

Sources of non-linearity in near infrared spectra of scattering samples

Donald J. Dahm

Department of Chemistry and Physics, Rowan University, Glassboro, New Jersey, USA

Introduction

The goal of our work is to convert spectra, which are readily obtained, to data that is a combination of linear functions of the various factors that affect the spectra.¹ Ideally, we would convert the spectra to the *absorbing power* of the material in the sample. The absorbing power of a material is the hypothetical fraction of incident light that would be absorbed if every absorption site, in some defined amount (usually given by a thickness) of material, were illuminated with the full incident intensity. The hypothetical fraction may be greater than 1.

In a mixture of absorbers, the absorbing power of the mixture is proportional to the absorbing power of each component times its concentration: that is, it follows Beer's law. In real samples, the incident light is not equal at each absorption site but, for a homogeneous material, the absorption power may be obtained from the Bouguer-Lambert law, which says that the intensity traveling through a material will fall off in accordance with the exponential function: $\exp(-kd)$, where k is the absorbing power and d is the distance travelled by the light (Figure 1). Taking a *logarithm* of this exponential yields a straight line, the slope of which is proportional to the absorbing power and called a *linear absorption coefficient*. The relationship is strictly true only for light travelling *within* a continuous material.

Examples and discussion of sources of non-linearity

Experimentally, we do not make a measurement within a material. When we make absorbance measurements on a real sample (especially a highly-scattering sample), a spectrum collected is not the

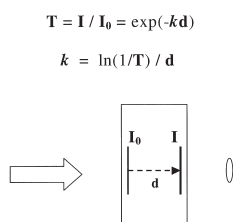


Figure 1. The transmitted intensity decreases as an exponential function of distance within a homogeneous particle. The absorbance (negative logarithm of the fraction of light transmitted) is a straight line. The slope of the line is proportional to k , which is called the absorbing power of the material making up the particle.

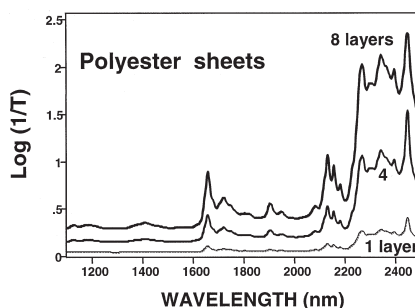


Figure 2. For samples consisting of plane parallel sheets, the absorbance $[\log(1/T)]$ in transmission is approximately proportional to the number of sheets in the sample.

absorbing power of the material and, generally, is not even linear with the absorbing power. [By the term “is linear with”, we mean “can be related by an *offset* and a *multiplier*”.] Below we will show how non-linearity can arise. [We mention in passing that the term *absorption coefficient* is also commonly used to mean the $\log(I/T)$ spectrum obtained from a material as it is presented in a particular transmission experiment. Usually, in a series of measurements the presentation is kept the same so that this difference between the experimental coefficient and absorption power may be corrected for by a calibration.]

Our first illustration of non-linearity involves increasing the number of layers in a transmission measurement. The spectra in Figure 2 show the $\log(I_0/I)$ [also referred to as $\log(I/T)$ or *absorbance*] spectra of 1, 4 and 8 identical polyester sheets, each with a rough, light diffusing surface. At first glance, the spectra appear to be approximately proportional to the sample thickness, given by the number of layers. However, as can be seen in Figure 3, where each spectrum is presented full scale, the shapes of the spectra are different. This is an indication of non-linearity.

If we had increased the thickness of the sample by increasing the thickness of a single layer, we would not have observed this. But by introducing the additional surfaces in the sample, we give the light the opportunity to reach the detector after it has bounced back and forth between the layers. This increases the effective path length within the sample. If there is very low absorption, the extent of this bouncing is determined by the amount of reflection or scatter that occurs at each surface. However, if there is very high absorption, there will be little increase in path length, because most of the light is consumed in the first pass through the layer. This leads us to our first generalisation:

Non-linearity will occur whenever the effective path length of light within a sample is a function of absorption.

Our second generalisation is:

Non-linearity will occur whenever light that has not been subjected to the absorption process reaches the detector.

Two very commonly encountered sources of such light are:

- in transmission: stray light; and
- in remission (reflectance): front surface reflection. (The reflection from the first surface encountered by the incident radiation has not been subject to absorption by the sample)

In Figure 4, we illustrate the effect of 10% of the incident light that has not been subjected to the absorption process striking the detector. Notice that the absorbance is suppressed, but the zero point is not moved (because there is no absorbance to suppress). The effect is to reduce the absorbance at the high absorption levels far more than at lower levels, introducing a non-linearity.

