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Standard Practice for General Techniques of Infrared Microanalysis[1]

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1. Scope

1.1 This practice covers techniques that are of general use in securing and analyzing microgram quantities of samples by infrared spectrophotometric techniques. This practice makes repetition of description of specific techniques unnecessary in individual infrared methods.

1.2 These recommendations are supplementary to Practices E 168, E 573, and E 1252, which should be referred to for theory, general techniques of sample preparation, and calculations.

2. Referenced Documents

2.1 *ASTM Standards:*

[1] This practice is under the jurisdiction of ASTM Committee E-13 on Molecular Spectroscopy and is the direct responsibility of Subcommittee E13.03 on Infrared Spectroscopy.

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E 131 Terminology Relating to Molecular Spectroscopy[2]
E 168 Practices for General Techniques of Infrared Quantitative Analysis[2]
E 573 Practices for Internal Reflection Spectroscopy[2]
E 1252 Practice for General Techniques for Obtaining Infrared Spectra for Qualitative Analysis[2]
E 1642 Practice for General Techniques of Gas Chromatography Infrared (GC/IR) Analysis[2]
E 2105 Practice for General Techniques of Thermogravimetric Analysis (TGA) Coupled with Infrared Analysis (TGA/IR)[2]
E 2106 Practice for General Techniques of Liquid Chromatography-Infrared (LC/IR) and Size Exclusion Chromatography (SEC/IR) Analysis[2]

3. Terminology

3.1 *Definitions and Symbols*—For definitions of terms and symbols, refer to Terminology E 131.

3.2 *Beam Condenser*—A specialized accessory designed for analysis of samples of a microgram or less, comprising an analyte area or volume of 2.0 mm diameter or less.

4. Contamination

4.1 Although the presence of contaminants is a general problem in any type of analysis, contamination can be particularly severe in micro work. For example, minor impurities in a solvent can become major components of a residue remaining after solvent evaporation. Materials extracted from thin-layer chromatographic materials, from the paper used in paper chromatography, and from solid adsorbents in general, may include particular contaminants of concern. It should also be noted that the gas-chromatographic stationary phase may lead to significant contamination. Consideration of these and other sources of contamination must always enter interpretation of results in microanalysis. Erroneous results can be minimized by the use of pure reagents, extreme care in sample handling, and the frequent use of "blanks" in the course of separation and subsequent recording of spectra.

5. General Microspectroscopic Techniques

5.1 Spectroscopic techniques used for the examination of microsamples are usually adaptations of comparable macro techniques, and many have been described in the literature **(1, 2)**.[3]

5.2 In computerized dispersive spectrometers or Fourier transform-infrared (FT-IR) instruments, computer routines for multiple scanning, signal averaging, absorbance subtraction, and scale expansion can be used very effectively to enhance the observed signal-to-noise ratio of weak bands and increase sensitivity **(3, 4)**. Absorbance subtraction is also commonly used to eliminate interfering bands from the sample matrix and thus lower the limits of detection (see Practice E 168).

5.3 *Use of Masking Apertures*—The aperture of sample holders used for microspectroscopic study (without the use of an infrared microscope) are usually significantly smaller than the beam at the sample position of the instrument. As a consequence of these small apertures, steps need to be taken to ensure that the best quality spectra be obtained, and the techniques used will depend on the type of spectrometer being used. In general, the use of a beam condensing accessory will greatly improve the results obtained (see 5.4).

5.3.1 When a double-beam dispersive spectrometer that is not equipped for control by minicomputer is used, the reference beam should be masked to a corresponding aperture. This can be accomplished by using an opaque sheet of stiff material punched with an appropriate opening, with reference screens, or with commercially available optical attenuators. Attenuation of the reference beam affects instrument performance, and appropriate adjustment of the instrument settings (that is, wider slits or higher gain) is necessary to produce reliable spectra at the lower energy levels. Enhancement of sensitivity can be attained by the ordinate scale expansion feature available on most spectrometers.

5.3.2 When using a single-beam spectrometer, the instrument background spectrum should be recorded through an aperture in the sample position that has dimensions no larger than those of the sample. Where appropriate, this can be done by using the empty sample holder itself.

5.3.3 On some FT-IR spectrometers, insertion of an aperture at the sample position will slightly change the observed frequency positions of bands, as a result of modification of the optical path. Hence, sample and reference aperture must be carefully aligned at the same position, particularly if computer differencing is to be done.

5.3.4 Some FT-IR spectrometers (especially those equipped with cooled mercury cadmium telluride (MCT) detectors) are so sensitive that under normal operating conditions (that is, when examining macro samples or recording the reference single beam spectrum) the energy throughput of the instrument needs to be restricted in order to avoid detector nonlinearity **(5)**. This is typically done by insertion of an aperture or wire screen into the path of the beam. However, when the same instrument is employed to examine microsamples using a sample holder, which is in itself an aperture, this throughput restriction should be removed.

5.3.5 When using an infrared microscope, it is normal to record the reference spectrum through the same aperture as is used for a particular sample. To accomplish this, it is most convenient to use visual observation to select the aperture size required to

[2] *Annual Book of ASTM Standards*, Vol 03.06.
[3] The boldface numbers in parentheses refer to a list of references at the end of this practice.

mask the sample area of interest. The single-beam spectrum of this sample area is recorded, and the reference single-beam background spectrum is then recorded afterwards. The transmittance (or absorbance) spectrum of the sample is obtained by using the instrument software to calculate the ratio of the two single-beam spectra.

5.4 Large energy losses because of beam attenuation may be avoided by the use of a beam-condensing accessory. This type of accessory is designed to condense the sample radiation beam to an analyte area of 2 mm or less, accommodating the smaller size of a microsample. A 4× beam condenser is adequate for most microsample analyses.

5.4.1 The heat produced by the concentrated beam may be injurious to some samples, especially in the case of some dispersive instruments. If this difficulty is encountered, a thin germanium wafer between the source beam and the sample, or a stream of cooling air directed upon the sample, will provide some protection for the ~~sample. A 4× beam condenser is adequate for most microsample analyses.~~ sample.

5.5 *Examination of Liquid Samples*— Direct examination of liquid samples can be accomplished by using sealed microcells or microcavity cells, which are commercially available and are characterized by small apertures and volumes of the order of a few microlitres. Beam-condensing accessories are available that can accommodate such microcells. The volume of demountable microcells that are suitable for liquids of low volatility is about 0.5 µL when assembled with a 0.1-mm spacer. Micro quantities of non-volatile liquids can be conveniently examined using micro internal reflection spectroscopy (IRS), (see Practices E 573). Sometimes the most convenient way to handle microquantities of a volatile liquid is to contain it in a gas cell having a large length-to-volume ratio, so that the material is examined in the vapor phase.

5.6 *Examination of Solid Samples*—The conventional techniques for handling macro amounts of solids are equally applicable for microgram quantities when scaled down accessories are used. Just as for liquids, compensation for the sample-beam attenuation or the use of a beam condenser is necessary for the recording of useful spectra; ordinate scale expansion, multiple scans, or signal averaging may be needed to enhance the sensitivity.

NOTE 1—A range of accessories such as micromull holders, micropellet holders, etc. are commercially available. Some are designed for specific instruments but others have general utility.

5.6.1 A small quantity of finely ground powder can be mulled in an agent such as mineral oil and smeared on a small sample plate about 3 by 5 by 1 mm. The sample plate is mounted in a holder as near as possible to the focal point of the converging sample radiation beam or in a beam-condensing unit.

5.6.2 Alkali halide disk or pellet techniques are of considerable importance in microsampling. Compromises in the usual recommended procedures may be required to permit analysis of ultra-micro samples. It is advantageous to use an alkali halide that has been maintained in a drying oven at 105 to 110°C. Blank samples of the stored alkali halide should be used to obtain frequent reference spectra, in order to guard against contamination.

