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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:*

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 Qualitative Infrared Analysis[2]

3. Terminology

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

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

[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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[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.

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 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. 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.

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 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). 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 in the reference beam or by computer subtraction.

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.

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). Micro IRS accessories are commercially available for use with FT-IR spectrometers.

5.6.13 For the case of intractible 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).

5.7 *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, 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 5—A suitable commercial version of the KBr triangle is marketed as the Wickstick®.[4]

[4] Available from Harshaw Cochran Rd., Solon, OH 44139.

5.7.6 A microcapillary brush may be made to handle small volumes of solvent (see Note 6) and can be used to cast a film on a remarkably small area of a salt crystal. When a microbrush 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 6—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, highperformance 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 performed in an off-line manner.

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 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 chromatographic column.

6.2 Sometimes, however, the GC fractions are trapped separately in the condensed phase for subsequent infrared analysis **(7)**. These fractions are usually recovered by passing the stream issuing from the vent line of the chromatograph through a solvent, a powdered solid, or a cold trap.

NOTE 8—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 factors must be considered when HPLC and FT-IR are to be combined. 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 be considered since there are distinct differences between normal- and reversed-phase HPLC. Another consideration is the capacity (and size) of the HPLC column 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 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 **(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 **(14)**.

7.2.2 A flow-through cell that uses a ZnSe IRE is commercially available **(15)**. The micro CIRcle ^{R} 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 detection limits are estimated to be between 250 and 500 µg **(16)**.

7.2.3 With careful selection of common normal-phase HPLC solvents, infrared data over specific frequency regions can be obtained. Hydrocarbon solvents may make it very difficult to observe the C-H stretches of the solutes, but with the development of microbore HPLC columns, it is now possible to take advantage of the reduced solvent volumes and use more expensive deuterated solvents to open up the C-H stretching region **(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 solvent-elimination have been incorporated into a single interface, and detection 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)**.

NOTE 9—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

[5] Available from Spectra-Tech, Inc., Stamford, CT.

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)**. 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)**. 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 be done with a free-standing TGA.

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

NOTE 10—Names that have been used referring to this technique include viewing infrared microspectroscopy, infrared microspectrometry, infrared microspectroscopy, and micro IR. Infrared ultramicrospectrometry (or = spectroscopy) 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 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 **(29)**. 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 **(29)**, 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,