Analysis of strongly absorbing chromophores by UV-visible ATR spectroscopy

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This paper illustrates the potential of the attenuated total reflectance (ATR) sampling technique for UV-visible analysis and explores ways in which this potential can be maximized. While UV-visible ATR is far from new, it has been largely neglected, perhaps due to the limited range of operating conditions used in previous work. By the appropriate choice of ATR optical design and operating parameters, we have been able to maximize the sensitivity of the technique for diverse sample conditions. We have also observed artifacts at high concentrations of some analytes. These are a result of refractive index dispersion resulting from strong electronic transitions in the vacuum UV. Of particular interest is the potential for using small amounts of solvent to shift the frequencies of these artifacts so as to optimize the analysis of minor constituents.

1. Introduction

UV-visible spectroscopy is widely used for the analysis of chromophores (groups of atoms characterized by strongly absorbing electronic transitions). The attractiveness of this method results from the simplicity of the spectra and their direct relationship to molecular functional groups — resulting in easy and robust calibrations. However, when using UV-visible transmission to analyze materials containing chromophores, it is often necessary to dilute the analyte by a factor between 1000 and 10,000 in a suitable non-absorbing solvent. This requirement is undesirable for a number of reasons, including: solvent-analyte interactions, errors in dilution, and the need to either store or dispose of significant amounts of material. The present paper is concerned with a potential solution to this problem based on the use of the attenuated total reflectance (ATR) sampling technique.

The utility of ATR for UV-visible chromophore analysis has been recognized for many years. However, based on a survey of the available literature, there appears to have been very little research done in this field. The one notable exception is the work of Harold Danigel and his colleagues at Ciba Geigy (now Novartis) who have applied the technique to online analysis of processes such as hydrogenation.1

To our knowledge, most of the previous work with UV-visible ATR has employed three reflection, sapphire tipped ATR probes with an angle of incidence of 60° at the element/analyte interface. This configuration is optically convenient since a succession of three reflections, each at a 60° incidence angle, will return an optical beam on a path parallel to its initial direction. For the work reported below, we used ATR flow cells with a range of incidence angles approaching 90°. This enabled us to employ fused silica as an ATR element, in turn providing considerably greater UV transmission and the ability to tailor the incidence angle and number of ATR reflections to optimize the performance for a given analysis.

2. Attenuated Total Reflectance Fundamentals

Attenuated total reflectance (ATR) spectroscopy is best known for its role as the almost universal sampling technique for mid-IR liquid analysis. This is due in part to the extremely strong fundamental functional group absorptions, which fall in this spectral region. The use of transmission sampling would require sample pathlengths of the order of a few µm. Such small pathlengths are impractical for most applications due to optical interference effects and the potential for obstruction of the flow path. ATR solves this problem by making use of the slight optical penetration that takes place when radiation propagating through a solid transparent material strikes the interface between this material and an adjacent layer of absorbing material.2 In effect, ATR provides the equivalent of a transmission cell having a pathlength of the order of the wavelength of the illuminating radiation. Most IR applications of ATR fall in the “fingerprint” spectral region (typically 400 to 2000 cm⁻¹), i.e. the region containing the fundamental functional

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group absorptions of organic molecules. At shorter infrared wavelengths, the technique is less often used due to the weakness of overtone and combination tone spectra combined with the fact that the ATR effective pathlength is proportional to wavelength.

Although the utility of ATR for the direct analysis of molecular vibrations decreases rapidly at shorter wavelengths, the situation becomes dramatically different for chromophores that have electronic transitions in the UV-visible spectral region. The absorptivities of chromophore electronic transitions are often orders of magnitude stronger than those of the molecular vibration fundamentals observed in the fingerprint region. Thus, even though the wavelengths are typically thirty times shorter, quite reasonable absorbance values are often obtained. Nevertheless, there are still limitations in applying ATR in the UV-visible region. These result from the limited selection of materials available for use as ATR elements combined with the requirement on the relationship between the refractive indices of the analyte and the ATR material. Section 3 treats the methods we are using to optimize ATR performance in the light of these limitations.

