the sample components of interest are entered, the program rotates the cryodisk so that the portion of the solid argon strip that contains each component is placed, in turn, at the focus of the infrared optics. An infrared absorption spectrum is measured and stored for each retention time that has been entered. One or more retention times, when the chromatogram indicates that no sample components are eluting, are selected and entered to be used for background subtraction.

RESULTS AND DISCUSSION

Infrared Absorbance Measurement Sensitivity. A key question to be answered for the GC/MI-IR technique concerns the obtainable level of infrared sensitivity. The infrared sensitivity should be sufficiently high to give an interpretable (e.g., all medium to strong absorption bands in the 700–4000 cm^{-1} region should be recorded with a minimum signal-to-noise ratio of 5:1) infrared absorption spectrum of the eluted compound of interest.

The question of infrared sensitivity can be dealt with in a rather straightforward manner. The two factors that together determine the level of infrared sensitivity are the noise level of the infrared measurement and the molar absorptivity of an appropriate infrared band for the compound being tested. If the Beer–Lambert law is assumed to apply to the matrix isolation spectra, it is possible to examine the question of infrared sensitivity more quantitatively.

The absorbance, A, observed for the selected infrared peak will be the product of the molar absorptivity, a, the sample (i.e., matrix) thickness, b; and the concentration of the sample, c. This relationship, the Beer–Lambert law, can be expressed as

$$A = abc = a\frac{V}{S}\frac{m}{M}\frac{1}{V} \quad (1)$$

where V is the volume of the matrix, S is the surface area covered by the matrix, and m and M are, respectively, the mass and molecular weight of the sample. If the area over which the sample is spread is assumed to be circular, then S is proportional to the square of the diameter, d. For a given compound a/M is constant, hence

$$A = (a/M)m/(\pi/4)d^2 \quad (2)$$

By taking the logarithm of both sides of this equation, we see that

$$\log A = \log (a/M) + \log m - 2 \log d + k \quad (3)$$

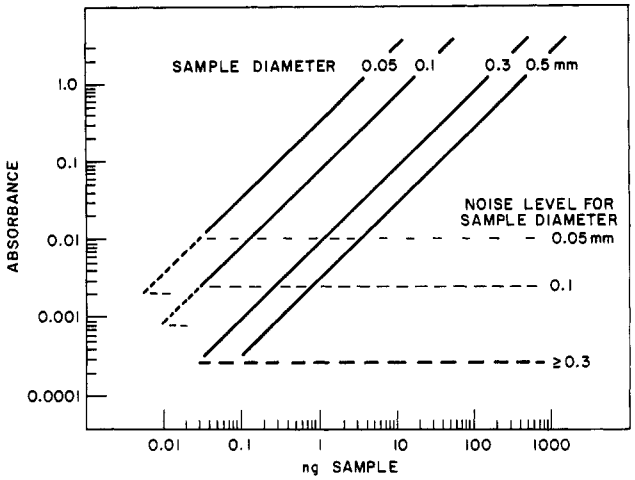

Figure 4. Absorbance and noise level to be expected for a given quantity of sample (see the text) according to the diameter of the area over which the sample is spread.

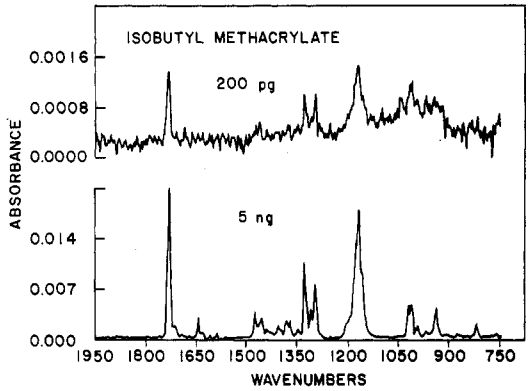

Figure 5. Infrared spectra of isobutyl methacrylate obtained in the evaluation of the GC/MI-IR apparatus.

where k is a constant. This equation is plotted in Figure 4, which illustrates the relationship between the quantity of sample, the diameter of the area over which the sample is spread, and the observed absorbance. The infrared beam is assumed to fall within the sample area. The value for (a/m) used to generate this plot is typical for the average of the two or three strongest peaks of a relatively strong absorber of modest molecular weight (100–200) like isobutyl methacrylate.

To read the plot, choose a given quantity of sample on the abscissa, for example, 1 ng, and then move vertically to the diagonal line defining the diameter of the sample area, for example, 0.3 mm; the absorbance is then read from the ordinate, 0.01 absorbance units in this example.