5.6.3 Commercial micropellet dies usually produce disks of either 0.5 or 1.5-mm diameter. A standard size 13-mm die may be adapted for micropellet work by punching a small aperture in a disk of, for example, tinfoil, manila folder, blotting paper, or filter paper about 0.1 mm thick. About one third the usual pressure should be used for pressing the micropellet. The tinfoil or paper serves as a holder for the pellet and can be positioned over the aperture of the micropellet holder or on the beam-condenser unit. Commercially available lead micro disks are also available.

NOTE 2—Stationery supply stores carry paper punches of assorted sizes and shapes that are suitable for making these apertures for micropellets.

NOTE 3—An aperture of 1 by 4 mm is about the minimum size on which some dispersive spectrometers can operate properly. If a beam condensing accessory is used, the minimum aperture is reduced to the order of 0.5 to 1.0 mm in diameter. Fourier transform instruments can obtain spectra through a 0.5-mm aperture, if necessary, without the use of a beam condenser.

5.6.4 A very small sample may be made transferable by rubbing or abrasion, or both, using dry potassium bromide (KBr) powder. Pellet grade KBr should be used, and subsequent grinding should be kept to the minimum necessary to disperse the sample. This technique is also valuable for removing a thin surface layer from a solid object.

5.6.5 A sample of a thin coating material may be obtained by rubbing the surface with glass-paper or silicon carbide paper. The spectrum of the sample on the surface of the paper is obtained by using the diffuse reflectance technique, with a clean piece of glass-paper or silicon carbide paper, as appropriate, being used as the reference.

5.6.6 Solid materials can be examined by first dissolving the material in a solvent (see 5.7). The resulting solution can be examined directly, or used to deposit the solute in a state more advantageous for analysis, such as a thin film or in a halide powder for the preparation of a KBr pellet or diffuse reflectance. The same solvent should be used to obtain a spectrum of the solvent blank, either directly or as a deposit, as appropriate. ~~NOTE 4—**Caution:** Solvent~~

5.6.6.1 **Warning:** Solvent or melt recrystallization or application of pressure to samples may cause changes in the crystalline structure of the material, and hence give changes to the observed spectrum.

5.6.7 Some solids can be heat-softened or melted by pressing between two small heated KBr plates and then examined in a demountable microcell holder (see ~~Note 4).~~ 5.6.6.1). It is often advantageous to perform the pressing operation with the sample between two sheets of aluminum foil first, so that more pressure can be exerted. The thin film is then peeled off the foil and examined between the salt windows. Some solid samples may be cut into thin wafers that may then be mounted in a micropellet holder for subsequent analysis.

5.6.8 Small flakes of material have been successfully examined by supporting them on a salt plate and then placing an aperture over the sample. Both salt plate and aperture are placed in the sample beam. Static forces may be used to hold very small samples

inside a pinhole aperture. Stray light may be observed under both types of sample mounting, since the sample does not normally fill the aperture completely. Improved spectral data are obtained by the use of a beam condenser (see 5.4) or, even better, an infrared transmitting microscope (see Section 11).

5.6.9 Samples can be held between two thin sheets of a polymeric material that has low infrared absorbance at the frequencies of interest, instead of being on the surface of a salt plate as in 5.6.6-5.6.8. Fluorocarbon tape may be used to obtain spectra over large portions of the mid-infrared region, while polyethylene film is particularly useful for far-infrared measurements. Both materials withstand the effects of many corrosive samples.

5.6.10 Another method for holding small solid samples in the beam is to stick them on a translucent adhesive tape and place an aperture over the sample. In this case, the spectrum of the adhesive tape should be compensated for, either by placing a ~~similar sample/aperture~~ similar aperture covered with adhesive tape in the reference beam or by computer subtraction of an adhesive tape spectrum collected in a manner similar to that of the sample.

5.6.11 To avoid the need to computer-subtract the spectrum of adhesive tape mentioned in 5.6.10, small pieces of salt window can be used to mount microsamples next to an aperture. The pieces of salt are cleaved from a used crystal by using a razor blade, and can be as small as 1 or 2 mm square. Transfer a few particles of adhesive from a (preferably old) piece of adhesive tape, using a probe, onto the extreme edges of this salt cover. Place the sample over the aperture, and cover with the salt plate. Pressure the salt cover onto the aperture so that the adhesive holds it in place. Adhesive from a used piece of tape will allow the cover to be removed more easily after sample collection is completed.

5.6.12 If using IRS with a small sample, ~~the~~ optimal results will be obtained if the small sample is placed across the width of the internal reflection element (IRE). With very small samples, optimal results will be obtained by placing the sample where the beam enters, so that the first reflection is concentrated at the sample position (see Practices E 573).

5.6.12.1 Micro IRS accessories are also commercially available and are generally referred to as "micr~~u~~o-ATR" accessories. The IRE of these accessories is only 1 to 3 mm in diameter with ~~FT~~ an effective sampling area of 0.5 to 2.0 mm in diameter, allowing analysis of smaller samples and, with a diamond IRE, greater contact pressures.

5.6.12.2 Particular cautions should be observed when using these types of accessories. Accessory design precludes control over the incident beam angle penetrating the IRE crystal surface, thus, a number of incident beam angles are directed onto the sample-crystal interface. The resultant spectra may not be directly comparable to spectra collected from a controlled incident angle IRE accessory or spectra collected by transmission. Additionally, if the active sampling area (0.5 to 2.0 mm) is not completely filled by the sample, that is, the sample is smaller than the crystal surface, stray-light effects can distort the spectrum. In both cases, the "standard" ATR-correction algorithm is not sufficient to account for these effects and may lead to even more erroneous results.

5.6.13 For the case of intract~~i~~able solid samples, the high-pressure diamond anvil cell may be used for squeezing samples to an appropriate thickness. While the cost of a diamond anvil cell is high, this is often the preferred method for reducing the thickness of samples that do not yield to simpler methods. The aperture of the cell is small, so it is necessary to use a beam condensing accessory, or better still, an infrared-transmitting microscope, to obtain the best quality spectra. Several comments should be made here, however. Diamond absorbs energy strongly between 1900 and 2300 cm^{-1}, which thus renders this accessory inappropriate for the study of samples that have significant absorptions in that region. On the other hand, diamond is a good far-infrared window material and allows spectra to be recorded down to below 50 cm^{-1}, using a beam-condenser and suitably equipped spectrometer. Squeezing the sample in the cell may change the morphology and any ordering in the structure of the sample (see ~~Note 4).~~ 5.6.6.1).

5.7 *Examination of Solutions*—In some instances, solutions of liquids or solids are advantageously used for recording spectra. The preparation of solutions in microquantities has inherent difficulties, and solvents usually obscure some portions of the spectrum. Some of these interferences can be eliminated by computer subtraction or double-beam techniques. Careful selection of the pathlength of the transmission cell or, with IRS, the type of IRE employed allows for dilute solutions (even in water) to be examined directly using an FT-IR spectrometer or a computer-assisted dispersive spectrometer. In general, solvent blank samples need to be examined in the same manner as the solutions generated, in order to identify the presence of contaminants.

5.7.1 A solution may be used to prepare a micro film of solute on a small window (approximately 8 by 8 by 2 mm) that has been gently scratched in order to contain the sample in a small area (3 by 3 mm, or less if using an FT-IR). It should be noted that the window must be made of a material that is not harmed by the solvent in use. Condensates from micro (capillary scale) pyrolysis can also be run in this manner. Alternatively, the deposit may be made directly onto a micro ~~IRE,~~ ATR and the spectrum obtained by IRS.

5.7.2 A small amount of a solution may be deposited onto a salt window using a capillary tube. In this case, the capillary action of the tube may be used to pick up a droplet of the solution. When the end of the tube is brought into contact with the window, the solution should partially flow onto the surface of the window. The solvent can then be evaporated to leave the residual solute as a micro film. If necessary, the capillary tube can be fitted with a small rubber bulb to allow more sample to be drawn into the tube, or a fine Pasteur pipette can be used.

