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Forensic Science Applications of Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS): I. Principles, Sampling Methods, and Advantages

ABSTRACT: Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) is a recently developed technique that offers several significant advantages over conventional methods of sampling solids. Some of these include: (1) minimal sample preparation (no sample preparation in a few cases); (2) a very wide range of sample concentrations which yield useful spectra; and (3) a very high sensitivity (less than 100 ng in some cases). DRIFTS has been applied to the analysis of a number of materials of forensic science interest. Specific applications for a variety of evidence types have been found; this work is therefore presented as a series. This paper presents the principles of the technique and examples of the sampling methods, including a microsampling method for liquids. Some of the unique advantages that DRIFTS offers for forensic science work, including better characterization of many compounds, are illustrated. In addition, an explanation of these DRIFTS features is discussed.

KEYWORDS: criminalistics, spectroscopic analysis, reflectance, Fourier transform, diffuse reflectance, DRIFTS, forensic science applications, infrared spectroscopy

Diffuse reflectance has been used for several decades in the ultraviolet, visible, and near-infrared regions as a special purpose sampling technique [*1–4*]. The optical accessories for such work have much lower throughput than conventional direct transmittance-type sampling arrangements, but intense sources and very sensitive detectors allowed such measurements to be performed readily. In contrast, before 1976 only very limited work had been done on diffuse reflectance sampling in the mid-infrared region with dispersive instruments [*5,6*], and these studies were concerned primarily with increasing the efficiencies of the various optical sampling devices or conducting certain specialized measurements. Following the advent of the commercial rapid-scanning Fourier transform infrared (FTIR) spectrophotometer, the possibility of performing routine diffuse reflectance measurements in the infrared region became apparent. In 1976, Willey [*7*] first demonstrated that a special purpose FTIR could be used for some types of diffuse reflectance measurements, albeit at low resolution with relatively long collection times. Most of the early pioneering work in the infrared, however, was performed (1978 to 1982) by Fuller and Griffiths [*8–13*], who coined the acro-

nym DRIFT (for diffuse reflectance infrared Fourier transform) spectroscopy (or DRIFTS as we refer to it) to describe this new technique.

Fuller and Griffiths showed that by using a commercial FTIR and a specially constructed, high-efficiency diffuse reflectance sampling accessory, good quality infrared spectra for a wide variety of solid samples could be obtained at medium resolution (2 to 4 cm^{-1}) and relatively short collection times (4 min or less). They investigated the effects of variations in a number of sampling parameters and conditions, such as choice of reference materials, particle size, thickness of samples and depth of penetration of the infrared beam, dilution factors, and so forth, on the resulting spectral data, and found optimum ranges of values for some of these.

For most solid samples, they found that the best results were obtained by mixing 1 to 10% of sample with finely powdered KBr or KCl and referencing this spectrum to that of powdered KBr or KCl. The diffuse reflectance spectra obtained from these samples, when converted into a form linear in sample concentration, were similar to the corresponding spectra (presented linear in absorbance) obtained from KBr pellets. The reflectance method, however, was found to be much more sensitive as Fuller and Griffiths observed a tenfold increase in band intensities of dilute samples (down to 53 ppm) when measured by DRIFTS, compared to those measured with conventional KBr pellets. The 13-mm-diameter pellets weighed approximately 200 mg, and the DRIFTS samples weighed approximately 20 mg; thus, one order of magnitude increase in spectral intensity was achieved with one order of magnitude decrease in amount of analyte. A detection limit of less than 100 ng was possible for some samples, and a recent review of infrared spectroscopy [14] states that although DRIFTS is not the most sensitive infrared method presently available, it is undoubtedly one of the most sensitive methods as far as routine techniques are concerned.

As an alternative method of sampling, Fuller and Griffiths suggested dissolving the analyte in a volatile solvent and adding powdered KBr. The solvent is then allowed to evaporate, leaving the KBr particles coated with the analyte. DRIFTS spectra obtained in this manner were found to have greater absorption intensities than those obtained when the same amounts of sample and KBr were simply ground together.

Fuller and Griffiths also presented a few examples of analytical applications of DRIFTS, including a brief section dealing with forensic science work; this section was limited, however, to the analysis of Empirin® and Valium® tablets [9,12,13]. Other applications that they investigated include both high performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) interfaces with DRIFTS [11,15]. Various other investigators have also demonstrated some specific applications of DRIFTS, including analyses of coal and geological samples [16,17], liquid stationary phases for gas-liquid chromatography [18], paint pigment photodecomposition [19], and cross-linking and degradation of acrylic coatings [20].

We have been assessing the use of the DRIFTS technique for crime laboratory analyses since 1982. It was found to be an extremely powerful tool that is well suited for many types of criminalistics work. This introductory paper presents the principles of the technique, examples of the various sampling methods, and some illustrations of the unique advantages that DRIFTS offers for forensic science analyses.

Theory

The physical processes involved in DRIFTS differ in some respects from those involved in conventional transmittance spectroscopy. It is therefore worthwhile to describe the principles involved. Most of this theory was developed by Vincent and Hunt [21] and Kubelka and Munk [22].

Specular Versus Diffuse Reflectance

When a collection of particles is irradiated, as depicted in Fig. 1, two different types of reflection can occur. Radiation that undergoes either single (for example, Photon 1) or multiple (Photons 2 and 3) reflections from the particle surfaces, but never actually penetrates the surface, constitutes specular reflectance. Even though each individual reflection has an angle of incidence equal to the angle of reflection, the surface irregularities and multiple reflections produce radiation reflected in all directions (mirror-type specular reflection only occurs when the surface irregularities have dimensions much smaller than the wavelengths of radiation).

Specular reflectance is described by the Fresnel equations, which for perpendicular incidence is given by:

$$R_s(\lambda) = \frac{(n - 1)^2 + n^2(\lambda^2/16 \; \pi^2) \; \alpha^2}{(n + 1)^2 + n^2(\lambda^2/16 \; \pi^2) \; \alpha^2} \tag{1}$$

Where $R_s(\lambda)$ = reflectivity of an incident ray, n = index of refraction of the sample, λ = wavelength of the incident radiation, and α = absorption coefficient of the particulate material. Note that both n and α are functions of λ. In the absence of absorption, the reflectivity reduces to $R = (n - 1)^2/(n + 1)^2$, and therefore, nonabsorbing materials generally reflect only a small fraction of the incident radiation.

For absorbing materials, α is nonzero, and for those cases in which α is quite large, Eq 1 approaches unity (which corresponds to complete reflection of the incident radiation); this is also the case for radiation that is not perpendicularly incident. Thus, strongly absorbing materials reflect more radiation than nonabsorbing ones, and the maximum specular reflection, as a function of wavelength, occurs near the absorption maximum. This maximum does not generally coincide with the absorption maximum, however, since the index of refraction changes rapidly ("anomalous dispersion") in the vicinity of the absorption band. This change is not symmetric about the absorption maximum; the refractive index peaks on the low frequency side [23]. A shift in the peak for the specularly reflected radiation relative to that observed for the absorption spectrum of the absorbing sample thus often results.