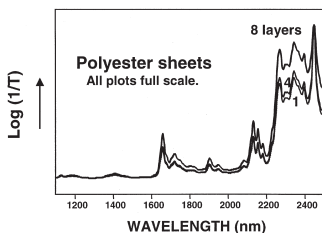


Figure 3. Displaying full scale the absorbance of each sample in Figure 2 shows that the presence of surfaces within the sample causes non-linearity in absorbance among the spectra, resulting in absorbance curves with different shapes for differing numbers of sheets.

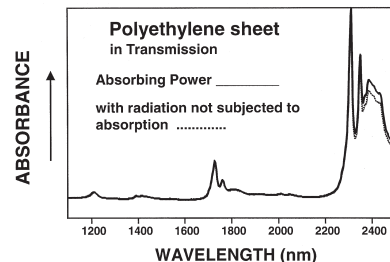


Figure 4. Illustration of non-linearity that would be exhibited in a transmission spectra of a single sheet, if 10% of the incident radiation reached the detector without being subjected to the absorption process.

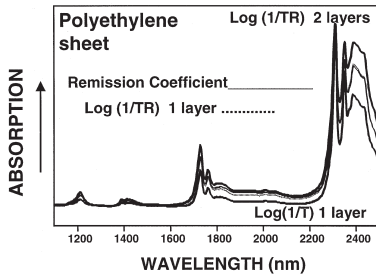


Figure 5. The reflection from the front surface observed in remission causes a change in shape when going from a transmission spectrum (bottom curve) to a transfectance spectrum (middle curve, dotted) of a single sheet. The absorption coefficient observed in remission (middle curve, solid), to a good approximation is proportional to the transfectance spectrum of a single sheet, but not to the transfectance spectrum of samples with additional sheets (top curve).

layer yields a spectrum that, to a good approximation, is proportional to the effective absorption coefficient of the sample as it would be observed in remission. This coefficient^{2,3} (obtained as described in Reference 2) is also displayed as one of the middle curves in Figure 5. The effective linear absorption coefficient obtained in remission is not a linear function either of the absorbing power or the absorbance spectrum obtained in transmission. In transfection, as in transmission, there is further non-linearity introduced by increasing the number of layers in the sample.

The extent of non-linearity due to surface reflectance may be calculated using the Stokes' formulas⁴ for absorption, transmission and remission by a single plane parallel layer. These results are compared in Figure 6 to the coefficient observed in remission.³ [The coefficients obtained in transmission and remission have been adjusted to the same scale using the assumption of the Kubelka–Munk theory,⁴ which is a simple factor of 2]. This illustrates that, for some simple cases, we can relate the absorbing power mathematically to the curves obtained in remission.

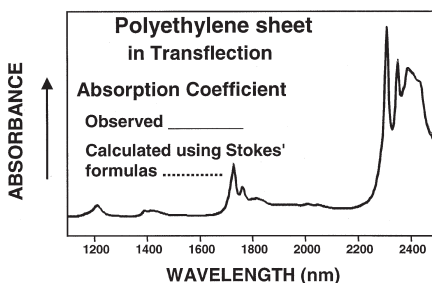


Figure 6. Using Stokes' formulas, developed in the 1860s, the spectrum observed from a single sheet in transfectance (solid) can be calculated (dotted) from the absorption coefficient observed in transmission.

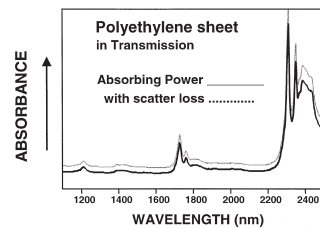


Figure 7. Illustration of the approximately linear change that would be exhibited in a transmission spectrum of a single sheet, if 10% of the incident radiation did not reach the detector (for example, due to scatter). Such a curve has essentially the same shape as that of the absorbing power of the material making up the sheet.

The effects of both these generalisations may be observed in Figure 5. Here we see that the absorbance spectrum in transmission [$\log(1/T)$] of a single layer (shown as the bottom curve) has a different shape than the absorbance spectrum obtained in transfection [$\log(1/TR)$] of the same sample (shown as one of the two middle curves). The number of layers for a sample observed in transfection is effectively twice that in transmission. Further, there is surface reflection observed in transfection. Thus the two curves are non-linear. The $\log(1/TR)$ spectrum of two layers (shown as the top curve) has yet a different shape, as further non-linearity is introduced by the increased number of layers.