5.7.3 A solution can be evaporated onto a powdered solid such as potassium chloride (KCl) for diffuse reflection techniques. The resulting powder is examined in a diffuse reflectance micro-cup.

5.7.4 Alternatively, the solution can be evaporated onto dry KBr powder which can then be used to prepare a micro KBr pellet (as in 5.6.2-5.6.4).

5.7.5 Another technique employs a porous triangle of pressed KBr in a capped glass vial having a small hole in the cap. The

solution is allowed to evaporate at the KBr triangle tip, leaving the solute concentrated there. This accomplishes filtration of adsorbent and deposition of the sample on KBr in a single step. The tip of the triangle (after evaporation of the solvent) is used to prepare a micro KBr pellet. If preferred, the diffuse reflectance technique can be used to obtain the spectrum of the solute in the KBr.

NOTE 54—A suitable commercial version of the KBr triangle is marketed as the Wickstick®.[4]

5.7.6 A microcapillary brush may be made to handle small volumes of solvent (see Note 6) 5) and can be used to cast a film on a remarkably small area of a salt crystal. When a microcapillary brush containing a solution of a volatile solvent and a less volatile solute is placed on the surface of a salt plate, the bristles of the microbrush hold the liquid in a small region. The non-volatile solute may thus be deposited in a restricted area of the salt plate, ready for analysis. Working under a stereo microscope, deposit the solvent on the crystal, touching only the glass fibers to the crystal **(6)**. Making a small indentation in the crystal with the point of a needle probe will help keep the solvent localized.

NOTE 65—Following is the procedure to make a microcapillary brush. Insert a bundle of 20 to 30 glass wool fibers into the end of a thin-walled microcapillary tube. Twirl the side of the tube near a micro burner flame until the fibers are fused to the side of the tube. (This may take a few tries since it is quite easy to singe the fibers if they get too near the flame.) Once the fibers are secured to the side of the tube, snip off all but a few millimetres of the fibers.

5.7.7 In practice, if there is a fair amount of residue in the solvent, it will tend to precipitate on the end of the fibers. This is just as well, as the solute can then be removed, rolled onto the surface of an infrared transmitting window, and placed over an aperture for examination. The "drop and suck" trick can be used with one of these brush capillaries. Use the brush to redeposit the solution on the crystal in a small area to maximize sensitivity. Use an aperture of appropriate size to mask the rest of the crystal or examine the sample using an infrared-transmitting microscope.

5.7.8 The technique of incorporating microgram samples into alkali halides by lyophilization (freeze drying) works well, although some additional precautions are necessary. Freeze drying is the removal of solvent from a mixture by low-temperature sublimation, normally done under vacuum conditions. Spectra of lyophilized materials often differ from those of the same material that is simply ground with the alkali halide. Precoating the lyophilization tube with a frozen layer of an alkali halide aqueous solution minimizes the loss of some types of samples because of adsorption on the glass surfaces. Contamination frequently arises from this procedure (for example, from pump backstreaming) and should be checked by using blanks of alkali halide powder alone. It should be noted that some solids have sufficient vapor pressure that a small sample will be reduced or even eliminated when being worked with during lyophilization.

5.8 *Micropyrolysis of Solid Samples*— Pyrolysis is often used to obtain spectra from materials like carbon-filled rubbers that are too opaque or heavily filled to yield spectra by other methods. The optimum method used to pyrolyze the sample will depend on its size.

5.8.1 The simplest method for micropyrolysis involves the use of a disposable pipette. The sample is inserted into the pipette and rolled to the neck region, and the large end is sealed in a small flame. When the sealed end cools, the polymer is tapped into that end. The sample is heated gently, producing pyrolysis products that condense on the walls of the pipette. The portion of the pipette containing the ash is then removed by scoring between the ash and the condensate and breaking the tube. A single droplet of solvent can then be added, washing the entire pyrolysate onto a salt plate for analysis.

5.8.2 Very small amounts of material can be pyrolyzed in a capillary tube instead of the pipette mentioned in 5.8.1.

5.8.3 A microcapillary brush (see 5.7.6) may be used to obtain a spectrum from a fragment that is too small to produce enough pyrolyzate by an ordinary pyrolysis analysis. Place the fragment in the end of the capillary brush that is away from the fibers and work the fragment toward the center of the tube. Seal the end of the tube. Then twirl the tube near a micro-flame in the area of the particle to pyrolyze the sample, being careful not to melt the tube. Cut off the sealed end of the tube containing the ash, draw a microdroplet of clean solvent up into the tube to dissolve the pyrolyzate, and then use the brush to deposit the solution onto a crystal.

5.9 Interest in coupling chromatographic methods with FT-IR spectroscopy arises from the need to separate and identify the components of mixtures. Chromatographic methods commonly used in conjunction with FT-IR analysis of the eluting components are gas chromatography, high performance liquid chromatography, supercritical fluid chromatography, and thin-layer paper chromatography (respectively known as GC, HPLC, SFC, and TLC), and paper chromatography. For GC and SFC the identification is usually performed in real-time using an FT-IR spectrometer, whereas the analysis of the compounds separated by other chromatographic techniques is may be performed in an off-line manner. For detailed guidelines concerning the practice of GC/IR and LC/IR, see Practices E 1642 and E 2106.

6. Analysis of Gas-Chromatographic Fractions (7-9)

6.1 Gas chromatographic fractions are normally examined directly as gases in a GC/FT-IR combination system in which the gas chromatograph is coupled directly to the FT-IR spectrometer and the separated components are analyzed in the gas phase as they

[4] Available from Harshaw

[4] The sole source of supply of the Wickstick known to the committee at this time is Harshaw, Cochran Rd., Solon, OH 44139. If you are aware of alternative suppliers, please provide this information to ASTM Headquarters, or the responsible technical committee.

emerge from the GC ~~column. To accomplish this, the hot gases are passed through a short, heated transfer line to an appropriate analysis cell, normally a light-pipe having a gold-coated interior. The optimum dimensions of the light-pipe depend upon the flow rate of the carrier gas being used and upon whether a packed~~ column ~~or capillary column is being used. Some commercial rapid-scan instruments are capable of providing identifiable infrared spectra on 10 to 20 ppm components of 1-µL injections; however, other instruments are capable of providing usable spectra of only major components of a sample. GC/IR units are commercially available as accessories for FT-IR instruments; these units give strong spectra of submicrogram amounts of some materials and may show some bands at levels of a few nanograms.~~

~~NOTE 7—The transfer line from the gas chromatograph to the infrared spectrometer should be heated to prevent condensation of sample components in the line. This transfer line should be as short as possible. In addition, it is important that the inside of the transfer line be made of a material that is inert to the chemicals eluting from the~~ (see Practice E 1642).

6.2 Gas chromatographic ~~column.~~

~~6.2 Sometimes, however, the GC~~ fractions ~~are~~ can also be trapped separately ~~in the condensed phase~~ for subsequent infrared analysis by passing the stream issuing from the vent line of the chromatograph through a solvent, a powdered solid, or a cold trap **(7)**. ~~These fractions are usually recovered by passing~~ Alternative procedures for GC/FT-IR detection involve the ~~stream issuing from the vent line~~ on-line trapping of submicrogram quantities of GC eluate at low temperatures **(10-12)**. Some commercially available cryogenic systems can provide detection limits at the ~~chromatograph through a solvent, a powdered solid, or a cold trap.~~ subnanogram level.

NOTE ~~8~~6—It must be assumed that all fractions obtained using a cold trap are multicomponent until proven otherwise.