Specular reflectance is illustrated in Fig. 2a and b which depict, respectively, the absorption and reflectance spectra of the meprobamate layer of an Equagesic® tablet (which consists of two distinct layers containing meprobamate and aspirin). The absorption spectrum was obtained from a KBr pellet containing tablet shavings while the reflectance spectrum was obtained directly from the intact tablet. The specularly reflected peaks may be seen to be
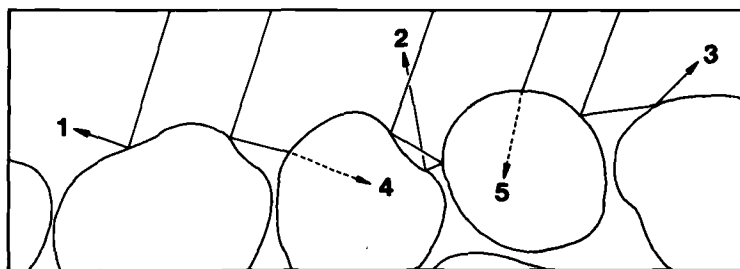

FIG. 1—*Specular versus diffuse reflectance for a collection of particles. The solid lines represent the incident and specularly reflected rays. while the dashed lines represent radiation that has penetrated the surfaces of the particles; the latter constitute the diffuse reflectance upon exiting from the sample.*
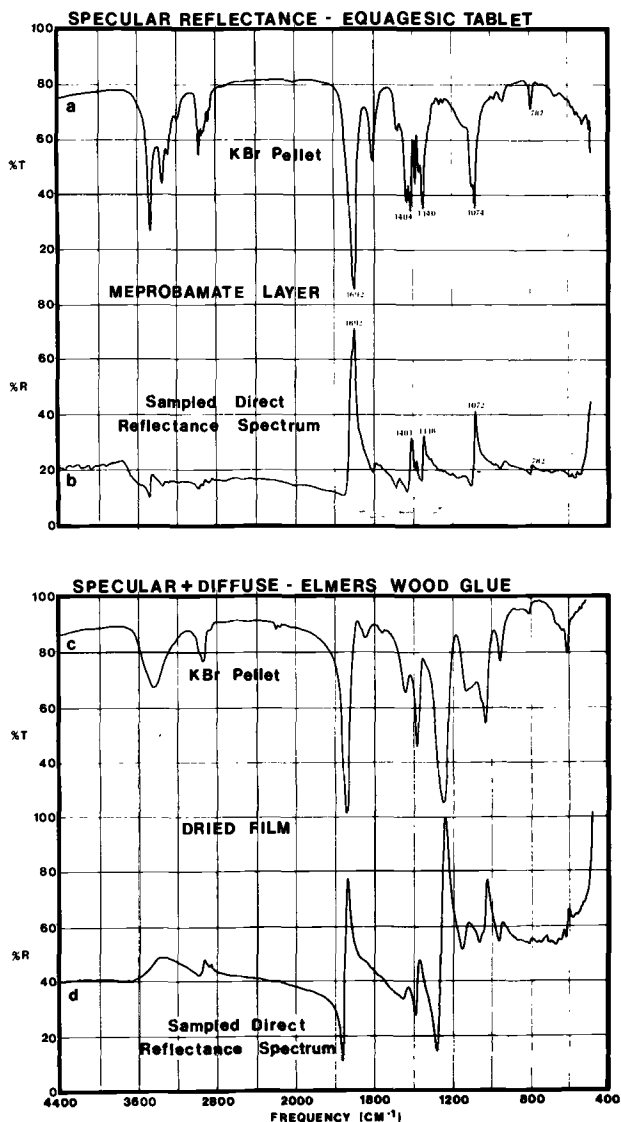
FIG. 2—(a) *Absorption spectrum of the meprobamate layer of an Equagesic tablet (KBr pellet).* (b) *Reflectance spectrum of an Equagesic tablet (meprobamate layer) sampled directly using DRIFTS.* (c) *Absorption spectrum of dried Elmers wood glue (KBr pellet).* (d) *Reflectance spectrum of dried Elmers wood glue sampled directly using DRIFTS.*

shifted (compare Fig. 2a and b) by 0 to 5 cm^{-1} to lower frequencies. In some cases, specularly reflected peaks may exhibit shifts to higher frequencies [21].

Diffuse reflectance is, by definition, those photons which emerge from the bulk sample after penetrating one or more particles. In addition to the initial refraction, these photons may have undergone a number of processes, including further refraction, scattering, diffraction, internal reflection, and even specular reflection (see Fig. 3). Those photons that are not absorbed will eventually exit from the macroscopic surface randomly in all directions. Any
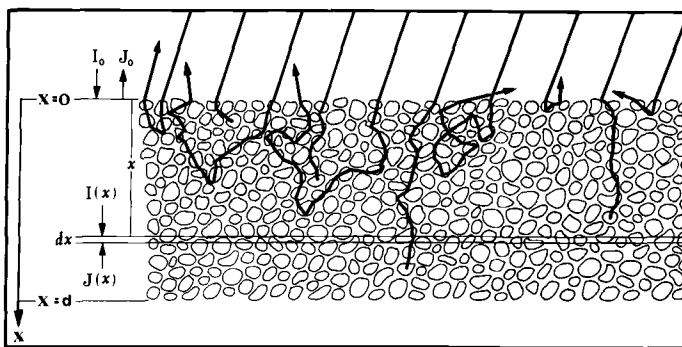
FIG. 3—*Schematic representation of the Kubelka-Munk model for diffuse reflectance from a homogeneous collection of absorbing and scattering particles.*

photon that penetrates a particle may be absorbed, as in transmittance spectroscopy. Every such particle can be considered a microscopic transmittance sample, with the photons absorbed according to the familiar exponential dependence on α.

Samples of moderate α, or those mixed with diluents of low α, allow most of the photons to penetrate at least one particle. For such samples, the dependence on α is far greater for absorption than for specular reflectance, so specular reflectance within the sample is relatively insignificant.

Spectra of a material which has significant amounts of both specular and diffuse reflection are shown in Fig. 2c and d. A piece of dried Elmers® wood glue (comprised primarily of poly[vinyl acetate]) was sampled directly giving the reflectance spectrum depicted in Fig. 2d; as a comparison, the absorption spectrum (KBr pellet) of this material is shown in Fig. 2c. The peak shifts produce, in this case, a "derivative" type split peak having a low frequency inverted component.

The radiation reflected from a collection of particles is thus comprised of two types, and it is important to remember that they exhibit opposite tendencies in regard to their spectral information. The stronger absorptions of a particular material generally produce more specular reflection than the weaker absorptions (since R in Eq 1 is quite small for the latter case and most of the radiation penetrates the particle surfaces). The relative amounts of specular and diffuse reflectance are also very strongly dependent on particle size. As particle size is decreased, specular reflection decreases; this arises from the fact that the smaller particles produce more multiple reflections, and this tends to reduce the amount of specularly reflected radiation. When a sample comprised of finely ground analyte diluted with powdered KBr is examined, for example, very little specular reflection occurs.

The Kubelka-Munk Model

Kubelka and Munk developed the most widely used model that describes the process of diffuse reflectance quantitatively [22]. In this model, it is assumed that a plane parallel layer of particles is illuminated randomly (isotropically) with monochromatic radiation. The particles, which have dimensions much smaller than the thickness of the layer, can both absorb and scatter the radiation (see Fig. 3; note that, for simplicity, the incident radiation in this figure is not isotropic). Specular reflectance (as defined above) is assumed to be negligible.

Let the vertical dimension (Fig. 3) be designated as the x axis, with $x = 0$ at the top surface, and $x = d$ at the bottom of the layer. The extension of the layer in the yz plane is assumed to be sufficiently large so that edge effects can be ignored. The intensity of the

radiation traveling in the positive x direction (downward) is represented by I, while that traveling in the opposite direction (upward) is denoted as J; the values of these at the surface ($x = 0$) are I_0 and J_0, respectively.

For an infinitesimal layer of thickness dx parallel to the surface and located a distance x from the surface, the intensity of the radiation incident upon this layer from above is $I(x)$, while that incident from below is $J(x)$. Since the radiation incident upon this layer is isotropic, the actual path length of a particular photon transversing dx is not dx, but $dx/\cos \emptyset$, where $\emptyset$ is the angle of incidence of the photon. Integration of this factor between $\emptyset = 0°$ and $\emptyset = 90°$ gives as the average pathlength a distance of $2dx$. The fact that $I(x)$ and $J(x)$ are actually isotropic is accounted for, to a good approximation, by using $2dx$ as the distance a photon crossing this layer travels.