To obtain a measure of infrared sensitivity, the noise level present in the measurement must be considered in relation to the peak absorbance. For the instrument design discussed here, the noise level is almost totally determined by the MCT infrared detector. In practice, short focal-length mirrors are used to focus the infrared beam at the sample. Under these conditions, sufficient infrared light will pass through a sample 0.3 mm in diameter to allow a narrow-band, high-sensitivity MCT detector to perform at the maximum signal-to-noise ratio of which it is capable.

Dashed horizontal lines were added to Figure 4 to indicate the noise level in absorbance units to be expected for spectra obtained with a sample spread over a given diameter. It is assumed that the focal length of the mirrors at the sample is fixed and that the adjustable iris for source image size within the spectrometer is set to make the source image at the sample match the size of the sample. The detector size is assumed optimum for the 0.3-mm sample diameter.

The addition of the horizontal lines to define the noise levels is quite informative in that it allows one to evaluate absorbance relative to noise and, hence, to determine the sensitivity for a range of operating conditions. For example, for 1 ng of sample spread over a 0.3-mm diameter area, the noise level would be 0.0003 absorbance units, which is equivalent to an absorbance-to-noise ratio of approximately 30 to 1.

Several important points should be noted here. First, the noise levels shown are determined largely by the detector and represent 15 s to 1 min of scanning time. Additional scanning time can improve the signal-to-noise ratio. Secondly, it is clear from eq 2 and Figure 4 that the absorbance increases dramatically as the diameter of the sample decreases. The apparatus was designed to take advantage of this principle. The sample area has been reduced to a practical minimum by directing the gases through a small diameter capillary tube placed very close to the collection surface. Consideration was also given to matrix volume, mirror aberrations, mechanical motion, and detector size. Some benefit can be obtained by decreasing the size of the MCT detector element but there is no gain when it is smaller than the sample image.

Figure 5 shows infrared absorption spectra measured for 5 ng (lower trace) and 200 pg (upper trace) of isobutyl methacrylate deposited on the cryodisk in argon.

Matrix Isolation. A question of interest to those familiar with matrix isolation spectrometry is whether matrix isolation is achievable under the condition of very rapid sample collection. The question arises because, traditionally, the formation of matrices containing individual compounds has been carried out slowly, over several hours, to achieve satisfactory matrix isolation. In the GC/MI-IR technique, however, compounds are deposited in an argon matrix as rapidly as they are eluted from the capillary column. This means that a typical deposition occurs in less than 10 s. It is important to achieve matrix isolation conditions because the analytical usefulness of GC/MI-IR is enhanced by the ability to measure very small differences in the infrared absorption spectra of similar compounds. These differences may be obscured if the molecules of a compound are not isolated adequately within an inert matrix. Intermolecular interactions can lead to some degree of absorption band broadening, wavenumber shifts, and band intensity variations that could obscure small differences between infrared absorption spectra.

The criteria that have been used routinely to confirm matrix isolation have included the widths of infrared absorbance peaks, and the wavenumber and intensity of various absorption bands sensitive to intermolecular interactions. In an earlier paper (*9*) on the subject of GC/MI-IR, the criterion used to test for matrix isolation was the width and wavenumber of the absorbance band belonging to the O–H stretching mode of the phenols. The observation of a sharp band at high wavenumbers close to the gas-phase wavenumber was interpreted to indicate the lack of interaction between sample molecules. Absorption bandwidth is a satisfactory criterion and can be applied to demonstrate the degree of matrix isolation achieved in the GC/MI-IR instrument described here.

However, a more stringent criterion has been demonstrated by W. O. George et al. in a recent publication (*12*). The spectral feature they use to evaluate the degree of isolation of the acrylonitrile molecules is a cluster of peaks at about 980 cm^{-1} that is due to the formation of a polymer. This cluster can be seen in Figure 6A, an example of unsatisfactory matrix isolation. Satisfactory matrix isolation can be achieved by a "slow spray-on" technique for forming the matrix as shown in Figure 6B where the polymer cluster is absent. A period of 4 h was used to deposit a gaseous mixture of 1 part acrylonitrile in 1000 parts argon on a cold surface. This same test can be applied in GC/MI-IR. Figure 6C shows a 0.5 cm^{-1}