~~6.3 Recently, alternative procedures for GC/FT-IR detection involving the on-line trapping of submicrogram quantities of solutes at low temperatures have been introduced. In one procedure the eluent gases are trapped in an argon matrix that is formed on a rotating cylinder maintained at cryogenic temperatures **(10)**. An alternative procedure involves direct deposition of the solute alone on a moving cold plate, which is maintained at sub-ambient temperatures by liquid nitrogen cooling **(11, 12)**. The resulting spectra exhibit very sharp bands, leading to detection limits that can be at the subnanogram level.~~

7. Analysis of Liquid Chromatography Fractions

~~7.1 A number of~~

7.1 There are many factors that must be considered when ~~HPLC~~ combining liquid chromatography and ~~FT-IR are to be combined.~~ FT-IR. The ~~most significant of these is the fact that the HPLC mobile phase will have a rich spectrum of its own which may obscure the spectrum of the analyte. The type of solvent (polar versus non-polar) must~~ LC effluent can be ~~considered since there are distinct differences between normal- and reversed-phase HPLC. Another consideration is the capacity (and size) of the HPLC column~~ coupled directly to ~~be used. Traditional analytical columns (3.9 to 4.6-mm inner diameters) pose problems that are very different from those associated with microbore columns (0.3 to 1.0-mm inner diameters). The choice of chromatographic parameters determines~~ the ~~applicability of HPLC/FT-IR as a viable microsampling tool.~~

~~7.2 Two types of HPLC/FT-IR interfaces (flow~~ FT-IR spectrometer using flow through ~~and solvent-elimination) have been developed. Because of the large relative concentration of mobile phase, solvent interference is a disadvantage of employing a flow-through cell for HPLC/FT-IR. Aqueous solvent systems create some of the worst problems for flow-through HPLC/FT-IR, since water is a very strong infrared absorber and can obscure large portions of spectrum.~~

~~7.2.1 Flow-through HPLC/FT-IR interfaces involving infrared transmission techniques have been developed. Because of interfering absorptions by the solvents, it has been estimated that the maximum pathlength in a transmission flow-through cell having plane-parallel windows should be 200 µm with hexane and only 25 µm when water is the mobile phase~~ cells ~~**(13)**. Another type of transmission cell that has been developed is a narrow bore tunnel along the axis of a cylindrical crystal. The solution flows through the tunnel in the crystal while the infrared beam is passed through the crystal at right angles and focussed onto the solution. This technique reduces the effective solvent thickness to below 20 µm and matches the cell volume to that of a microbore HPLC column~~ or specialized IRE accessories **(14)**.

~~7.2.2 A flow-through cell that uses a ZnSe IRE is commercially available~~. Alternatively, the LC effluent may be deposited onto infrared transmissive powders **(15)**. ~~The micro CIRcle ® cell[5] has a relatively large volume for HPLC work (24 µL), but reasonable results have been obtained with normal-phase and aqueous reversed-phase solvents for solute concentration of 1 to 2 % (w/v). Better sensitivity is obtained with normal-phase solvents, but~~ or moving substrates **(16)** for analysis by diffuse reflection or reflection absorption spectroscopy. The commercially available LC/IR systems can offer detection limits ~~are estimated to be between 250 and 500 µg **(16)**~~.

~~7.2.3 With careful selection~~ at the microgram level for a broad range of ~~common normal-phase HPLC solvents, infrared data over specific frequency regions can~~ separations. (see Practice E 2106).

7.2 Caution must be ~~obtained. Hydrocarbon solvents~~ taken when interpreting LC/IR results as solvent interferences may ~~make it very difficult to observe the C-H stretches~~ obscure critical areas of the ~~solutes, but with the development~~ spectrum necessary for correct identification or interpretation of ~~microbore HPLC columns, it is now possible to take advantage of~~ the ~~reduced solvent volumes~~ analyte spectra. Cross contamination and ~~use~~ peak tailing is also more ~~expensive deuterated solvents~~ prevalent in LC/IR interfaces.

7.3 Supercritical fluid chromatography (SFC) can also be coupled to ~~open up the C-H stretching region~~ an FT-IR spectrometer

(17). For aqueous reversed-phase chromatography, Jinno *et al.* used a PTFE cell and a CD_3CN/D_2O solvent system with a microbore column to observe carbonyl and C-H stretches **(18)**. In a reversed-phase interface for analytical columns the solute was extracted from the aqueous phase into an organic solvent, which diffused through a hydrophobic membrane and was eventually sent to the infrared flow-through cell **(19)**.

7.3 Solvent-elimination HPLC/FT-IR has been accomplished with solute deposition on KCl powder **(20)** or a moving KBr plate **(21)** for diffuse reflectance or transmission spectroscopy, respectively. In the presence of aqueous solvents, it is necessary to use on-line extraction with an organic solvent **(22)**, post-column reaction of water with 2,2-dimethoxypropane **(23)**, or direct elimination of water **(24)** followed by diffuse reflectance spectroscopy (on KCl or diamond powder) or reflection-absorption spectroscopy. A simple method for solvent elimination is to manually collect several fractions of the eluent into small glass containers, add a small amount of potassium bromide or diamond powder, and allow the solvent to evaporate, using gentle heat, if necessary. The resulting powder is then examined using a diffuse reflectance or pressed pellet method.

7.4 Supercritical fluid chromatography (SFC) has recently been coupled to an FT-IR spectrometer **(25)**. High-pressure flow-through cells and or solvent-elimination have been incorporated into a single interface, and detection systems exist, offering dection limits for the system are between 10 and 40 ng.

8. Analysis of Thin-Layer Chromatographic Fractions

8.1 The spots containing the components of interest, plus the associated absorbent, are generally collected by scraping them from the plate; the components can be recovered by extraction with a suitable infrared solvent, and the spectrum of the solution can be determined by the usual methods. If preferred, the spectrum of the analyte may be obtained after transference to a porous triangle of KBr (see 5.7.5).

8.2 Extraction of the spot is usually required before spectral determination of the component of interest because the common TLC absorbants (silica gel and alumina) are infrared absorbers. Potassium bromide (KBr) can be used as an absorbant for some systems. When the areas of KBr containing the components of interest have been located, the adsorbent is recovered as before and either a KBr pellet is prepared in the conventional manner, or a spectrum is obtained by the diffuse reflectance method. An automated extraction system for analysis by diffuse reflectance has been described **(26)(18)**.

NOTE 97—The quantity of analyte available from any one spot may be insufficient to produce a usable spectrum. In this case it is usually necessary to stripe the sample onto preparative TLC plates and to recover the total eluted band in which the sought components are located. Programmed multiple development, a form of TLC in which the chromatography is performed using several developments, often concentrates the TLC spots of sample so that sufficient quantities of material are present to give identifiable IR spectra.

8.3 Quantitative or semiquantitative estimates of concentrations may be obtained from direct comparison of values for an unknown sample with those obtained for a standard sample.

9. Analysis of Paper Chromatographic Fractions

9.1 The areas of interest in paper chromatograms are cut from paper. These fractions may be recovered by solvent extractions, as in 8.2, or may be examined in-situ using infrared reflectance techniques. With the latter method, spectral subtraction is used to eliminate contributions from the paper substrate. The reference spectrum used for subtraction should be obtained from a piece of the paper that has been treated with the solvent used.

10. Analysis of the Gases Evolved from a Thermogravimetric Analyzer

10.1 As a sample is heated under a controlled atmosphere in a TGA experiment, gases may be evolved from the sample during times of weight loss. Various methods have been devised to allow for the analysis of these gases by infrared spectroscopy. The evolved gases are generally mixtures of volatiles, which could be decomposition products, water of crystallization, residual solvent or monomer, or even the gases evolved during an in-situ reaction (for example, polymer curing). The composition of the mixture evolved from a particular sample depends greatly on the nature of the surrounding atmosphere and other variables such as the heating rate and sample morphology. Detection of µg amounts of evolved gases can be achieved with an FT-IR spectrometer, which represents a 0.01 % weight loss from a 10-mg sample.

10.2 The evolved gases can be trapped in the condensed phase for subsequent infrared analysis. The total purge stream, which includes the evolved gases, is normally passed through a cold trap or a solvent. The resulting condensed phase can be examined directly, or more commonly by GC/FT-IR analysis (see Section 6).