Let the absorption coefficient of the absorbing material be designated by k and let s be a scattering coefficient. The latter represents the fraction of incident radiation that is *back-scattered* per unit distance. Consider now the decrease in intensity of $I(x)$ as it passes through the layer dx. The fraction $kI(x)2dx$ is lost through absorption, while the fraction $sI(x)2dx$ is lost by radiation scattered back toward the surface. At the same time, however, backscattering of radiation incident from below, $J(x)$, contributes an amount of radiation $sJ(x)2dx$ to $I(x)$. The total net intensity change in $I(x)$ as it passes through dx is thus:

$$dI(x) = -kI(x)2dx - sI(x)2dx + sJ(x)2dx \qquad (2)$$

Likewise, the net intensity change in $J(x)$ can be expressed as:

$$-dJ(x) = -kJ(x)2dx - sJ(x)2dx + sI(x)2dx \qquad (3)$$

Note that the two equations have opposite signs since they represent radiation traveling in opposite directions.

When these two differential equations are solved, expressions for the transmittance of radiation through the layer ($T = I_d/I_0$) and the diffuse reflectance of the layer ($R = J_0/I_0$) are obtained, as functions of k, s, and d. For an infinitely thick layer (a situation realized for infrared radiation at depths of 3 mm or more for finely powdered samples [13]), the diffuse reflectance, designated as R_∞, becomes:

$$\frac{(1 - R_\infty)^2}{2 R_\infty} = \frac{k}{s} \qquad (4)$$

This expression is known as the Kubelka-Munk equation, and the term on the left is usually denoted as $F(R_\infty)$.

It is worth noting that in the absence of scattering ($s = 0$), and for finite values of d, Eq 2 reduces to the Beer-Lambert law. Like the Beer-Lambert law, the absorption coefficient k is proportional to the product of the absorbtivity a and concentration c. Thus:

$$F(R_\infty) = \frac{(1 - R_\infty)^2}{2 R_\infty} = k' ac \qquad (5)$$

assuming that the scattering coefficient is constant. This expression thus relates the reflectance of the sample to its concentration in the same manner that Beer's Law relates the absorbance A of a sample to its concentration. The dependence of R_∞ on c is quite different from conventional transmittance sampling, however, and some of the consequences of this are discussed later. Also, it usually is not possible to measure R_∞ directly and the reflectance of a sample relative to a reference material, such as powdered KBr, is normally obtained.

Experimental Procedure

Instrumentation and Sampling

An Analect FX-6200 FTIR, equipped with a liquid nitrogen-cooled broadband mercury cadmium telluride (MCT) detector, was used to collect spectral data. Collimated radiation, produced by Analect FXA-501 collimating mirrors, was used for one of the two sampling beams of this instrument. A Hanning (raised cosine) apodization function operating in the normal resolution scan mode (which together provided a resolution of $5.2 \ cm^{-1}$) was used in all cases, unless otherwise specified. For most work, the instrument optical housing was purged with dry nitrogen.

Reflectance spectra were obtained using an Analect FXA-530 aspheric reflectance accessory; a schematic diagram of this accessory is depicted in Fig. 4. All spectra were obtained in the normal (uninverted mode). Although this accessory was designed to be used with collimated radiation, it was found to work nearly as well using the focused sampling beam, with minimal loss of sensitivity. Spectra were obtained using both the collimated and focused sample beams.

For the analysis of solid powdered samples, the Analect 12- and 2-mm diffuse reflectance sample cups were found to be either too large or too small for normal usage. The former required an excessively large amount (~ 530 mg) of powder, while the latter presented difficulty in achieving a lateral alignment (although the collimated beam is designed to focus to a spot approximately 1 mm in diameter, absorptions of aluminum oxide were usually observed when using the 2-mm cups). Well diameters of 4 mm were found to give optimal results for both the focused and collimated beams; these were used for all the powdered samples. All spectra were referenced to powdered KBr, and unless otherwise specified, interferometer gains of $4R$ ($R = \sqrt{2}$) were used for these samples.

Spectra of KBr pellets were acquired using the focused sampling beam. For these, an attenuater screen was also inserted into the beam, and a gain of 1 was usually used. Diamond anvil cell (DAC) spectra were obtained using a High Pressure Diamond Optics, Inc. (Tuscon, AZ) cell, together with a specially modified Analect ×4 beam condensor used in the collimated sample beam. Depending upon the sample and accessory used, 10 to 250 scans were collected (with the latter requiring approximately 4 min).

Presentation of Spectral Data

A number of different spectral formats have been used by Fuller and Griffiths, as well as others, for presentation of DRIFTS data. Some of these include: (1) the "raw" reflectance
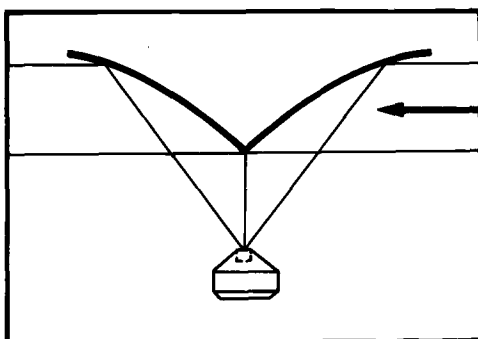

FIG. 4—*Schematic diagram of the Analect aspheric reflectance accessory used in this work. The 4-mm cup used for powdered samples is also shown.*

spectrum itself (or more correctly, the *relative* reflectance spectrum); (2) the Kubelka-Munk format of this data (see Eq 4); and (3) a log format presented as $\log_{10}$ (1/reflectance). The latter undoubtedly was chosen by analogy to the Beer-Lambert formula, $\log_{10}$ $(1/T)$ = $A = abc$; for DRIFTS data, however, this form has no particular significance as the Beer-Lambert law applies only to direct transmittance sampling.

The Kubelka-Munk format, in contrast, presents the data in a form linear in concentration, very similar to an absorbance spectrum. Kubelka-Munk spectra are thus generally presented in an "inverted" format (for example, Fig. 5*b*) in line with the convention used for absorbance spectra. This spectrum can then be converted into a "percent transmittance" (%*T*) spectrum.
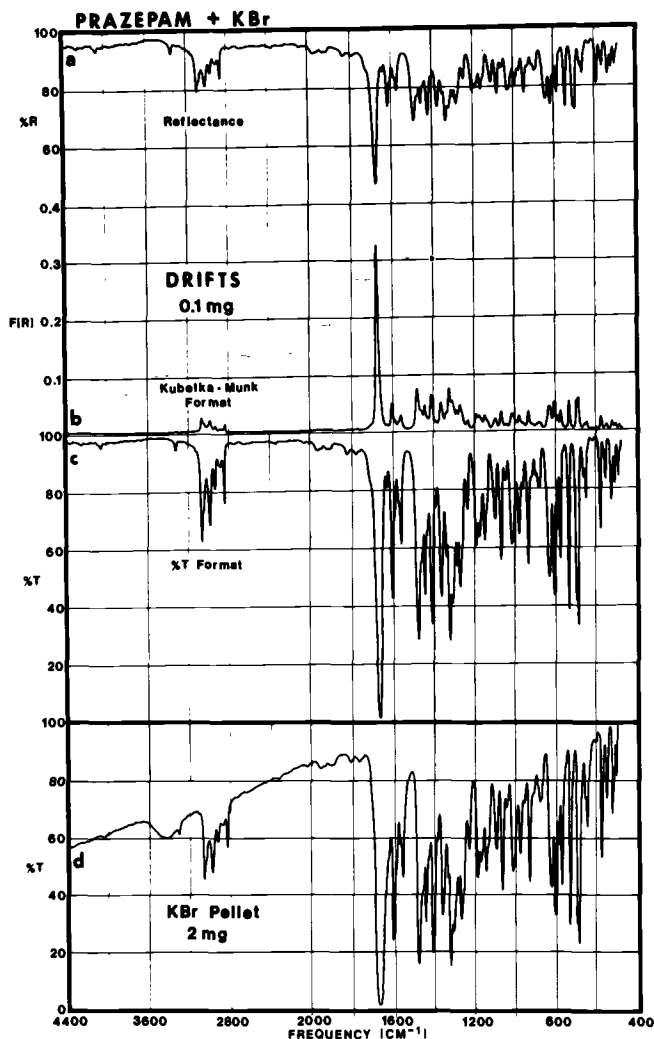