In transmission, the absorbance spectrum of a single plane parallel layer yields an absorption coefficient that is a good approximation to the absorbing power. In transfection, the absorbance spectrum of a single plane parallel

The above situation may be contrasted to a case that does not introduce significant non-linearity. In an absorbance measurement, any incident light that is not detected contributes to the measured absorbance. In Figure 7, we illustrate what would happen in an experiment if some of the incident radiation is lost through a mechanism other than the absorption process. The solid line represents the absorbing power of polyethylene. Displayed on the same scale is a spectrum (shown by the dotted line) that illustrates the effect of 10% of the incident light being scattered and missing the detector without having been absorbed. The absorbance curve is lifted up. If we displayed this second curve full scale, it would lie beneath the solid line, indicating that the shape remains essentially unchanged. This effect has introduced a change that yields a spectrum that is linear with the absorbing power.

Let us assume that this case is a transmission experiment and it is scatter that caused the intensity loss. The amount that the curve is lifted up is a measure of the amount of scatter loss. In this example it is 10%. It may be corrected for by:

subtracting an amount from the transmittance spectrum corresponding to 10% or 0.1, and then dividing the remaining transmittance by 0.9 to put it on the scale of the incident intensity.

This is an example of a way to obtain the absorbing power of a material from a transmission measurement in the presence of scatter. Similar techniques have been shown to be useful in linearising a response in preparation for linear regression.⁵

There are, in fact, other mechanisms that introduce linear changes in the absorbance spectrum. An example is the change in void fraction in a sample. Of course, such a change in the presence of another effect that produces non-linearity, such as stray light, may produce two spectra that are non-linear with each other.

Since we have said above that:

(a) in transmission, the absorbance is a linear function of layer thickness; and
 (b) changes in void fraction do not, in themselves, cause non-linearity in a transmission spectrum; it is tempting to conclude that a single layer of particles, in transmission, will yield a spectrum proportional to the absorbing power of the material making up the particles. We would like to now illustrate that particles do not behave as plane parallel layers and that this assumption is not correct.

In Figure 8 are two spectra of a very thin sample (essentially a mono-layer of particles, with spaces between them). The difference in the spectra is that one has the detector closer to the sample than the other. The loss due to scatter is greater for the one further away. In Figure 9, both of these are shown full scale. They have essentially identical shapes. This would seem to indicate that we have an absorbance curve, unaffected by scatter and, therefore, have a measure proportional to the absorbing power of the material. In Figure 10, the absorbance spectra of the particles is displayed full scale along with the

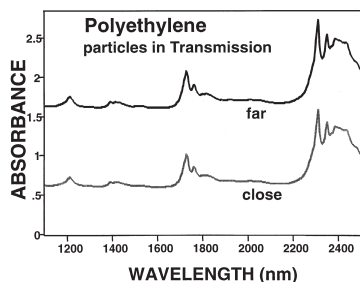


Figure 8. There is an observed increase in absorbance from a thin sample of particles when the distance from sample to detector is increased.

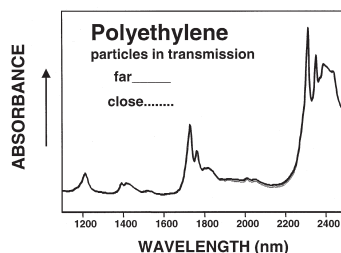


Figure 9. The shape of the spectra in Figure 8 are relatively unaffected by the change in distance from the sample to the detector and the curve has the shape, approximately, of the effective absorption coefficient for a particulate sample.

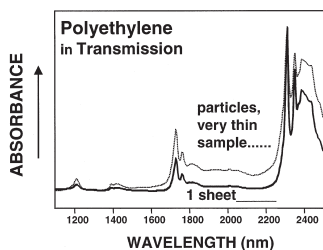


Figure 10. The effective absorption coefficient of a particulate sample is not the same as that of the material making up the particles, because a significant fraction of the light striking the surface of a particle reaches the detector (through the process of diffraction) without having been subjected to the absorption process.