10.3 The evolved gases can be passed through a transfer line into a gas analysis cell **(27, 28)(19, 20)**. **Both the transfer line and cell need to be heated to avoid condensation of high-boiling materials. Using a dispersive spectrometer, it is necessary to stop or divert the flow for the necessary analysis time. An FT-IR spectrometer can record spectra continuously during the experiment without the need to alter the normal flow rate. TGA/FT-IR accessories are available for FT-IR spectrometers, and some combined TGA/FT-IR instruments are also available (28). available. With such equipment, it is possible to measure the evolution of some individual gases, even though they are evolved as part of a mixture. This cannot Detection of µg amounts of evolved gases can be done achieved with an FT-IR spectrometer, which represents a free-standing TGA. 0.01 % weight loss from a 10-mg sample. (see Practice E 2105)**

11. Infrared Spectroscopy Using a Microscope (29-31) (21-23)

NOTE 108—Names that have been used referring to this technique include viewing infrared microspectroscopy, infrared microspectrometry, infrared

microspectroscopy, and micro IR. Infrared ultramicrospectrometry (or= ultramicrospectroscopy) refers to a special method in which the sample is physically masked to below the diffraction limit (smaller than 20 µm).

11.1 Spectra collected with infrared transmitting microscope accessories can differ from conventional spectra in several important aspects. Therefore, care should be taken to carefully document the experimental conditions used when spectra are obtained by infrared microspectroscopy. The most important difference is the fact that the spectra may be affected by the diffraction properties of infrared radiation. The cross sections of the samples being measured can be similar in size to the wavelength of radiation used to analyze them. Since the sample area is defined by masking at an image plane, and diffraction of the radiation affects the spectra recorded, this can show distortions in band shape or in relative intensity, or in both.

11.1.1 The experimental parameters to be recorded when publishing results of an infrared study using a microscope are: (*1*) the area of the specimen being analyzed, (*2*) the size and type of the detector element, (*3*) whether the spectra were obtained using the transmittance or reflectance mode, (*4*) the specimen geometry and method of preparation, and (*5*) the shape, location, and type of image plane masks used. Important instrumental conditions also to be recorded are the spectral resolution, the data collection time, and the nature of the reference background spectrum. It should be remembered that it is also critical to report any computer manipulation of the spectrum, such as baseline correction or subtraction.

11.1.2 The spatial definition of the sampling area obtainable with a microscope using infrared radiation is limited by diffraction effects arising from the relatively long wavelengths of radiation involved. This diffraction effect is wavelength dependent and thus is particularly noticeable below a frequency of about 1000 cm^{-1}(10 µm). The area of the specimen from which the radiation is collected increases with wavelength, and thus the spectrum obtained represents an increasingly larger area as the wavelength increases.

NOTE ~~11—The~~ 9—The energy from a point, when imaged by an optical system, does not come to a point, but rather to a central bright spot followed by a succession of dark and bright rings **(291)**. The bright rings are called lobes or pods, and they contain energy from the original point. For any unobscured optical imaging system, roughly 85 % of the energy is in the central maximum of the pattern. (The objectives used for infrared microscopes have a central obscuration, which lowers the apparent energy in this region, typically by some 10 %.) The remainder of the infrared energy lies in the bright rings, which will be outside of the optical image and thus may be absorbed by unexpected parts of any sample that is larger than the aperture used. To illustrate what the implications of the resolution limit are for infrared microspectrometry, consider the longest infrared wavelength of interest, for example, 20 µm (a frequency of 500 cm^{-1}). When this wavelength is used in the diffraction equation **(291)**, along with a numerical aperture of 0.5, the calculations indicate that *for a point source* the first dark ring occurs at 24 µm from the sample edge. Successive dark rings occur at 44, 64 and 84 µm. Roughly 5 % of the energy from the point source is still present beyond the fourth dark ring. In practice, of course, the source used must have significant size.

11.2 Microscope attachments are commercially available that allow for spectra to be recorded in a transmittance mode, where the beam passes through the specimen plane, or in a reflectance mode, where the beam reflects at the specimen plane. Reflectance may occur at the specimen surface, from a reflective support, or sometimes at both planes.

11.3 All commercially available microscope attachments for infrared microspectroscopy allow for the positioning of an aperture of variable size at a specimen image plane, or planes, in the optical path of the microscope. The function of this aperture is to limit the area of specimen being studied. The image plane where the remote aperture is placed is an optical conjugate of the specimen plane, related in size through the magnification of the optical system. Thus, a relatively large aperture can be used to mask a small dimension of the specimen. Round and rectangular variable apertures are available to the user. The aperture geometry should be selected so as to match the shape of the desired specimen area as closely as possible. This is particularly important for photometric accuracy when recording spectra of small samples. Radiation reaching the detector that does not pass through the sample will cause distortions in relative absorption intensities.

11.4 An additional remote image mask may be placed at an image plane before the specimen, so that there is an aperture before and after the specimen. This aperture needs to be the same size as the limiting aperture mentioned in 11.3. When using the reflectance mode of a microscope equipped with dual remote image masking, the radiation normally passes through the same aperture before hitting the sample as it does after reflecting off the sample surface (that is, the one aperture serves both functions).

11.5 It is very important that the optical alignment of an infrared-transmitting microscope be well maintained in order to obtain good results. Both the infrared and the visible beam paths need to be co-linear and co-focal at all times; otherwise spectra can be recorded from an area different from the one visually examined. The alignment procedure for a microscope operating in the transmission mode involves the use of a small aperture, typically a 100-µm pinhole, at the sample position. With this 100-µm aperture installed at the sample position, insert one or both remote apertures having equivalent size to that in the sample plane, and align these apertures visually so that they all appear coincident. Switch to the infrared mode and maximize the infrared energy through these apertures, following the manufacturer's instructions. Check that the visible light and the infrared radiation are still collinear after this adjustment, and at regular intervals.

11.5.1 Of particular importance is the concentration of the primary and secondary mirrors of the Cassegrain objective or the condenser, or both. Unless absolutely necessary, ~~this adjustment~~ adjustments to the optical system of an infrared microscope should not be made without specific instructions available to achieve proper alignment.

11.6 *Sample Handling Considerations*:

11.6.1 While the use of a microscope for IR sampling simplifies the analysis of many samples, sample preparation is critical to obtaining the desired spectral measurement.

11.6.2 The collection, handling, and mounting of microscopic samples must be considered in terms of the sample geometry

needed for IR spectral measurements. For IR spectral measurement of organic materials in the transmission mode, a sample thickness from 5 to 20 µm is most desirable. Thick samples cause both loss of detail in regions of strong absorption and distortions of absorption ratios. While sample areas 10 by 10 µm can be analyzed, larger areas provide spectral data with higher signal-to-noise ratios. To minimize diffraction effects, the smallest sample dimension should be approximately five times the wavelength of interest when using a single mask and two times the wavelength when dual remote image masks are used (see Note 12~~0~~). When possible, a thin, uniform sample with as large an area as is practical should be selected. The maximum sample area is determined by the microscope/spectrometer optics and the detector element size. In most current IR microscopy systems the maximum sample area is 250 by 250 µm, even though the specimens may be many times this size.

NOTE 12~~0~~—The actual values of sample dimensions for minimizing the diffraction contributions are dependent on the numerical aperture of the microscope objective used. For example, with a numerical aperture of 0.5, a sample size of five times the wavelength for a single aperture system and 2.5 times the wavelength for a microscope with dual remote image masks will lead to approximately 95 % of the incident radiance passing through the defined sample area. With a 0.25 numerical aperture objective, the same conditions will be met when the sample dimensions are approximately ten and five times the wavelength, respectively.

11.6.3 Collecting, mounting, and thinning of samples for microspectral measurement require special techniques and tools. Since the largest sample areas that can be analyzed by IR microscopy are just resolved by the unaided human eye, sample preparation is aided greatly by using a low-power stereo binocular microscope. Magnifications of 7 to 20× are most useful for locating and mounting samples for analysis. Fine needles, tweezers, spear pointed probes, razor blades, and scalpels are valuable tools to extract and manipulate samples. Rollers, presses, compression stages, and microtomes are used to reduce sample thickness. These tools and techniques are described in detail in the following sections.