FIG. 5—(a) *Reflectance spectrum of 0.1 mg of prazepam diluted with KBr (1 ; 400 dilution by weight), 100 scans.* (b) *Kubelka-Munk format of the above spectrum.* (c) %T *format of the above spectrum.* (d) *Absorption spectrum of 2 mg of prazepam in a 13-mm KBr pellet (1 : 100 dilution by weight), 100 scans.*

While the %T format of the DRIFTS data has no physical significance, we have chosen to present most of our DRIFTS spectral data in this manner in addition to the reflectance spectrum, as it offers several significant advantages over the Kubelka-Munk format. Some of these include:

(1) the ability to observe simultaneously both the weakly absorbing and strongly absorbing peaks in the same spectrum (for example, compare Fig. 5b and c) as a result of the logarithmic nature of the %T presentation;

(2) facilitation of the comparison of DRIFTS results to conventional infrared data, the overwhelming majority of which is presented in %T; and

(3) this method provides a convenient standard format for the presentation of data obtained from both very low and very high concentration samples. These extremes in concentration are possible because of the very wide range of DRIFTS; the reflectance spectra of these samples, however, lack discernible detail for many of the spectral features of interest.

Materials

The following materials were used: Equagesic tablets (containing the newer formulation consisting of meprobamate and aspirin) and Elmers wood glue, purchased locally; Spencer Kellogg Spenkel F5826-M-SS oil-modified alkyd resin, Olympic Paint Company; prazepam, U.S.P.C., Inc.; alprazolam, the Upjohn Company; 2-methylbutane (*iso*-pentane) and potassium bromide (infrared grade), J. T. Baker; 1-piperidinocyclohaxanecarbonitrile (PCC), Drug Enforcement Administration San Francisco Laboratory; and n-docosane ($C_{22}H_{46}$), n-tricosane ($C_{23}H_{48}$), n-tetracosane ($C_{24}H_{50}$), n-pentacosane ($C_{25}H_{52}$), and n-hexacosane ($C_{26}H_{54}$), Alltech Associates.

Sampling Methods, Features, and Advantages

As mentioned, Fuller and Griffiths described two methods for the sampling of solids using DRIFTS. In addition, they demonstrated an example of direct sampling in which no sample preparation was required. While Fuller and Griffiths did not examine materials other than solids, we have found DRIFTS to be quite useful for the analysis of many liquids. Examples illustrating each of these sampling methods are presented below, including discussion of several modifications of the methods used by Fuller and Griffiths that we have found to be beneficial. In addition, the numerous advantages of DRIFTS (many of which were not discussed by Fuller and Griffiths) will be described, with emphasis on those features of particular significance for forensic science analyses.

Powdered Samples

DRIFTS spectra obtained for a typical powdered solid sample diluted with KBr are shown in Fig. 5. Figure 5a is the reflectance spectrum of prazepam diluted with KBr; a 1:400 dilution (by weight) was used, and as the sample cups hold approximately 40 mg of powder, 0.1 mg of prazepam was sampled. The Kubelka-Munk format[2] of this data is shown in Fig.

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[2]When using the Analect program to obtain the Kubelka-Munk spectrum, a threshold value, in percent reflectance ($\%R_t$) is required. This reflectance value generally is chosen to be less than that of the reflectance of the strongest peak. The Kubelka-Munk spectrum, $F(R) = (1 - R)^2/2R$, is then displayed on the screen with the highest value in this spectrum corresponding to $F(R_t)$, where R_t is the threshold reflectance (which is actually $\%R_t$ divided by 100, since R in the Kubelka-Munk Eq 5 assumes a value between 0 and 1). The maximum value of this presentation also corresponds to an absorbance value of 3, and as the Kubelka-Munk spectrum is treated as an absorbance spectrum in the conversion to the %T format, the original threshold value serves, in effect, as an expansion factor for this transformation.

5b; note its similarity to an absorbance spectrum. Using an absorbance to transmittance conversion program, the %T spectrum shown in Fig. 5c was obtained.

As a comparison to conventional transmittance sampling, the spectrum of 2 mg of praze-pam in a 13-mm KBr pellet (1:100 dilution) is shown in Fig. 5d. While the spectral results obtained using these two different methods can be seen to be very similar, the DRIFTS technique required less sample preparation, and its greater sensitivity is also evident (both spectra represent the average of 100 scans). For dilute samples using DRIFTS, absorbed water (which gives the broad absorption near 3400 cm⁻¹ in Fig. 5d) is generally compensated for by referencing the sample to KBr ground to approximately the same extent as the sample plus KBr. While the same can be done for pellet samples, this requires preparation of a reference blank pellet containing the same amount of KBr and absorbed water—a tedious task.

Scattering effects, which cause the sloping baseline in some pellet spectra (such as observed in Fig. 5d) rarely occur with DRIFTS. Scattering in pellets arises from either poor sampling techniques or large differences between the indices of refraction of the analyte and matrix material (that is, KBr). To illustrate an example of poor sample preparation, PCC base and excess KBr were mixed together, ground only slightly, then pressed into a pellet. PCC base, with its relatively low melting point, presents an additional difficulty if it is not sufficiently ground because it tends to anneal (which increases scattering) while scanning. The spectral results for this preparation are depicted in Fig. 6b, which shows the low throughput obtained for this nearly opaque pellet. The same mixture used to form this pellet was then sampled using DRIFTS, with results shown in Fig. 6a and c. As may be seen, even with insufficient grinding, scattering is not a problem when using DRIFTS (better DRIFTS spectra would, in fact, be obtained with more grinding, however).

A second example of a high scattering sample is depicted in Fig. 6e, which shows the spectrum of candlewax in a KBr pellet. Although this mixture was ground more thoroughly than the PCC sample, scattering is still a problem for the KBr pellet spectrum. For candlewax, even after very thorough grinding, a pellet spectrum free from scattering effects is ex-
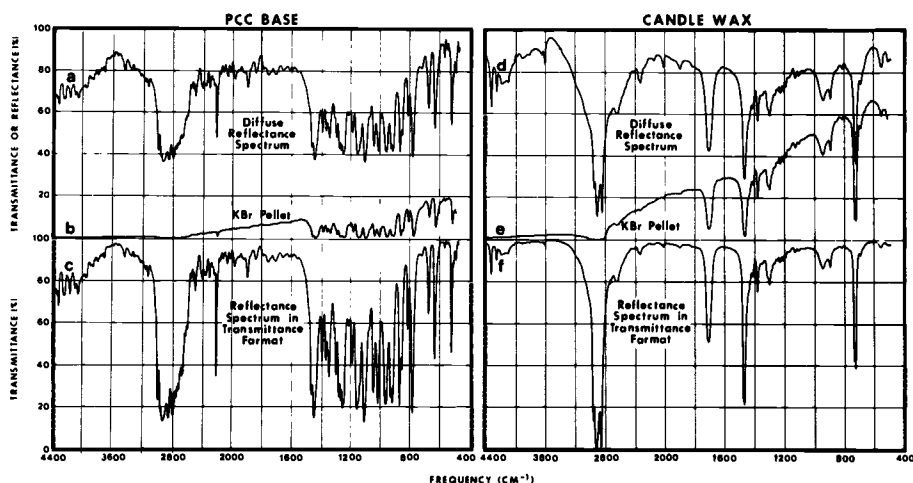

FIG. 6—(a) *Reflectance spectrum of PCC base mixed with excess KBr and ground only slightly, 100 scans. (b) KBr pellet spectrum of the above mixture, 100 scans. (c) %T format of the above (a) spectrum. (d) Reflectance spectrum of candlewax mixed with excess KBr, 100 scans. (e) KBr pellet spectrum of the above candlewax mixture, 100 scans. (f) %T format of the above (d) spectrum.*

tremely difficult to achieve, presumably as a result of refractive index differences. The DRIFTS results for this same mixture (Fig. 6d and f) further demonstrate the suitability of this method for the sampling of highly scattering or opaque materials.