A simple ATR measurement geometry is shown in Figure 1. Here, a transparent ATR element having a refractive index equal to \( n \), is shown with one surface in contact with a sample (refractive index \( n_s \)) and the other in contact with air (index \( n_a \)). In the absence of sample absorption, radiation propagating within the element will be either partially or totally reflected at the two interfaces depending on the relationship between the angle of incidence (\( \theta \)) and the ratio of the two refractive indices at each interface. This relationship is shown in Figure 2, a plot of internal reflectance versus angle for a given value of \( n_s/n_a \). As illustrated, the radiation will be totally reflected at the interface as long as the angle of incidence is greater than a critical value, given by \( \sin \theta_c = n_a/n_s \). However, even though the radiation is totally reflected, its electric field penetrates into the second medium by a small amount proportional to its wavelength. As a result, if the sample happens to be an absorber, the radiation will be partly attenuated. Hence the term “Attenuated Total Reflection”.

The materials used for ATR elements in the mid-IR have high refractive indices (e.g. \( n = 2.4 \) for ZnSe). This enables the use of a convenient angle of incidence (e.g. 45°) with samples having refractive indices as high as 1.5. As a result, commercial ATR devices often use multiple reflections (typically 10 to 12) and thus provide high absorbance sensitivity. Numerous ATR sampling devices have been designed over the years for mid-IR analysis. One of these is the Axiom Tunnel Cell shown in Figure 3. This device provides optimum linearity and repeatability for flowing liquid analysis by using a non-focussing optical geometry which insures that the radiation propagates within a narrow range of angles within the ATR element. The key to this design is the use of a cylindrical rod of ATR material in conjunction with a pair of reflecting cones. The first cone converts axial (parallel) rays into rays all of which propagate at the same angle with respect to the axis of the rod. The second cone returns the rays to their original axial direction. Optical stops at each end of the rod eliminate rays which would follow undesired paths by virtue of having entered the rod without first being reflected by the cone.
3. Optimizing ATR Performance for UV-visible Applications

In applying ATR in the UV-visible region, the primary concern is the lack of suitable high index materials for use as ATR elements, especially at wavelengths less than roughly 300 nm. The highest index material suitable for use in most of this region is sapphire, with an index of \( n = 1.8 \) at 340 nm. However, sapphire must be specially treated for use at less than 250 nm and even then is usable only in limited thicknesses. A much more desirable material from the viewpoint of UV transmission is UV-grade fused silica (Suprasil™), with an index of 1.5 at 270 nm.

Previous ATR devices for use in the UV-visible region have generally used sapphire elements with a 60° angle of incidence. This arrangement is particularly convenient for use in a probe form, since a three reflection, 60° ATR element can function as a retroreflector. We have developed both probes and flow cells which eliminate this restriction on angle of incidence. By employing incidence angles closer to 90°, these designs allow the use of low index materials such as fused silica for many applications. The practical advantages of this approach will be illustrated below.

All of the internal reflectance data to be discussed in this paper were obtained by using a modified Axiom model TNL-120 Tunnel Cell mounted in the sample compartment of a Hewlett Packard 8453 UV-visible diode array spectrometer. This combination is quite convenient since the 8453 sampling geometry is in the form of a well collimated beam somewhat smaller in diameter than the TNL-120 ATR element. This enables us to use refraction at the end cone of the ATR rod to determine the angle of incidence and number of reflections in the rod. This situation is shown in Figure 4 for a single reflection fused silica element mounted in a TNL-120 (rod diameter 3.175 mm, length at the shoulders: 36 mm). For this mode of operation, the optical stop which normally blocks radiation from entering the rod directly was removed.

The calculated characteristics of 1, 2, 3, and 4 reflection fused silica and 5 and 6 reflection sapphire rods are given in Table I. In calculating the cone angles needed to obtain the desired number of reflections, we used the indicated values of rod refractive indices. These values are only strictly true for the indicated wavelengths. The effect of index dispersion will be to alter the angle of incidence and reduce the transmission as one moves away from the chosen optimum wavelength.

The last column of Table I lists values of \( n_c \), the critical refractive index. This is the value of sample refractive index for which the angle of incidence is equal to the critical angle. Absorbance sensitivity for a given analysis can be enhanced by selecting the rod parameters which maximize the number of reflections while providing a critical index only slightly higher than the maximum expected sample index. It should be noted, of course, that the refractive index will vary considerably in the vicinity of strong absorption bands. One result of this is that the bands will become distorted as the critical index is approached.

Figure 5 gives the transmission spectra of the Tunnel Cell with three of the fused silica and one of the sapphire rods. Note that even though the sapphire rod has been annealed to maximize its UV transmission, the propagating distance within the rod was sufficient to render it nearly opaque beyond 250 nm.

Although the data for this paper were obtained with a sample compartment version of the UV-visible Tunnel Cell, we have also constructed a fiber-optic coupled version. Figure 6 shows this cell coupled to the 8453 sample compartment by means of the Axiom model FOI-5HP sample compartment interface.