11.6.4 Liquid and solid samples are often mounted on thin salt windows. It has been observed that the best spectra are obtained when the windows are quite thin, as this has the least effect on the optical system of the microscope. Typically salt or KCl can be cleaved to produce windows with surface dimensions of a few millimetres and a thickness of 1 to 2 mm. These salt plates are easily cleaved to size with a single edged razor blade and tapping tool such as a screwdriver handle. The razor blade is placed on the surface of the salt parallel to a prominent fracture edge. The handle of the screwdriver is then gently tapped onto the back edge of the razor blade, causing the razor blade to cleave the crystal. It is very convenient to mount these small crystals over a hole in a piece of cardboard or manila folder using a small amount of nitrocellulose cement, or over a hole in a thin aluminum plate with a small amount of rubber cement.

11.6.5 Solid particles are easily placed on the salt plate with a fine pointed tungsten needle (see 11.6.10). If the particles are quite thin, they need only be placed on the surface of the salt plate. It is quite useful to score the surface of the salt plate with the needle to produce a simple map such that the particle can easily be found unequivocally under the microscope. If the small sample is quite thick, a variety of different techniques can be used to reduce it in thickness.

11.6.6 Micropipettes are useful for applying small amounts of reagents, adhesive, or solvents to salt plates for particle manipulation. Glass micropipettes can be purchased from several suppliers or produced by reducing the diameter of a glass tubing, using typical glass-blowing techniques. Micropipettes are also useful for applying small amounts of liquid to the surface of the salt plate for analysis. An alternative technique for placing a liquid on a salt plate is to evaporate a solution of the liquid on the surface of the salt plate from a microbrush (see 5.7.6).

11.6.7 When small amounts of liquids are to be analyzed, they have to be kept in a restricted area of the salt plate, or the analysis is extremely difficult. This can be accomplished in several ways. One procedure is to rupture the surface of a salt plate in a small area with repeated jabbing of that region with the end of a small microprobe. The small salt crystals that are produced are allowed to remain in the small well, and the liquid is added to that well. The capillary spaces between the particles retain the liquid in situ, minimizing spreading.

11.6.8 Another useful procedure for analyzing small amounts of liquid is to place a micro flat of salt on the surface of the somewhat larger salt plate. The liquid is then allowed to flow between these salt plates by capillary action, the liquid being applied to the edge of the junction of the two salt plates with a micropipette.

11.6.9 Spreading of droplets of liquid can be minimized by placing the salt plate on the gently heated surface of a small metal washer. This applies additional heat at the outside of the salt plate and somewhat less heat near the center of the salt plate. The result is that the droplet is forced toward the center. This technique can often minimize spreading sufficiently so that good infrared spectra can be produced from very small amounts of liquid.

11.6.10 *Fine-Pointed Tungsten Needles*:

11.6.10.1 Fine-point tungsten wire needles are very useful for the extracting and handling of microscopic samples for IR microscopy. Tungsten microprobes can be purchased from ~~at least one source.[5] It~~ several sources. It is moderately simple to produce or resharpen such microprobes. A tungsten wire of approximately 22 gage is inserted into a pin vise. For ease of use this pin vise should not exceed about 6 in. in length, and 5 in. is probably a better dimension for most users. The tungsten needles are sharpened by chemical or electrolytic etching.

11.6.10.2 *Method 1*: Chemical Etching—Tungsten wire is chemically etched by sodium (or ammonium) ~~nitrite (**Precaution**—see Note 13)~~ nitrite) to produce a smooth, fine, stiff, pointed needle **(Warning:** see 11.6.10.3). Start by placing a few grams of sodium nitrite into a small crucible. Because the reaction between the sodium nitrite and tungsten is highly exothermic, this crucible should have a handle or be held with tongs. One end of a short length, 5 cm (2 in.), of 18 to 22 gage tungsten wire is

heated to red heat in a flame and then thrust into the sodium nitrite. When the etching reaction occurs, a sustained glow will be seen. Repeated dipping of the wire into the glowing melt created by this reaction will produce a fine point. This can also be accomplished by placing a fragment of sodium nitrite on a Meker-type burner and allowing the salt to melt. The needle is then heated in the flame and dragged through the molten sodium nitrite. Another procedure is to hold a sodium nitrite stick near the edge of the flame and, after heating the needle in the flame, to drag it through the molten end of the sodium nitrite stick. Salt residue remaining on the needle tip can be removed by washing with water. ~~NOTE 13—~~**Precaution:** ~~The~~

11.6.10.3 **Warning:** The reaction between tungsten and sodium nitrite is strongly exothermic and may cause spattering of hot material. Eye protection is essential.

11.6.10.3~~4~~ *Method 2: Electrolytic Etching*— Tungsten wire will etch to a fine-pointed tip in sodium or potassium hydroxide electrolyte under mild conditions of 6 to 12 volts DC or AC. If DC, the positive lead from the power supply is attached to the tungsten wire while a carbon electrode serves best as the negative electrode. Etching rates are controlled by the current flow. Repeated dipping is recommended until the desired tip is formed. After several minutes a needle with a rather fine tip will be produced.

11.6.10.4~~5~~ In use, these needles are usually mounted in a needle holder. For convenience it is recommended that both ends of the wire be pointed so that the needle can be reversed in its mount when the first tip becomes damaged.

11.6.11 *Microbrushes*—Microbrushes are very useful for handling small amounts of liquid. See 5.7.6 for their usage and manufacture.

11.7 *Procedures for Thinning Samples*—Microparticles are commonly too thick to give good quality infrared spectra. If this is the case, it is desirable to thin them until their infrared absorbances of interest have a maximum of about one. Samples that are too thick may often be squeezed to a more suitable thickness for infrared spectroscopy. Since the length and width dimensions of samples for microspectroscopic analysis are usually quite small, only moderate forces are necessary to cause the materials to be flattened. This can often be done under a stereo microscope using a microprobe. If the sample is tacky, this should be done on a small salt window. If the sample is hard, the rolling should be carried out on a hard surface and then transferred to a suitable mount. Metal (or foil) is a useful surface for this purpose, but if the sample is light in color, a flat black phenolic jar cap allows for better visual observation during the operation. It should be noted that any attempt to thin a sample by application of pressure may result in morphological changes to the sample.

11.7.1 *Pressing Between Polished Metal Plates*—The sample may be placed between the polished anvils of a KBr pellet press, without KBr, for squeezing. The thinned sample can either be peeled from the platen with a needle or scalpel and placed on a sample mount for transmission analysis, or measured in a reflection mode directly on the anvil. A clean area of the anvil is used as the reference and a reflection/absorption spectrum of the sample is recorded.

11.7.2 *Squeezing in a Diamond Anvil Cell*— See 5.6.13.

11.7.3 *Compressing Samples Between IR Transmitting Windows*—Since microscopic samples are small, little force is required to compress samples to reduce their thickness for spectral measurement. Many infrared transmitting window materials may be used to compress micro samples. Barium fluoride, KRS-5, potassium bromide, sodium chloride, zinc selenide, and zinc sulfide have been used successfully with samples of rubbers, plastic, fibers, and organic crystalline materials. Simply pressing two windows together with the sample between them compresses the sample and provides optical contact between the windows and the sample, thus reducing surface losses. For this method 2-mm thick barium fluoride windows are recommended for harder materials and water solutions. While 2 mm thick windows of sodium chloride, potassium bromide, and KRS-5 provide an extended transmission range, they are softer and have reduced applications for compressing samples. If an elastic sample is to be examined and one does not have available a device for continuously applying pressure to a salt plate, another technique is usable. The elastic sample is placed between two salt plates. The plates are pressed with the heel of a probe, causing the sample to thin. While pressure is maintained on the sample, small amounts of an adhesive, such as a viscous nitrocellulose solution, can be applied to the edges of the salt plates. After the nitrocellulose adhesive has dried, the sample between the salt plates will then remain in compression and can be readily analyzed.