One of the most significant advantages that DRIFTS offers over conventional transmittance sampling arises from its large sampling range. This feature is especially useful where a very wide variety of different types of solid materials are sampled frequently, such as occurs in many forensic science laboratories. This wide range of DRIFTS is illustrated in Fig. 7, which depicts spectral results for alprazolam at two extremes of concentration. Figure 7a and b are the reflectance and %T format spectra, respectively, of alprazolam diluted with KBr at a ratio of 1:2000 (representing approximately 20 μg of alprazolam); Fig. 7d and e depict these same spectra for neat (that is, pure or undiluted) alprazolam. A KBr pellet spectrum (Fig. 7c) is also shown. The alprazolam powder did not require any preparation, but other samples, depending upon their particle sizes and absorption coefficients, may need to be ground to a fine powder (to minimize specular reflection) before neat sampling.

Fuller and Griffiths presented spectra of the neat powders that they sampled in either the Kubelka-Munk format or the reflectance format (but not both). As may be seen in Fig. 7d, the pronounced scale compression for the stronger absorptions in the reflectance spectrum tends to both distort and obscure much of the detail of these peaks. This detail is observed much more clearly, however, when this data is presented in the %T format (Fig. 7e). The weaker absorptions, in contrast, are seen clearly in the reflectance spectrum, but hardly at all in the %T format. This behavior results from the extremely wide absorbtivity/concentration range observable in a reflectance spectrum; two formats of the spectral data (reflectance and %T) are thus necessary to best view all of the features present for these concentrated or neat samples.

Fuller and Griffiths provided a partial explanation for the wide range of DRIFTS from their examination of the behavior of the Kubelka-Munk equation for very low sample con-
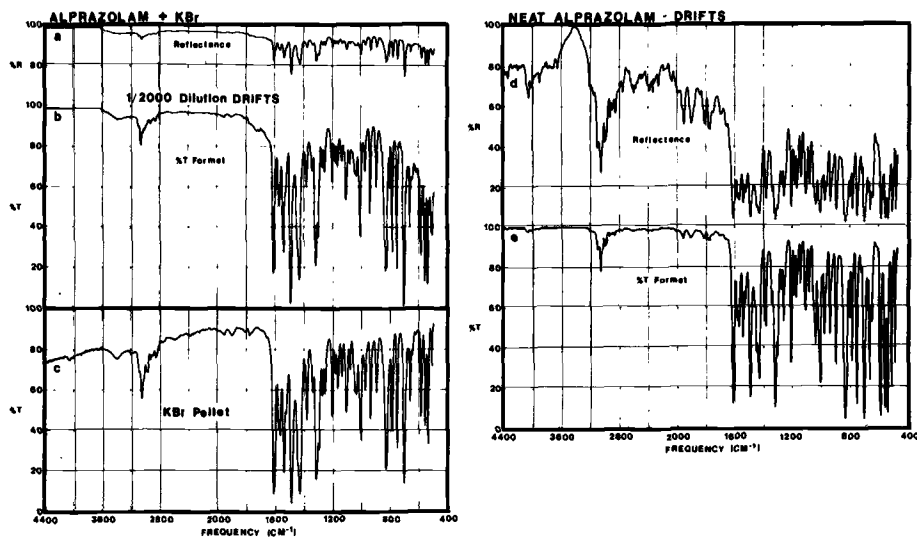

FIG. 7—(a) Reflectance spectrum of approximately 20 μg of alprazolam diluted with KBr (1:2000 dilution by weight), 250 scans. (b) %T format of the above spectrum. (c) Absorption spectrum of alprazolam in a KBr pellet, 100 scans. (d) Reflectance spectrum of neat alprazolam, 250 scans. (e) %T format of the above (d) spectrum.

centrations. For such cases, they showed[3] that the signal-to-noise ratio is proportional to the square root of the sample concentration, unlike conventional transmittance sampling where it is linearly proportional to concentration. DRIFTS measurements are thus inherently more sensitive than those obtainable by transmittance methods.[4]

This feature of DRIFTS is illustrated in Fig. 8, which is a plot of the reflectance (R_∞) versus k/s (which is proportional to concentration) according to the Kubelka-Munk formula (Eq 4). The very steep decline in (R_∞) for small values of k/s graphically illustrates the sensitivity of DRIFTS. The very wide range of this technique, as well as its ability to sample neat powders, can also be seen from this graph. Assuming that the region between $R_\infty = 0.9$ (90% reflectance) and 0.01 (1% reflectance) yields useful spectral data, values of k/s $(= k'ac)$ from 0.0056 to 49.00 are obtained; this corresponds to nearly four orders of magnitude. In contrast, a plot of transmittance versus A $(= abc)$ according to the Beer-Lambert equation (shown as dashed lines in Fig. 8) shows that for transmittance sampling, a corresponding range of A from 0.0458 to 2.0 (less than two orders of magnitude) is obtained.

Although Fuller and Griffiths (or others) did not provide a physical explanation for the sensitivity of DRIFTS (or its very wide range), we believe these features can be rationalized from the differential pathlengths that are sampled in the diffuse reflectance process. Unlike conventional transmittance sampling, in which all of the transmitted radiation traverses the same pathlength (and hence "sees" the same amount of analyte), a number of different individual pathlengths are possible in DRIFTS sampling (see Fig. 3). The average
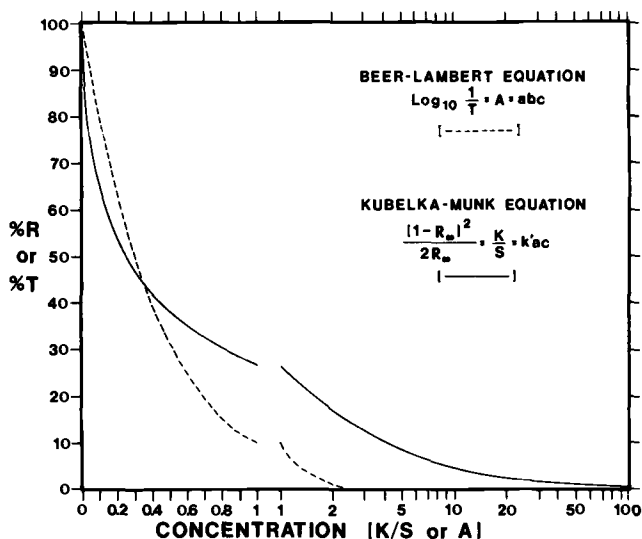

FIG. 8—*Plots of R_∞ versus k/s for the Kubelka-Munk equation* (solid line) *and T versus A for the Beer-Lambert equation* (dashed lines). *Note that for k/s (or A) from 1 to 100, a logarithmic scale is used.*

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[3]For very small values of c, R_∞ approaches unity; thus, Eq 5 becomes $(1 - R_\infty)^2/2 = k'ac$. The sample absorption is $(1 - R_\infty)$ which is now $(2k'ac)^{1/2}$. The signal to noise ratio is therefore proportional to the square root of the sample concentration.

[4]In principle, the significantly lower efficiencies of DRIFTS accessories (which typically have throughputs of 5 to 15%) might be expected largely to offset this advantage, as higher interferometer gains are required. In practice, we have found that even with an FTIR, sampling of low concentrations with KBr pellets is impractical because of impurities in the KBr (which tends to absorb organic vapors and water) and scattering effects. As already discussed, these are much less of a problem when using DRIFTS.

pathlengths experienced by strongly absorbed wavelengths, for example, would be expected to be quite small. Even for strongly absorbed wavelengths, however, some radiation will be diffusely reflected as they will have penetrated only a short distance through the sample before being backscattered to the surface. The weakly absorbed wavelengths, on the other hand, may penetrate quite deeply into the sample (in addition to undergoing "multipass"-type paths), effectively sampling a much greater amount of material. As such, these wavelengths will be absorbed much more than might be expected. This enhancement is quite evident in Fig. 7d, where the weaker absorptions above 1700 cm^{-1} have much greater intensities, relative to the stronger absorptions, than observed for transmittance sampling (Fig. 7c).