Fig. 4. TNL-120U UV-Visible Tunnel Cell configured for operation using a single reflection ATR element.

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### TABLE I

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<th>UV-VISIBLE ATR ELEMENTS FOR USE IN TNL-120 SERIES TUNNEL CELLS</th>
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4. Experimental Results

To illustrate the potential of UV-visible ATR, we have chosen three examples: an aqueous (low refractive index) dye, a mixture of additives in a dispersive (high refractive index) polyol, and a neat aromatic. These examples are representative of three major categories of samples encountered in UV-visible ATR analysis.

Case A: Analysis of Photographic Dyes

The primary motivation for the using ATR in chromophore analysis is the desire to eliminate, or at least minimize, the need for sample dilution. The situation is illustrated in Figure 7. Traces A, B, and C are spectra of a neat red dye obtained by using the Axiom FST-500 Rack and Pinion Sampler, a fiber-optic coupled variable path sampling device. The pathlengths of traces “A” and “B” are 10 mm and 1 mm, respectively. For spectrum “C”, the sampling rod of the FST was brought into contact with the bottom of the sample containing beaker. At this point, the residual transmission pathlength provided by the unevenness of the beaker was much less than the resolution of the FST’s electronic pathlength readout (0.01 mm). Although a measurable spectrum can be obtained with this short path, it would be difficult, if not impossible, to insure accuracy under these conditions. Trace “D” corresponds to the same dye run in the TNL-120 with a three reflection fused silica rod. In this case, the major bands fall in an ideal range for quantitative measurement.

Figure 8 illustrates the dependence of sensitivity on the ATR rod parameters. For water based samples and other ma-
materials with similarly low refractive indices, one of the fused silica rods will usually prove optimum. In the case of the dye used for this example, the three reflection fused silica rod would be the best choice. (In general, it can be shown that for an analysis carried out in the presence of fixed background noise, the greatest sensitivity to changes in concentration will be obtained with the effective pathlength set for an absorbance value of 0.434 AU). This figure also illustrates the band shifts and associated non-linearity that occur as the critical refractive index is approached. These are due to the dispersion of the sample refractive index in the region of a strong absorption. However, given the capabilities of modern chemometrics, such departures from the Beer-Lambert law are no longer a major concern for most analyses.

Case B: Aromatic Additives

Aromatic compounds are some of the most strongly absorbing chromophores. This is due to the presence of low lying electronic transitions which result from the effects of double bond conjugation and aromatic ring resonance. As a result, even when aromatics are used as additives at part per thousand levels, it is often necessary to dilute the samples by another three orders of magnitude in order to measure the concentrations by transmission.

The data presented in this section were all obtained using customer supplied samples of a propylene based polyol containing a mixture of three aromatic additives with total concentrations ranging from 0.33 % to 0.66 %. These are representative of materials that are normally diluted 3,000:1 in methanol and then measured in transmission as part of a routine QC procedure. Under these conditions, the additive concentrations are easily measured with no interference from polyol absorptions.

In contrast to aqueous solutions such as the dye analyzed above, many organics have refractive indices close to or above that of fused silica. This is particularly true in the high frequency (UV) region of the spectrum since the refractive indices of most substances increase with frequency due to the presence of strong electronic transitions in the vacuum UV. Thus, although the reference value of the refractive index of the polyol (1.454) is lower than the critical index for a single reflection fused silica rod, we found the transmission of the rod to be less than 3% over most of the UV-visible spectrum when the cell was filled with any of the neat polyol samples.

In order to reduce the refractive index to a more amenable value, we added various amounts of solvents having lower refractive indices. Similar results were obtained with hexane and with methanol. The latter are shown in Figure 9 for the sample having the highest concentration of additives. The most obvious feature of each of these spectra is a fairly sharp transmission cut-off, which shifts to shorter wavelengths as the solvent concentration is increased. This cut-off starts approximately at the point where the sample index reaches the critical value ($n_s = 1.467$). The minimum transmission occurs when the index of the sample is equal to that of the ATR element (1.48).

In addition to the transmission cut-off caused by refractive index variation, the spectra of Figure 9 exhibit features resulting from the presence of the additives. Indeed, at a solvent concentration of 25%, the cut-off has shifted so that at least two of the additive bands become clearly distinct (see Figure 10). However, when the dilution is increased to 50%, the benefit of any further shift is negated by a marked reduction in band strength as the refractive index is reduced well below the critical value.