11.7.4 *Rolling with a Polished Bearing*— A stainless steel bearing, mounted on a suitable axle, can be used as a roller device to thin samples. Such a device can conveniently be attached to the far end of a probe, or scalpel, providing a dual purpose tool. The total force required to roll an organic material into a flattened sample is very small, while the pressure exerted on the sample can be very high. These roller devices are very effective and have been used with both organic and inorganic powders. They are particularly useful for flattening a single fiber for spectral identification. Samples can be rolled onto a metallic surface and examined in reflection or onto infrared transparent windows for transmission. Fibers are often flattened on a glass microscope slide, then peeled off and mounted over a small hole in a metal plate. The roller bearing is one of the more convenient sample flattening devices for micro samples.

11.7.5 *Pressing with the Heel of a Probe or Side of a Needle*:

11.7.5.1 A small sample can be placed on a surface and pressure applied with the flat end of the needle holder handle. Even moderate pressure will usually produce considerable thinning of the sample. A needle or spear point probe can also be used to reduce the sample thickness. This thinning is accomplished because the forces per unit area on a very small particle can be quite high, even with moderate applied force, because of the small area of contact. If the sample is fairly hard, it can often be thinned by pressing on it with the side of a sewing needle which is held in a pin vise. Again, the small contact area of the side of the sewing

needle increases the force per unit area considerably. This needle can be rolled across the sample, producing a thin flake.

11.7.5.2 Materials with a high concentration of fillers, such as polymers, can be analyzed with infrared spectroscopy after thinning the sample with pressure or by cutting a very thin wedge of the sample with a sharp blade. Often the filler is not uniformly dispersed and clear regions can be found for analysis.

11.8 Micropyrolysis (see 5.8) is another method for the examination of highly filled polymers. The very small amount of pyrolyzate obtained is most conveniently analyzed using the infrared transmitting microscope, especially if it has been localized in an indentation in a salt window using a microcapillary brush, as discussed in 5.7.6.

11.9 *Refractive Index Matching Mounts and their Limitations*—A thin film of mineral oil and perfluorinated hydrocarbon oil on the sample mount acts to retain powdered samples, as well as to match their refractive index. This aids the analysis by reducing reflection problems. Both mineral oil and perfluorinated hydrocarbon oil are used in infrared spectroscopy to suspend fine particles of a solid sample as a mull because they have few absorption bands. When used in series, the full spectrum of the sample can be recorded. The major function of the mountant in IR microspectroscopy is to reduce or eliminate the surface reflections from a solid sample that can cause distortion of absorption measurements. These mountants also reduce reflective or scattering losses and can thus improve spectral measurements. The difficulty in using an index matching mountant lies in correcting for its presence. An interactive subtraction of the mountant spectrum may be necessary. The film should be thin enough so that its spectrum will allow for a good subtraction. Regions of the spectra where the absorption bands of the mountant spectrum exceed 1 A should not be considered reliable after spectral subtraction.

11.10 *Microtoming and Mounting Materials*:

11.10.1 Microtoming, that is, the cutting of thin sections, is a recognized microscopical technique that applies well to infrared microspectroscopy. Microtomes are commercially available in many styles: they consist of a means of moving a sample relative to a knife in order to cut a thin layer of material from the sample. The sample can be repositioned and the cutting process repeated. For light microscopy, sections 0.5 to 20 µm in thickness are common, and ~~these same thicknesses are also good~~ this sample thickness works well for IR spectroscopy.

11.10.2 While samples can be sectioned directly, supporting the sample during sectioning is more common. Paraffin wax is the preferred embedding medium for IR microspectroscopy. Wax cuts well and is generally easily removed with warm xylene. It is chemically similar to Nujol and thus also has minimal interferences in IR spectroscopy. A similar material used is beta-pinene wax. Other embedding materials commonly used for light microscopy are acrylic and epoxy resins. These are difficult to remove after sectioning and can cause spectral interferences, and hence are not recommended for the IR application. The size and porosity of the sample will often determine if plastic embedding can be used. Care in selection of embedding materials is necessary since these materials can alter the sample by reacting with it, dissolving it, or contaminating it. An alternative method for supporting the sample is to employ a thin, free-standing polymeric material that may be easily removed, if desired, after the microtoming has been completed.

11.11 An infrared transmitting microscope equipped with a polarizer can be used for studying the dichroic properties of materials.

11.12 *Reflectance Infrared Microspectroscopy*:

11.12.1 Many samples that are inconvenient to examine by transmittance using an infrared transmitting microscope can often be examined in the reflectance mode. This is a convenient way of recording spectra of samples that are too thick for good quality transmittance spectra, or are on a non-transmitting substrate. In general the technique requires no sample preparation and is nondestructive.

11.12.2 Different types of reflectance spectroscopy are used to obtain spectra, depending on the type of sample surface being examined. If the sample is a thin film on a reflective surface, then infrared reflectance/absorbance spectroscopy (IRRAS) is used **(3~~2~~4)**. Other types of samples are examined by diffuse or specular reflectance.

11.12.3 Infrared reflectance/absorbance spectroscopy can be used to study films having a thickness in the approximate range from 0.2 to 20 µm **(3)** that are covering a reflective surface. The infrared energy passes through the sample twice, since it travels through the sample, is reflected at the interface, and exists along a reflective path. The actual distance the beam travels through the sample depends on the microscope objective being used, but it is more than twice the sample thickness since the beam is focusing at the reflective surface. The reference background spectrum is recorded using a clean area of the reflective surface, or if necessary, a standard reference mirror. Typical samples include coatings on metal beverage cans, coatings on high-refractive-index materials such as silicon, and thin lubricant films on metals.

11.12.4 Materials that have a reasonably flat or shiny surface may reflect sufficient infrared energy to obtain spectra in the reflectance mode. Typical samples include polymers (films, pellets, and molded or extruded parts), contaminants on electronic components, and hard substances such as teeth, minerals, and paints. It is possible to obtain spectra of surfaces that are apparently rough, by diffuse reflectance.

11.12.5 The actual spectrum obtained from samples will often arise from both front-surface specular reflection and diffuse reflection. The actual ratio of these two effects is a function of surface roughness; diffuse reflection will occur most when the roughness spacing is similar in size to the infrared wavelengths of interest. As a consequence the observed specular reflectance/diffuse reflectance ratio may vary across the spectrum.

11.12.6 The penetration depth of front-surface reflectance is very small and is similar to that achieved using IRS. Spectra

recorded by front-surface reflectance will show significant differences from spectra recorded by transmission microspectroscopy, or spectra from bulk samples. The effective depth of penetration is a function of wavelength, and increases as the wavelength increases. In addition to this physical effect, other chemical effects, such as surface oxidation, surface contamination, or migration of plasticizer or other additive to the surface, may alter the spectrum of the material.

11.12.7 The spectra observed by front-surface reflectance (especially those from highly reflective surfaces) commonly show band distortions, such as band shifts, inversions, or derivatization. These effects arise from an absorption of a dielectric material being accompanied by a change in the refleractive index of the material at the same frequency. The observed spectrum is related to n', which is a complex function **(33)** including the ~~pseudo-absorbance~~ absorbance (k) and refractive (n) index components:

~~$$n' = n - ik \quad (1)$$~~

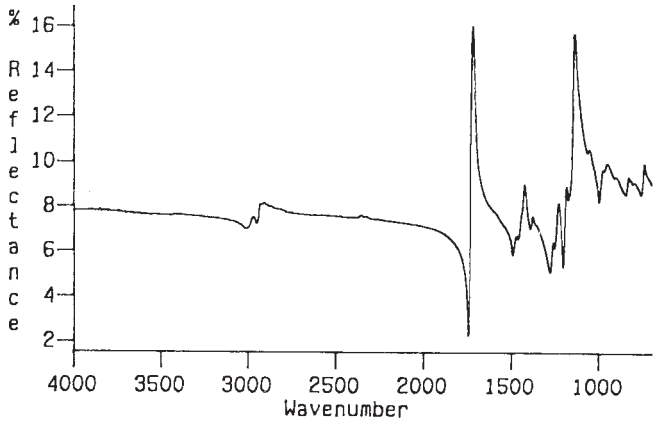
$$n' = n + ik \quad (1)$$

In the case of pure specular reflectance, the Kramers-Kronig transform **~~(33, 34)~~(25, 26)** may be used to calculate both the real and imaginary parts of this complex function, and thus extract terms that are related to both the absorbance spectrum and the refractive index as a function of frequency. Computer software to perform these calculations is available from some instrument vendors and software houses.