This latter feature of DRIFTS, together with the lack of appreciable scattering effects, can be quite useful for distinguishing between closely related compounds. Members of many homologous series, for example, cannot be differentiated by conventional transmittance infrared spectroscopy. This is illustrated in Fig. 9, which shows KBr pellet spectra for five straight chain saturated hydrocarbons ($C_{22}H_{46}$ to $C_{26}H_{54}$). Note in particular the similarities between members in the even-numbered or odd-numbered subseries. The odd-numbered hydrocarbons exhibit doublets for the 1470- and 720-cm^{-1} absorptions, and the very weak absorptions between these two features are also very similar for these.

The reflectance spectra of these same hydrocarbons, which were diluted with KBr at a ratio of approximately $1:3$, are shown in Fig. 10. The weaker absorptions can be seen clearly in these spectra, and although they too are quite similar for all five compounds, there are distinguishing features, especially in the region from 720 to 1200 cm^{-1}. We believe these differences to be real rather than arising from impurities in the hydrocarbons, as this same spectral region was found to have distinguishing features in differentiating the four normal alkanes n-pentane to n-octane based upon their Raman spectra [24]. DRIFTS therefore can provide more spectral data to characterize or individualize a particular compound than normally obtainable through conventional means (although pellet or film spectra can be expanded, there are practical limits to the useful information that can be obtained from this as discussed in Footnote 4).

Solution Deposits

The second method of sampling of solids involves evaporation of a volatile solution of the solid in the presence of KBr, followed by transfer of this now coated KBr to a sample cup. Alternatively, the solution can be deposited directly onto KBr prepacked in the sample cup. Results obtained using the latter method are shown in Fig. 11 for 20 μg of prazepam deposited as four drops (approximately 40 μL) of a dichloromethane solution. The reflectance and %T format spectra are depicted in Fig. 11a and b, respectively, and as a comparison, the absorption spectrum of a film of prazepam (also deposited from a dichloromethane solution) on a KBr plate is shown in Fig. 11c. As the latter required several milligrams of sample, the increased sensitivity of the DRIFTS method is again evident.

We have found this direct deposit method to be more sensitive than the method of evaporating the solvent in the presence of KBr (which Fuller and Griffiths found, in turn, to be more sensitive than simply mixing the same amounts of sample and KBr together). This undoubtedly is due to the fact that for the former, the analyte is concentrated near the surface of the KBr where most of the sampling in DRIFTS occurs. The direct deposit method is a much more convenient means of sampling, and spectra of solutes in organic solvent extracts can be obtained in a matter of minutes following deposition. This method is thus ideally suited for the analysis of many organic compounds and for microsampling.

We have observed, however, that two factors may sometimes limit the usefulness of this technique or dictate that caution be exercised in its application. The first of these results from the inhomogeneity in the analyte concentration. Since the Kubelka-Munk model as-

FIG. 9—*Absorption spectra of five normal hydrocarbons in KBr pellets:* (a) *hexacosane,* (b) *pentaco-sane,* (c) *tetracosane,* (d) *tricosane, and* (e) *docosane.*

sumes a homogeneous sample of infinite depth, the fact that the analyte may be concentrated near the surface sometimes results in significant relative peak intensity differences from those observed for homogeneous samples. This usually results in decreased intensities for the weaker absorptions of the sample relative to the stronger absorptions. Since the weaker absorbing wavelengths tend to experience longer average pathlengths in DRIFTS sampling than the strongly absorbing ones (as discussed previously), they will penetrate deeper into the sample. It is therefore reasonable to expect these weaker absorbing wavelengths to experience attenuated relative intensities when the analyte is concentrated near the surface. This problem can be minimized by depositing a larger volume of a more dilute solution to increase the depth of the zone in which the analyte is deposited.
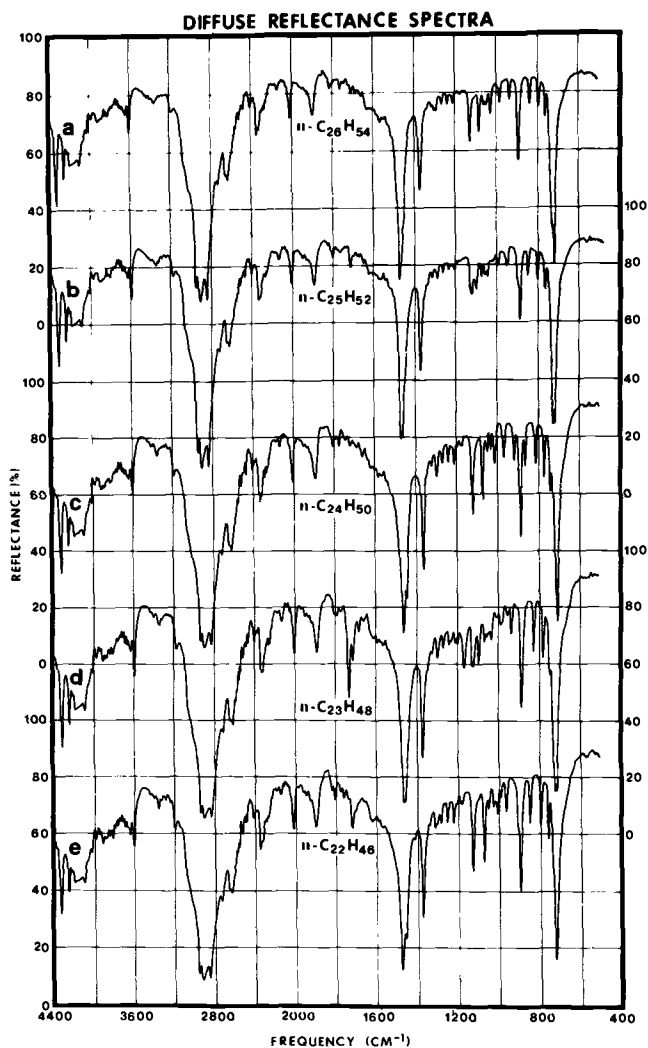
DIFFUSE REFLECTANCE SPECTRA

FIG. 10—*Reflectance spectra of five normal hydrocarbons mixed with KBr (at approximate dilution ratios of 1 : 3):* (a) *hexacosane,* (b) *pentacosane,* (c) *tetracosane,* (d) *tricosane, and* (e) *docosane.*