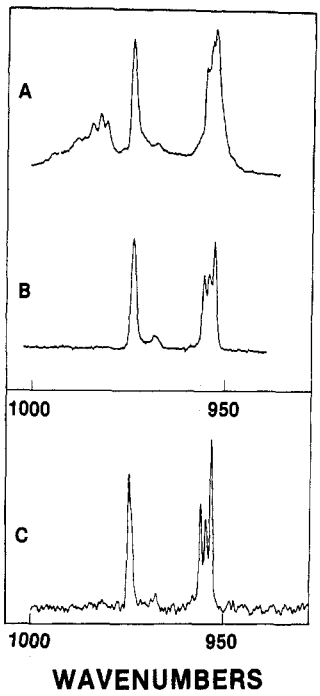

Figure 6. Matrix isolation infrared spectra of acrylonitrile: (a) incomplete isolation of acrylonitrile molecules resulting in polymer bands at ~980 cm^{-1} (*12*); (b) illustration of good matrix isolation (*12*); (c) GC/MI-IR spectrum of acrylonitrile (see text).

resolution spectrum obtained with the apparatus described in this paper. An injection of 0.5 μg of acrylonitrile in hexane was split 30 to 1 at the injector of the gas chromatograph. The effluent acrylonitrile peak, constituting approximately 15 ng in a 6-s half-width, was carried in a stream of 5% argon in helium. Acrylonitrile in the matrix was scanned for 72 s to give the 0.5 cm^{-1} resolution spectrum shown in Figure 6C. Comparison of spectrum C with spectra A and B shows that under these conditions no intermolecular interactions are occurring. Thus, it is possible to use GC/MI-IR under properly chosen conditions to obtain high-resolution infrared absorption spectra of the quality required for spectrometric studies of molecules. Such spectral information can be useful in assigning the absorption bands among the theoretically predicted vibrational modes of a molecule.

Carrier Gas Composition. The proper choice of carrier gas composition for GC/MI-IR can be determined from the quantity of compound present in each chromatographic peak, the width of the peak, and the rate of carrier flow. This choice is illustrated below, first for one set of operating conditions and then in a more general way for a broad range of operating conditions.

Assume a rate of carrier gas flow through a capillary gas chromatographic column of 1 mL/min carrying a component of molecular weight 150. Assume also that the total mass of the separated component is 15 ng (0.1×10^{-9} mol) and that this mass is concentrated into a peak nominally 6 s wide. We would like to ultimately collect this separated component in a small volume of solid argon for spectrometric observation. For optimum spectrometric observation under matrix isolation conditions, where each molecule of the compound is fully surrounded by argon atoms, we might reasonably choose to have the ratio of argon atoms to sample molecules be 1000 to 1. Following this choice, we can calculate the rate of flow of argon that will give the selected ratio of argon atoms to sample molecules. The average rate of flow of the compound over the 6-s interval is 1×10^{-10} mol per 0.1 min or 1×10^{-9} mol/min. The chosen rate of argon flow would be 1000 times higher or 1×10^{-6} mol/min. If the desired rate of argon flow is compared to the molar rate of helium flow through the

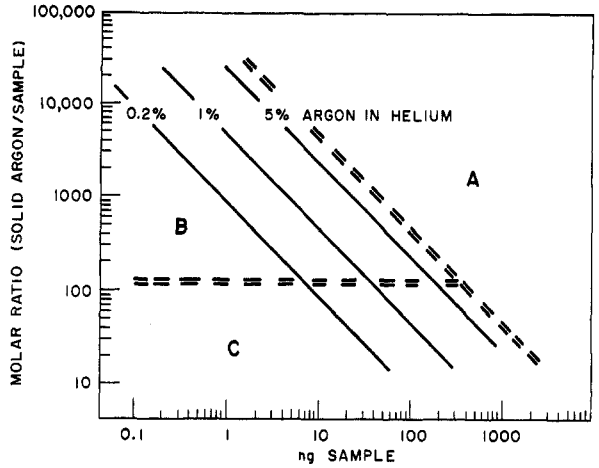

Figure 7. Molar ratio of argon to sample in the matrix as determined by the quantity of sample (see text) and the ratio of argon to helium in the carrier gas: (region A) excessive matrix volume; (region B) matrix isolation; (region C) cryogenic solid solution.

chromatograph, the ratio of rates is approximately 1:40. This ratio can be obtained by using as the carrier a mixture of 2.5% argon in helium. Gas chromatographic performance is essentially unchanged from that obtained with 100% helium as the carrier.