The above result suggests the possibility of optimizing the analysis by first increasing the solvent concentration to further displace the transmission cut-off, and then increasing the measurement sensitivity by changing to an ATR rod having more reflections and a lower critical index. This is illustrated by Figure 11, which includes spectra obtained for all three of the additive concentrations using a three reflection fused silica rod. At a methanol concentration of 65%, the polyol peak has

Fig. 9. ATR "transmission" spectra of one of the polyol mixtures, both neat and at four dilutions in methanol using single reflection fused silica element.

Fig. 10. ATR "absorbance" spectra of neat methanol and the dilutions of polyol in 25% and 50% methanol in Figure 9 expanded vertically to reveal the additive bands.
been shifted enough so that at least three additive peaks can be observed. This result also exhibits a direct relationship between additive concentration and absorbance, indicating the possibility of developing a calibration under these conditions.

Although the above approach to additive analysis does use a solvent to tune the measurement conditions, the concentration of solvent is far lower than that needed for transmission analysis. In addition, the sample volume is much lower since the TNL-120 requires only about 40 µl of sample, compared to typically 1 ml for the transmission measurement. The total savings in solvent consumption, storage, and disposal could be quite significant in routine QC applications.

**Case C: Neat Aromatics**

In the above illustration, refractive index dispersion of the major component in the mixture had a marked impact on the ability to measure concentrations of additives — even though this component does not absorb in the spectral region of interest. For our final illustration, we have selected benzene, a material which is both highly dispersive and absorbing in the UV-visible region. Benzene is useful for illustration since it is the basis for a large group of aromatic chemicals, many of which have similar UV-visible spectra.

Figure 12 includes spectra obtained with a single reflection fused silica ATR element for four mixtures of methanol and benzene. In this case, the major contributor to dispersion is an extremely strong absorption band not far beyond the end of the measurable spectrum. As a result, the strengths of the observable bands are highly influenced by solvent concentration but the bands are not particularly distorted as long as they are below the transmission cut-off. The behavior at frequencies above the cut-off is marked by two interesting characteristics. First, the transmission of the ATR cell increases with frequency in this region. This is due to the fact that the refractive index of the sample is higher than that of the element, giving rise to first surface “external” reflection rather than attenuated total reflection, (ATR). Second, the group of absorption bands centered at 200 nm is inverted when it is on the high frequency side of the cut-off. This is consistent with the behavior expected in this sub-critical (non-ATR) region, as illustrated by Figure 2.

**5. Conclusions**

UV-visible chromophore analysis is not a universal analytical technique. This is due both to the paucity of allowed bands and the fact that the bands that do occur fall in a narrow spectral region and are broadly overlapping. However, in the cases where it does apply, UV-visible analysis offers the advantages of economical hardware and simple, straight forward calibrations. Both its advantages and its limitation are the result of the simplicity of UV-visible spectra. Although there are relatively few strong chromophore bands, those that exist are a direct measure of the presence of specific functional groups and thus can be used to monitor fundamental changes in molecular structure.

UV-visible ATR makes it possible to monitor high concentrations of strongly absorbing chromophores, thereby minimizing the need for diluting solvents. This can result in improved accuracy and the elimination of the need to store or dispose of large quantities of materials. This paper has illustrated the potential for broadening the areas of application of UV-visible ATR by optimizing the measurement conditions for individual tasks and, in particular, by selecting the optimum angle of incidence and number of ATR reflections.

If a mixture being studied has a refractive index near that of the ATR element, a high frequency transmission cut-off can occur due to dispersion of its refractive index. We have demonstrated that this cut-off can be shifted to higher frequencies by adding moderate amounts of a solvent having a lower refractive index. Furthermore, the sensitivity of the analysis...
for weak bands can be maximized by selecting the fractional dilution and ATR element parameters to place the transmission cut-off slightly to the high frequency side of the bands of interest.

It should be pointed out, of course, that any measurements made near the critical angle can be adversely affected by any variables — such as temperature or the nature of the feedstock — which can affect the refractive index of the solution. In making such measurements, it is thus crucial to control the cell temperature and to calibrate for all other significant variables.

The results given above indicate the potential for applying UV-visible ATR to a wide variety of measurement tasks while minimizing the need for the dilutions often required in transmission analysis. For situations requiring no dilutions, this offers the possibility of continuous on-line analysis. Even in cases where moderate dilution is required in order to reduce the refractive index, it may be possible to divert a sample to a side stream, meter-in a controlled amount of solvent, and return the mixture to the process with minimal impact on process conditions.

References