The solution method of sampling (using both techniques) on occasion produces spectra somewhat different from those obtained when the solid is mixed with KBr and sampled. This difference is usually manifested as a general peak broadening and loss of some detail (compare, for example, Figs. 5c and 11b), and these spectra often closely resemble those obtained for cast films of the same material. It is thus evident that for these samples, this method produces a noncrystalline (or partially crystalline) glass of the analyte. This appears to be more of a problem when dilute solutions are deposited, and is also somewhat dependent on the particular solvent used. Because of this feature, as well as the inhomogeneity that may occur, spectra of standards sampled in the same manner (including separate standard spectra for microtechniques, if necessary) should be collected for reference purposes.
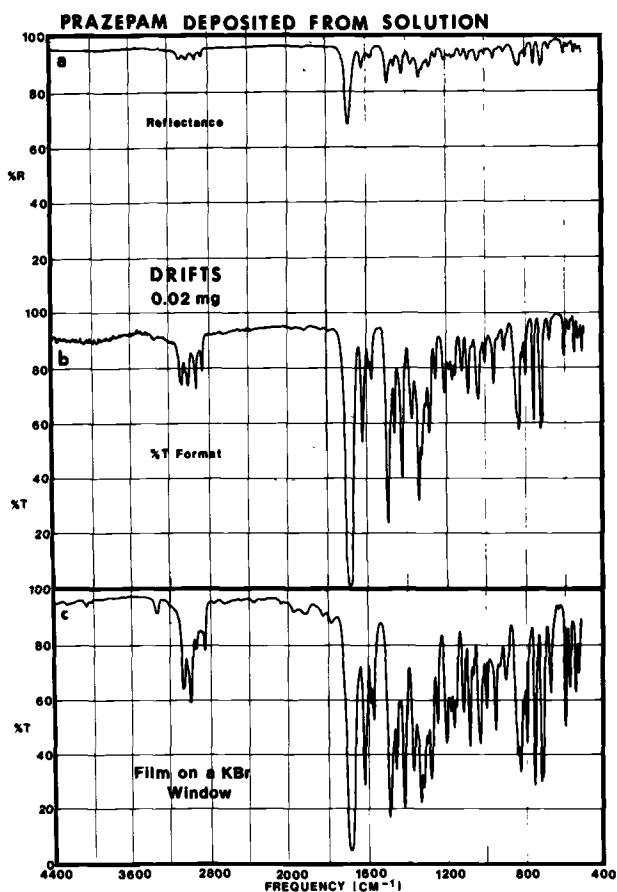
FIG. 11—(a) *Reflectance spectrum of 20 μg of prazepam deposited as four drops (approximately 40 μL) of a dichloromethane solution, 100 scans. (b) %T format of the above spectrum. (c) Absorption spectrum of a film of prazepam deposited onto a KBr window from a dichloromethane solution, 100 scans.*

Direct Sampling

Because of the very wide sampling range of DRIFTS, some bulk organic materials can be sampled directly with no sample preparation. Willey [7] first demonstrated some examples of direct sampling using DRIFTS, which included analysis of starch, wool cloth, paper, and plant leaf. Fuller and Griffiths applied this method to the direct analysis of an intact Empirin tablet. Further applications of this technique to the analysis of tablets of forensic science interest are described in the second paper of this series. Direct analysis offers obvious advantages for forensic science work as it is completely nondestructive, and it requires no sample preparation.

An example of direct sampling is illustrated in Fig. 12. The unfinished surface of a piece of cedar wood siding was positioned at the focal point of the DRIFTS accessory giving the reflectance spectrum depicted in Fig. 12b. Note the low reflectance of this material which is a feature normally observed in direct sampling. Willey presented his direct sampling data as $\log_{10}$ (1/reflectance), whereas Fuller and Griffiths presented their reflectance spectrum de-
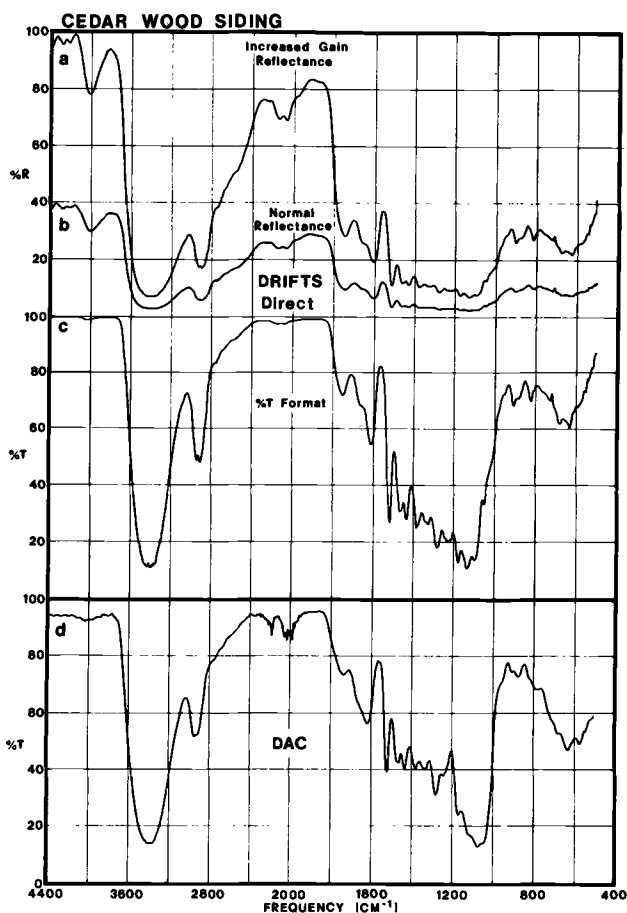
FIG. 12—(a) *Reflectance spectrum of a piece of cedar wood siding sampled directly; sample gain 16, reference (powdered KBr) gain 4R, 250 scans.* (b) *Reflectance spectrum of the above sample; sample and reference gains 4R, 250 scans.* (c) %T *format of the above* (a) *spectrum.* (d) *Diamond anvil cell (DAC) spectrum of the above siding sample; gain 8, 250 scans.*

picted full scale between the highest and lowest points of the spectrum. Both of these presentations circumvented, to some extent, the problem of low reflectances of these samples. As in the case of neat powders, however, we have found that presentation of direct sampling data in the %T format greatly facilitates observation of most spectral features.

To compensate for the low reflectances of these samples, increased instrument gains for the samples, relative to those used for the background, are used. This serves simply to raise the entire spectrum by a constant amount. The increased gain reflectance spectrum of the siding sample is depicted in Fig. 12a, and the %T format of this spectrum is shown in Fig. 12c. As a comparison, the absorption spectrum obtained for this siding in the diamond anvil cell (DAC) is shown in Fig. 12d.

Liquids

Liquids can also be sampled using DRIFTS, including volatile liquids and viscous oils. Results of this method applied to the analysis of a volatile liquid, 2-methylbutane (boiling

point [b.p.] = 28°C) are illustrated in Fig. 13. Several drops of 2-methylbutane were deposited onto finely ground KBr (prepacked in the sample cup), giving the reflectance and %T format spectra shown in Fig. 13a and b, respectively. The absorption spectrum of this liquid in a 0.025-mm liquid cell is shown in Fig. 13c. Both the DRIFTS and the liquid cell spectra displayed fairly rapid decreases in band intensities in time as a result of sample evaporation.[5] Because of the speed of the FTIR, however, good quality spectra were still obtainable.

The relative band intensity differences obtained for these two methods (compare Fig. 13b and c) are worth noting; they arise primarily from two factors. For DRIFTS sampling, the
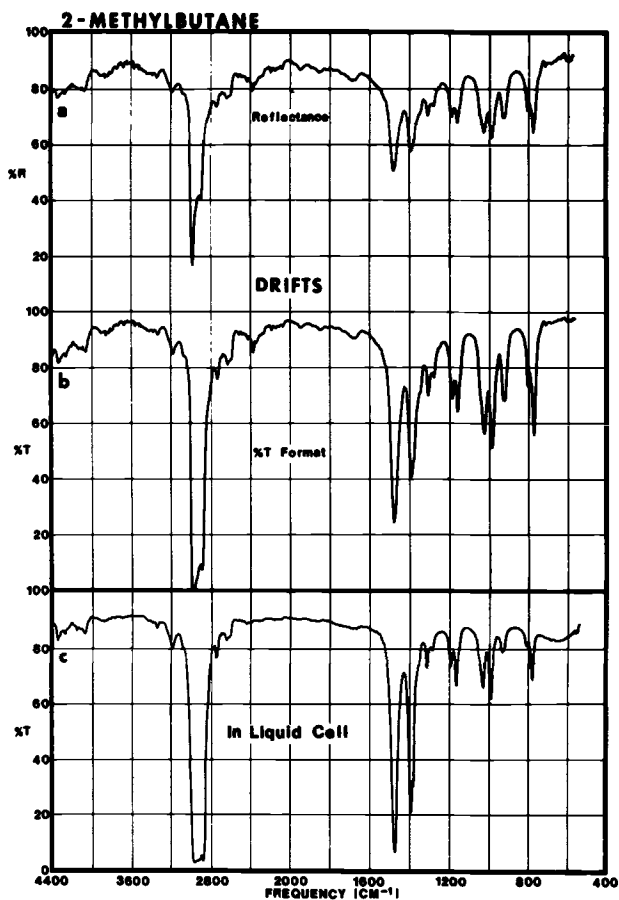