In a more general way, the relationship between the percent argon in the carrier (P) and the ratio of argon to sample (A/S) in a matrix is given approximately by

$$A/S = (P/100)(R/22400)t\ (M/m) \qquad (4)$$

where $R/22400$ is the molar rate of carrier flow when R is in units of standard cm^3/min, t is the peak width (full width at half height) in units of minutes, and m/M is the number of moles of sample for the compound being collected. If values are chosen for R, t, and M, we can write the above equation as

$$\log (A/S) = \log P - \log m + k' \qquad (5)$$

where k' is a constant.

It is instructive to plot this relationship as shown in Figure 7. The values chosen for this plot were $R = 1.0$ cm^3/min, $t = 6$ s (0.1 min), and $M = 100$ daltons. The abscissa is the mass of a given sample component in nanograms. The ordinate is the molar ratio of argon to sample component at a point on the cold surface where a compound has been collected. The plot is read by choosing a quantity of compound, say 10 ng, and moving upward to the desired percent argon in the carrier gas, indicated by the diagonal lines, and then moving left to read the ratio of argon-to-sample in the matrix.

Figure 7 is divided into three regions by dashed lines. Region A represents conditions of high sample abundance in combination with a high ratio of argon to sample in the matrix. Operation in this region with a relatively high ratio of argon to helium in the carrier is generally not desirable since the high dilution of minor sample componets in the solid argon reduces the achievable infrared sensitivity for these components. This consideration was apparent in the earlier discussion of infrared sensivitity. Also, it is difficult to accomodate the large volume of argon in a compact area on the cryodisk surface. An ultimate limit for the minimum surface area upon which an effluent compound can be collected under matrix isolation conditions is determined by the volume of the matrix. For example, 100 ng of a compound of molecular weight 100 constitutes 1×10^{-9} mol of sample. If this quantity of sample were to be diluted in solid argon at a mole ratio of 1 to 10 000, the amount of solid argon present would be 1×10^{-5} mol or 0.23 mm^3 (at 1.76 g/cm^3). This volume of solid

argon covering a circular area 0.5 mm in diameter results in a matrix thickness of 1.0 mm. Clearly, this level of dilution yields a greater volume of argon than can be deposited in a compact area. An excessive rate of argon flow can produce a matrix that is cracked and that adheres poorly to the cryodisk.

Region B represents the optimum range for the collection of gas chromatographically separated compounds under matrix isolation conditions. The lower boundary at about the 100:1 ratio of argon to sample represents a somewhat arbitrary dilution below which sample molecules can no longer be considered to be matrix isolated. Under stringent criteria with certain compounds (e.g., highly polar), this level may be set as high as 1000:1. (See earlier discussion of the acrylonitrile spectrum.)

Region C encompasses the conditions where the argon-to-sample ratio is sufficiently low that the matrix should be considered simply a solid solution of argon and sample. Nevertheless, high-quality infrared spectra with narrow, well-resolved vibrational bands can often be obtained for nonpolar molecules under these conditions of relatively low dilution. By working in regions B and C the dynamic range of sample abundance over which infrared absorption spectra can be obtained covers 3 to 4 orders of magnitude for any one ratio of argon to helium in the carrier gas.

Separation of Sample Components. Capillary chromatography is an excellent way to separate the components of very complex mixtures of volatile compounds. Figure 8a shows a portion of the chromatogram (FID) produced when 2 μL of a sample from a coal gasification product stream was analyzed on a DB-5 capillary column with 2% argon in helium as the carrier gas. It is important, in a GC/MI-IR instrument, to maintain the separation of sample components during the cryogenic deposition step. This will ensure than an infrared absorption spectrum can be measured for each component (peak) in the chromatogram without contamination by infrared absorbing compounds eluting nearby. Two factors, the cryodisk motion and the nozzle position, must be controlled to maintain, on the cryodisk, separations achieved chromatographically while retaining high IR sensitivity. The cryodisk surface must move just fast enough during deposition to avoid having a chromatographically resolved component deposit on top of a previously eluting component. This will be the optimum rate of cryodisk rotation because, although faster cryodisk motion will spread the components more widely on the cryodisk surface, the more diffuse deposition of each component will reduce IR sensitivity. It has been found empirically that the nozzle should be positioned approximately one nozzle diameter above the cryodisk surface (see Figure 3) during matrix deposition. This arrangement produces the most precise and compact deposit on the cryodisk, again helping to retain component separation and IR sensitivity. Figure 8, parts B and C, shows infrared absorption spectra recorded for two adjacent components in the chromatogram of Figure 8A. These components eluted only 12 s apart, but the separation has been preserved as indicated by the absence of absorption bands common to both Figure 8B and Figure 8C.