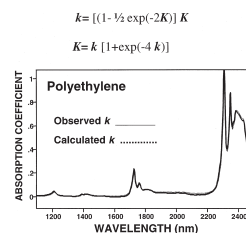


Figure 11. For a particular experimental arrangement, the absorption coefficient observed in transmission, k , can be related to the absorption coefficient observed in remission, K , by a function containing a single exponential term. The observed absorption coefficient has the same shape as the absorbance curve of a very thin sample.

transmission spectrum of a single sheet. Notice that they do not have the same shape. Why not? Particles do not reflect light, either externally or internally, in the same manner as plane parallel sheets. For example, small particles, unlike sheets, exhibit a significant amount of diffraction around the sphere.⁶ Some of the light that strikes the surface of the particle continues toward a transmission detector without being subjected to the absorption process (analogous to the case of front surface reflection in remission). This is yet another source of non-linearity in a particulate sample.⁷

Correcting spectra for non-linearity

Now we would like discuss an approach to relate spectra obtained under one set of conditions to spectra obtained under another set. For example, if the effective linear absorption coefficient, K , obtained in remission is not the linear with the one obtained in transmission, k , can they be related by a simple function? In Figure 11 are shown the functions that relate such data as obtained on a Foss NIRSystems 6500. These are empirical functions, and were reported previously.⁷

So, if these functions can relate the absorption coefficients obtained in remission and transmission: Can they be used to more generally relate data obtained in remission to that obtained in transmission on the same sample? In Figure 12, we see data on an “infinitely thick” sample of polyethylene particles. The top and bottom curves are the absorbance spectra obtained in transmission and remission. The curve in the middle is that calculated by the formula at the top of the figure (which has the same form as the bottom one shown on the Figure 11). The simple one term function no longer works, and we need to add another term. The top curve is actually a bold spectrum observed in transmission along with a dashed curve that was calculated from the remis-

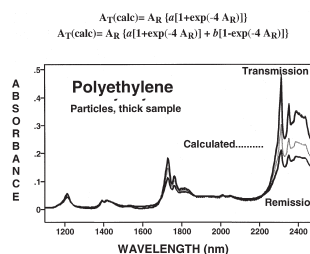


Figure 12. For the same experimental arrangement as in Figure 11, the absorbance observed in transmission (top curve, solid) can not be obtained from the absorbance observed in remission (bottom curve, solid) with a function containing a single exponential term (middle curve, dashed), but can be obtained with a function containing two exponential terms (top curve, dashed).

sion spectrum and the bottom formula in Figure 12. In general, we can relate curves obtained under various conditions by a two-term relationship, where:

- one term has full effect at very high absorbance values, and no effect at low absorbance values, and
- the other term has full effect at very low absorbance values, and no effect at high absorbance values.

We are now doing theoretical work to guide us in the selection of the form of these functions, but what is important is that:

If we adjust the a and b factors to fit the ends of the data, it seems possible to find a factor in the exponential (replacing the “4”) that will fit the data in between.

Conclusion

So where does that leave us in the quest for linear spectral data?

We hope to be able to use the measurements at low absorbance levels to obtain effective linear absorption and remission coefficients. (It is not always possible to obtain such data at higher absorbance levels, when a single particle is “infinitely thick”.) Then by a combination of theoretical relationships and empirical functions (obtained for a particular experimental arrangement), relate an absorbance curve collected either in remission or transmission to the absorbing power of the material.

We presume that if we had such linear data, principle component analysis would work better, and that results of calculations of “distance” between spectra would change significantly. The assumed advantages in linear regressions include: fewer PLS factors (comparable to the number of components) being required to adequately describe a data set, and eliminating the need to limit the spectral range to get a more linear response to amount of analyte.

Acknowledgment

The work described here was performed as part of a larger effort jointly with Kevin Dahm and Karl Norris. Experimental measurements were performed in the laboratories of Foss NIRSystems.

References

1. D.J. Dahm and K.D. Dahm, *J. Near Infrared Spectrosc.* **3**, 53 (1995).
2. D.J. Dahm and K.D. Dahm, *Appl. Spectrosc.* **53**, 647 (1999).
3. D.J. Dahm, K.D. Dahm and K.H. Norris, *J. Near Infrared Spectrosc.* **8**, 171 (2000).
4. D.J. Dahm, K.D. Dahm and K.H. Norris, *J. Near Infrared Spectrosc.* **10**, 1 (2001).
5. K.H. Norris, *NIR news* **12(3)**, 6 (2001).
6. H.C. van de Hulst; *Light Scattering by Small Particles*. Dover Publications 200 (1981).
7. K.D. Dahm and D.J. Dahm; *Practical Limitations Imposed by Particle Size in Determination of Absorption Coefficients*. International Diffuse Reflectance Conference, Chambersburg, Pennsylvania, USA, August 14, (2000).