FIG. 13—(a) *Reflectance spectrum of several drops of 2-methylbutane deposited onto powdered KBr, 10 scans.* (b) %T *format of the above spectrum.* (c) *Absorption spectrum of 2-methylbutane in a 0.025-mm liquid cell, 10 scans.*

[5]The Analect FX-6200 performs "on-the-fly" Fourier transforms of interferograms; thus, this sample evaporation process can be observed on the display screen. This feature is especially useful for monitoring the progress of solvent evaporation for the previously discussed solution deposit method. The FX-6200 also normally employs an interferogram monitoring program that automatically excludes from averaging those interferograms (or strictly speaking, transforms of interferograms, since these are actually what are averaged) that are considered unsuitable. Since the basis for this discrimination is deviation from the "norm," this program is not used for the sampling of volatile liquids as this involves averaging spectra having progressively less intense absorptions.

observed reflectances are dependent not only upon an absorption coefficient, but also upon a scattering coefficient (see Eq 4). Were this scattering coefficient constant for all wavelengths, the relative peak intensities for DRIFTS spectra would be expected to be very similar to those observed for transmittance sampling. The scattering coefficient does, in fact, vary somewhat with wavelength, decreasing with increasing wavelength (this is the reason why opaque KBr pellets produce an "upward" drifting baseline with increasing wavelength).

According to the Kubelka-Munk theory, radiation experiencing a decrease in the scattering coefficient should penetrate deeper into the sample. In line with this, Fuller and Griffiths demonstrated experimentally that longer wavelengths penetrate deeper into the sample than shorter wavelengths. Because of this, the low-frequency absorptions in DRIFTS generally have somewhat greater relative intensities in comparison to transmittance spectra. This feature is quite evident in Fig. 13b, and can be seen, to a lesser extent, for some of the other DRIFTS results. The reason for the pronounced enhancement effect for this liquid sample probably arises from its high volatility. This undoubtedly produces a depletion of analyte near the surface of the packed KBr, which in turn would be expected to amplify the effects of differences in penetration depths (note that this inhomogeneity is just the opposite to that previously discussed). Supporting this, Fuller and Griffiths observed a similar low-frequency enhancement when a 0.5-mm layer of KBr was placed on top of a powdered sample.

While some liquids can be sampled neat with DRIFTS, better results are obtained for others (especially viscous liquids) if they are first diluted. This is illustrated for a very viscous alkyd resin in Fig. 14. The reflectance spectra of neat and diluted deposits are depicted in Fig. 14b and a, respectively. The %T format spectrum of the latter is shown in Fig. 14c. For comparison purposes, the absorption spectrum of a film of this same resin is shown in Fig. 14d.

For the diluted sample, 1 μL of 1% resin solution in hexane was deposited; this corresponds to 10 nL of the alkyd resin. As the spectral absorptions for this deposit are fairly strong (Fig. 14a), it is clear that useful spectra of sample volumes much less than 10 nL are possible. Because alternative methods for the convenient microsampling of liquids are lacking, DRIFTS may well be one of the best methods presently available for nanolitre samples of liquids of low volatility.

Comparisons to Other Techniques

For the samples discussed above, the DRIFTS spectral results have been compared to those obtained by conventional transmittance techniques. Summarizing, the advantages of the DRIFTS technique over these other methods include:

(1) minimal sample preparation (no sample preparation in a few cases);

(2) a very wide range of sample concentrations which yield useful spectra (from parts per million to neat, undiluted materials in some cases; the latter includes the ability to obtain spectra directly on some bulk materials run intact);

(3) a very high sensitivity (less than 100 ng in some cases) together with a convenient method of microsampling;

(4) alleviation of most sample scattering problems;

(5) suitability for highly opaque materials;

(6) suitability for very weakly absorbing samples;

(7) fewer problems associated with the KBr pellet matrix effects such as ion exchange, sample reaction with KBr, effects of pelleting pressure, and so forth;

(8) fewer problems associated with KBr impurities such as absorbed organic materials and especially absorbed water;

(9) better characterization and individualization of some samples based upon the presence of very weak spectral features not normally observed;
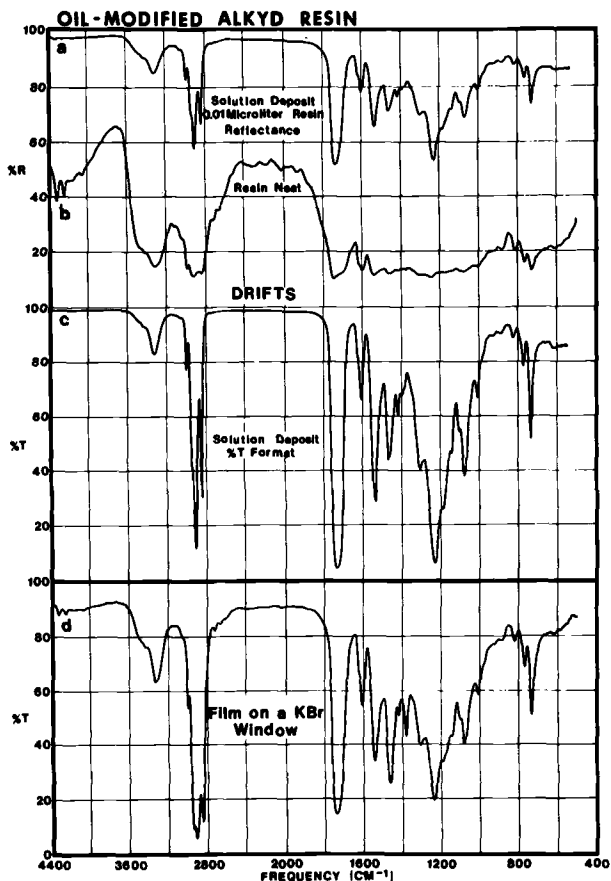
FIG. 14—(a) *Reflectance spectrum of 0.01 µL of an oil-modified alkyd resin deposited as a 1-µL hexane solution, 200 scans.* (b) *Reflectance spectrum of one drop (approximately 5 µL) of the above resin deposited neat onto KBr, 200 scans.* (c) *%T format of the above (a) spectrum.* (d) *Absorption spectrum of a thin film of the above resin squeezed between two KBr plates, 100 scans.*

(10) less postsampling cleanup required than for cast films (following DRIFTS sampling, one simply discards the powder from the sample cup); and

(11) suitable for liquids, which include a very convenient means of microsampling (less than 1 nL in some cases).

A few of the disadvantages of DRIFTS include:

1. The requirement for a FTIR. Although the feasibility of using a diffuse reflectance accessory on a computerized dispersive instrument has recently been demonstrated [25], most of the applications that we will be presenting cannot be performed on dispersive instruments.

2. The cost of an accessory. Most commercial diffuse reflectance accessories cost several thousand dollars.

3. Spectral variability. DRIFTS generally is a quite reproducible technique for a given set of sampling conditions. As previously discussed, the direct solvent deposit method (which is one of the more convenient and sensitive of the DRIFTS techniques) does produce some

spectral variability when nonhomogeneous analyte concentrations are sampled; the origin and nature of these variabilities, however, are readily explained through consideration of the DRIFTS sampling process.

4. Low frequency limitations. The low throughputs for diffuse reflectance accessories necessitate the use of sensitive MCT detectors. These, however, are quite ineffective below 400 to 500 cm^{-1}; the low frequency region thus generally cannot be examined using DRIFTS (through the use of special detectors, this can be achieved [26], but DRIFTS is not well suited for this purpose).

Acknowledgments

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