A glance at the chromatogram in Figure 8A shows that even capillary GC is incapable of resolving all components of a complex mixture. Fortunately, the GC/MI-IR instrument has proven capable of recording excellent infrared absorption spectra for compounds that are only partially resolved. This is accomplished by spectra background correction. Figure 8D shows an infrared spectrum recorded in this way for a component eluting in a complex region of the chromatogram. The analysis of this and other samples has proven that GC/MI-IR is a powerful technique for analyzing the complex types of

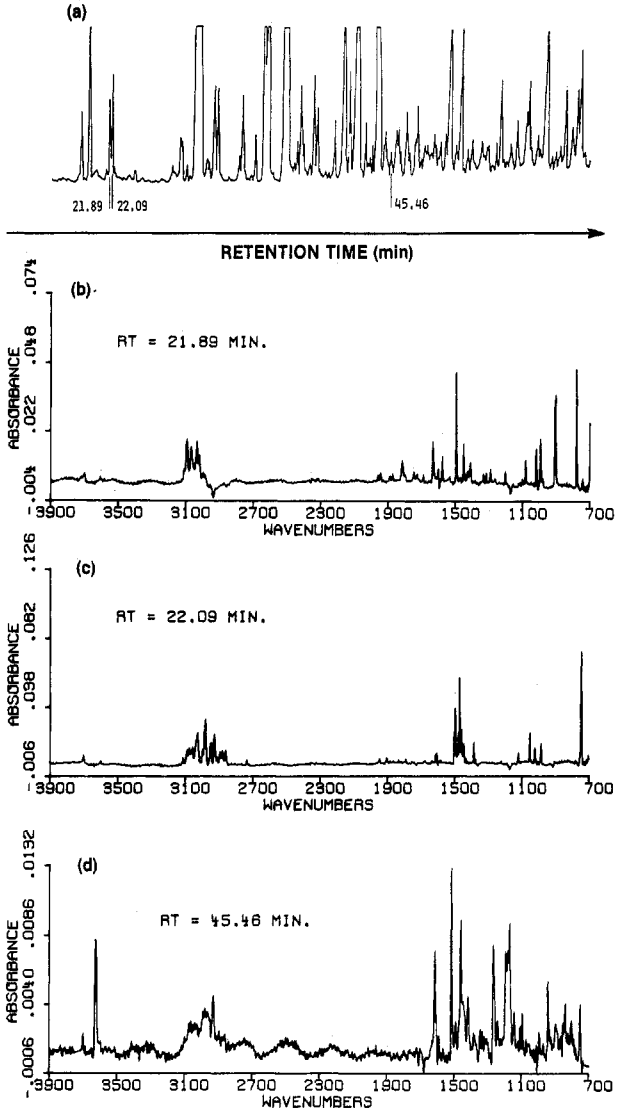

Figure 8. Application of GC/MI-IR in a coal toxicology study: (a) a portion of the gas chromatogram for the analyte; (b and c) infrared spectra for two compounds separated in retention time by 0.2 min; (d) infrared spectrum corresponding to one of many very minor peaks in the chromatogram.

samples that one often encounters in the analytical chemistry laboratory.

Specificity of Matrix Isolation Infrared Spectra. The infrared absorption spectrum of a matrix isolated substance not only is unique for every chemical compound but also is a good source of information about the functional groups present in, and the structure of, the molecules of each compound. Thus the technique of GC/MI-IR will be of great use to analytical chemists. For example, in the analysis of environmental samples for polychlorinated biphenyls (PCBs) and chlorinated dioxins, positive identification of specific molecular isomers is very important. Small differences in molecular structure may lead to large differences in toxicity. Infrared spectrometry, particularly of matrix isolated molecules, is well suited to distinguishing between closely related compounds. In mass spectrometry identifications of chemical compounds are based on fragmentation patterns and these may be similar for a variety of molecules. Infrared spectra are measured for intact molecules and any change in the identity or position of a substituent on a molecule alters the vibration modes of the molecule, resulting in distinctive infrared spectral features.

Figure 9 shows the infrared spectra of two isomeric chlorinated biphenyls measured under matrix isolation conditions.