11.12.8 To demonstrate this relationship, Fig. 1 shows the microreflection spectrum from a poly (methyl methacrylate) sample, in units of percent reflectance, versus a gold mirror reference. This spectrum includes a contribution arising from reflection at the back (inside) surface of the sample, as well as the front surface external reflection. The back surface reflection gives rise to double-pass transmission of energy, and so the spectrum includes some features due to absorbtion inside the sample. The computed n component (which includes the refractive index) is shown in Fig. 2, and the k component (which includes the absorbance spectrum) is shown in Fig. 3. Due to the second surface reflection, the experiment does not yield quantitatively correct n and k spectra. These components may be used for qualitative purposes, but care must be taken in their numerical interpretation. The n values in Fig. 2 are about 50 % higher than the correct value for the refractive index.

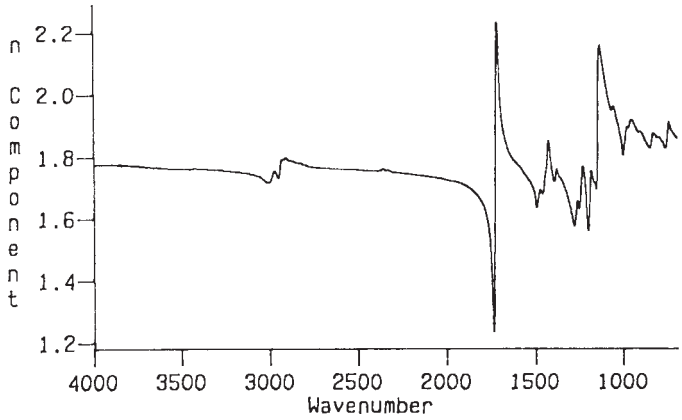
11.13 *Spatial Mapping of Spectral Features*—Some infrared microspectroscopy systems may include computer control of the microscope sample stage. This computer control may be used to obtain an array of infrared spectra which are recorded as a function of sample position within the area defined by the movement of the sample stage. This data array may be composed of spectra sampled along a straight line, a 'linemap', or sampled as an *x-y* grid. The movement of the sample stage is controlled by the computer which uses a setup file created by the user. This file will include: (*1*) the step size between sampling points, and (*2*) the overall dimensions of the line or area to be ~~'~~ "mapped~~'~~". Computer analysis of the data array may be utilized to extract information on the distribution of chemical functional groups across the mapped area, accomplished by measurement of the absorbance of a selected frequency or frequencies in the array of spectra. The processed information may then be used to show the distribution of a particular peak or peaks as a function of position in the mapped area. These computer presentations take the form of a contour map, a false-color map based on the contour map, an intensity map, or a 3-D display of the data array. The spatial resolution obtained in the computer images results from: (*1*) the step size, (*2*) the actual sample variation acrosss the mapped area, and (*3*) the spatial definition of the sampling area as defined by the microscope aperture(s). It must be emphasized that the apparent spatial resolution obtained will be a function of wavenumber when using small aperture settings (see 11.1.2 and Note 9).

11.13.1 An extension of the spectral mapping experiment is accomplished by imaging of the sample analysis beam onto a focal plane array composed of detector materials such as InSb or MCT. The sample is interrogated with a single or series of wavelengths per "image," accomplishing much the same as outlined in 11.13, but without physically moving the sample and collecting sample



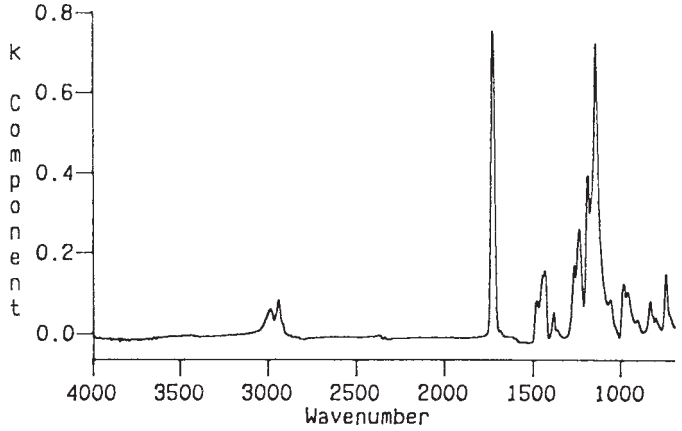
NOTE 1—Low values of percent reflectance are to be expected using this technique.

FIG. 1 Observed Microreflectance Spectrum of Poly (Methyl Methacrylate) Ratioed Against the Spectrum of a Reference Gold Mirror



NOTE 1—This component is related to the refractive index of the material as a function of frequency.

FIG. 2 Real Component (*n* portion) Resulting from the Kramers-Kronig Transform of the Poly (Methyl Methacrylate) Microreflectance Spectrum



NOTE 1— This component is related to the absorbance spectrum of the material, but is not corrected for the wavelength dependence of the intensity due to varying depth of penetration.

FIG. 3 Imaginary Component (*k* portion) Resulting from the Kramers-Kronig Transform of the Poly (Methyl Methacrylate) Microreflectance Spectrum

data from a series of discrete points in the sample. A relatively large area (0.5 to 2.0 mm) of a sample is imaged in one detector exposure, with the spatial resolution of the sample determined by the spacing of the detector array elements and the optical characteristics of the instrumentation.

11.14 *Internal Reflection Microspectroscopy*—Some infrared microscopes may use an internal reflectance sampling optic. After obtaining a suitable background spectrum, the optic is allowed to come into contact with the sample, enabling the collection of an internal reflectance spectrum from a small surface area. The ~~area~~ sample area is defined by the geometry of the internal reflectance element, in essence the ~~contact~~ area of the sample that contacts the internal ~~reflectance~~ reflection element. Common IRE materials are zinc selenide (ZnSe), diamond, germanium, and silicon. Diamond and ZnSe may allow the restricted visual observation of the sampling area through the IRE itself, whereas silicon and germanium objectives do not allow this visual aspect, and thus must be positioned most carefully to ensure that the selected area of the sample is investigated. Damage to the IRE may easily occur, so the IRE should be examined periodically using visual magnification. In addition, the small size of the IRE may result in high pressures being exerted onto the sample area and possible changes to chemical or physical properties, or both, of the sample. Also, migration of sample material to the IRE may occur. The IRE requires frequent examination to prevent cross contamination and damage to the IRE objective. It may also be possible to ratio a single beam spectrum of the objective, while not in contact with sample, versus the previous background spectrum to detect contamination of the IRE. The examination process may prevent the carryover of one sample to another as the IRE is being used.

11.15 *Low Angle Specular Reflectance Microspectroscopy:*—:

11.15.1 Some infrared microscope system may be equipped with a low angle (ISV70°) (~70°) specular reflectance ojective (see Note 141).

NOTE 141—This objective is sometimes referred to as a grazing angle objective, although the term 'grazing angle' generally refers to an angle of 85° or more.

11.15.2 This objective may be useful for recording spectra of small areas of reflecting surfaces and may provide an advantage over the reflectance method outlined in 11.12. It should be pointed out that the low angle of incidence results in an elliptical sampling area. Definition of the actual analysis area may be difficult to perform.

12. Keywords

12.1 infrared; microanalysis; molecular spectroscopy

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