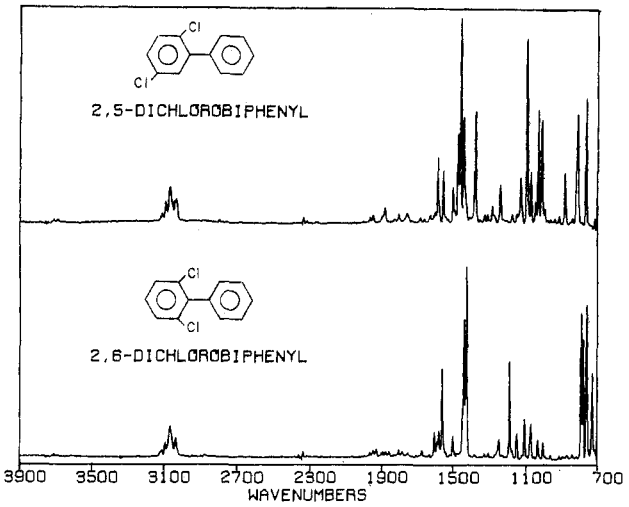

Figure 9. GC/MI-IR spectra illustrating the distinct difference in the spectra for two closely related PCB isomers.

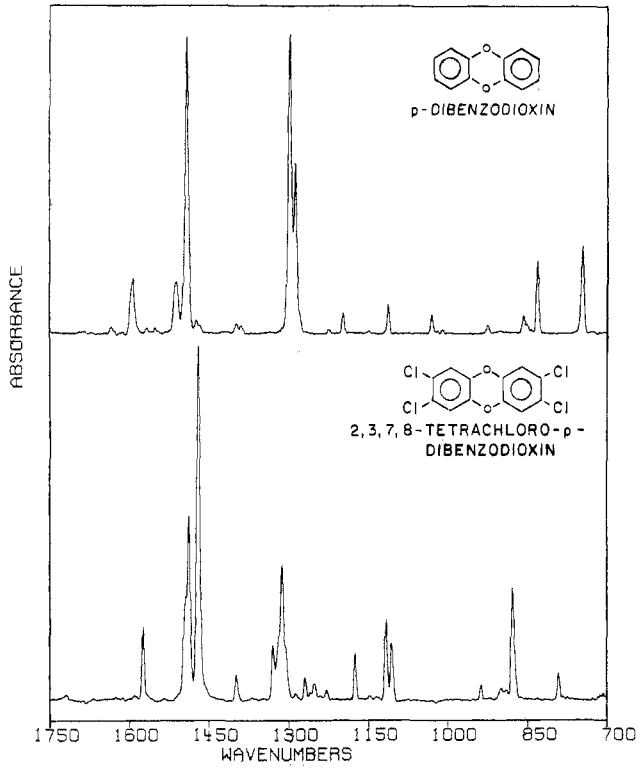

Figure 10. GC/MI-IR spectra for approximately 60-ng quantities of two dioxins.

The excellent specificity of these spectra is evident upon close examination. In Figure 10 the matrix isolation infrared spectra of dioxin and the highly toxic 2,3,7,8-tetrachloro-*p*-dibenzodioxin are shown to illustrate how reliably they can be identified. Clearly, a computerized library matching program based on the infrared spectra of matrix isolated compounds can become a routine tool for the positive identification of substances in a wide variety of samples.

There are certain classes of compounds, such as the straight-chain hydrocarbons, for which vapor or liquid phase infrared spectra alone are not very useful for positive identification. The compounds are very nonpolar and consequently show low levels of infrared absorption. Also, the homologous series varies only in the number of $-CH_2-$ units in the chain and so the infrared spectra of different compounds are quite similar. Infrared spectra of these molecules under matrix isolation conditions, on the other hand, show well-resolved and unique band structure that can be used for

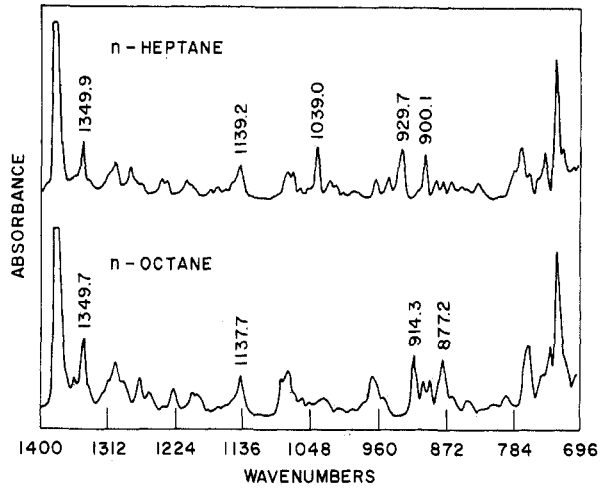

Figure 11. Discrimination between two straight chain hydrocarbons by GC/MI-IR.

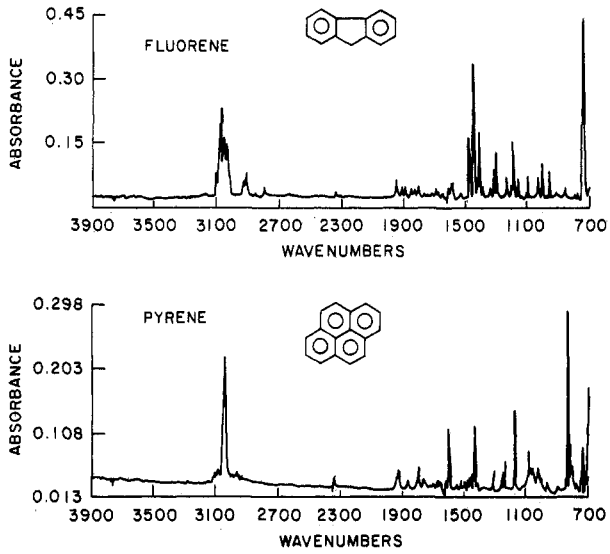

Figure 12. GC/MI-IR spectra for 50-ng quantities of two polycyclic aromatic hydrocarbons (PAH's), illustrating the ability to examine low volatility substances.

positive identification as shown in Figure 11 for the *n*-heptane and *n*-octane. The absorption bands of the matrix isolated hydrocarbons are narrower and therefore more intense, as a rule, than those for vapor-phase or liquid-phase hydrocarbons. This feature combined with efficient sample examination results in increased sensitivity that complements the high specificity.

Sampling Versatility. Under matrix isolation conditions all but the simplest chemical compounds have infrared absorption spectra that can be measured and used for identification purposes. GC/MI-IR can be applied to the analysis of any complex mixture made up of components that are sufficiently volatile and stable to undergo gas chromatographic separation. In GC/IR using a light pipe, the infrared throughput of the light pipe is degraded at higher temperatures leading to high detection limits for compounds of low volatility. A detection limit for fluorene (bp 293 °C) by GC/IR (light pipe) of 2.2 μg has been reported (*13*). This problem does not occur in GC/MI-IR because temperatures in the separation portion of the instrument have no effect on the infrared absorbance measurement process. Temperatures of the injector, column, and interface line can be raised as high as necessary and practical to achieve a chromatographic separation. Figure 12 shows an infrared spectrum, obtained by GC/MI-IR, of 50 ng of fluorene as well as a spectrum of

50 ng of pyrene (bp 393 °C).

CONCLUSIONS

Gas chromatography/matrix isolation infrared spectrometry will be a very useful and practical tool for analytical chemistry. The technique is applicable over the range of sample sizes normally chromatographed on capillary columns and gives infrared spectra with excellent signal-to-noise ratios. A particular virtue of GC/MI-IR is that the specificity is high, allowing straightforward discrimination among closely related chemical compounds.

The hardware is more sophisticated and requires more precise construction than the lightpipe GC/IR interface but is conceptually simple and has been automated for easy use. The preceding discussion has indicated how the operating parameters affecting sample collection can be carefully defined and controlled.

GC/MI-IR should find numerous applications in laboratories analyzing complex mixtures of organic compounds for which capillary column gas chromatography is required. Determinations of pesticides, polynuclear aromatic hydrocarbons, flavor and fragrance compounds, and trace materials of forensic interest are some examples. Recently, a commercial instrument (*14*, *15*) has become available that efficiently incorporates matrix isolation as an interface between a gas chromatograph and an infrared spectrometer.

Registry No. Isobutyl methacrylate, 97-86-9; acrylonitrile, 107-13-1; 2,6-dichlorobiphenyl, 33146-45-1; 2,5-dichlorobiphenyl, 34883-39-1; *p*-dibenzodioxin, 262-12-4; 2,3,7,8-tetrachloro-*p*-dibenzodioxin, 1746-01-6; *n*-octane, 111-65-9; *n*-heptane, 142-82-5; fluorene, 86-73-7; pyrene, 129-00-0.

LITERATURE CITED

(1) Barnes, A. J., Orville-Thomas, W. J., Muller, A., Gaufres, R., Eds. "Matrix Isolation Spectroscopy"; Reidel: Boston, 1981.
(2) Green, D. W.; Reedy, G. T. "Fourier Transform Infrared Spectroscopy: Applications to Chemical Systems"; Ferraro, J. R., Basile, L. J., Eds.; Academic Press: New York, 1978; Vol. 1, pp 1–59.
(3) Hallam, H. E., Ed. "Vibrational Spectroscopy of Trapped Species"; Wiley: New York, 1973.
(4) Meyer, B. "Low Temperature Spectroscopy"; American Elsevier: New York, 1971.
(5) Dunkin, I. R. *Chem. Soc. Rev.* **1980**, *9*, 1–23.
(6) Andrews, L., "Advances in Infrared and Raman Spectroscopy"; Heyden: Philadelphia, PA, 1980; Vol. 7, pp 59–82.
(7) Burdett, J. K.; Poliakoff, M.; Turner, J. J.; Dubost, H. "Advances in Infrared and Raman Spectroscopy"; Heyden: Philadelphia, PA, 1976; Vol. 2, p 152.
(8) Mamantov, G.; Garrison, A. A.; Wehry, E. L. *Appl. Spectrosc.* **1982**, *36* (4), 339–347.
(9) Reedy, G. T.; Bourne, S.; Cunningham, P. T. *Anal. Chem.* **1979**, *51*, 1535–1540.
(10) Bourne, S.; Reedy, G. T.; Cunningham, P. T. *J. Chromatogr. Sci.* **1979**, *17*, 460–464.
(11) Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy; Paper No. 609, 1983; No. 84, 300, 363, and 931, 1984.
(12) George, W. O.; Lewis, E. N.; Williams, D. A.; Maddams, W. F. *Appl. Spectrosc.* **1982**, *36* (5), 592–596.
(13) Gurka, D. F.; Hiatt, M.; Titus, R. *Anal. Chem.* **1984**, *56*, 1102–1110.
(14) Borman, S. A. *Anal. Chem.* **1984**, *56*, 936A–938A.
(15) Bourne, S.; Reedy, G.; Coffey, P.; Mattson, D. *Am. Lab.* (*Fairfield, Conn.*) **1984**, *16* (June), 90–101.

RECEIVED for review December 18, 1984. Accepted March 15, 1985. This work was performed under the auspices of the U.S. Department of Energy.

Hierarchical Tree Based Storage, Retrieval, and Interpretation of Infrared Spectra

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An information system for large files of full-curve infrared spectra is described. The retrieval and update of spectra are based on a hierarchical tree generated during the setup of the spectral bank using the three-distance clustering technique. The hierarchical tree can be easily updated and enables retrieval of any library spectrum in a number of comparisons proportional to $\log_2 N$, N being the number of spectra in the tree. The parameters (dimension of the measurement space, number of spectra, updating sequence, safety wall) necessary to "optimize" the generation of the hierarchical tree are discussed. The clustering of structural features in a tree of 219 infrared spectra and the application of the tree to the prediction of such structural features in a test set of 242 spectra are described. The prediction ability of the tree is analyzed. The use of such a tree in an automated spectrum interpretation system and as a large scale retrieval system is suggested.

With the rapidly increasing use of computerized spectrometers that can be linked to laboratory (or main-frame) computers, the possibility of recording and storing thousands of spectra has become a reality in many laboratories throughout the world. Regretably, the effort expended to accomplish this has been small compared to that required for full and meaningfull utilization of these acquired data. The most common purpose for storing the recorded spectra is for furture needs such as comparisons, references, searches for similar spectra, etc. A more sophisticated, but less easily attained goal is the application of the existing data to the automated inference of structural features from the spectrum of a compound of unknown structure. The above tasks can be performed efficiently only if all spectra are accessible on-line. Additionally, the algorithms for handling spectra (i.e., search, retrieval, comparison, extraction of structural information) should be very fast since thousands of spectra have to be scanned or considered every time.

The work described here is part of a broad research program directed to the development of a computer model of the process by which the structure of an organic compound is deduced from its spectra properties. The CASE (computer-assisted structure elucidation) program consists of three major modules: INTERPRET, ASSEMBLE, and SIMULATE (*1*). ASSEMBLE accepts a molecular formula and a broad range of structural inferences: structural fragments and other information that cannot be expressed in that way. This program then generates all topologically different molecular structures

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