Optimal absorbance measured under constant detector noise and in the presence of stray light

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Introduction

Spectra should, if possible, be measured at an absorbance level where the signal-to-noise ratio (*S/N*) is optimal i.e. at maximum *S/N* values. In practice, choosing the optimal light pathway or the optimal concentration of the constituent can in some cases accommodate this. In other cases, such as diffuse reflectance measurements of samples, it may be difficult to alter the sample, then the instrumental or the instrumental setup can sometimes be optimised, such that absorbance can be measured close to optimum.

Stray light appears to some degree in all filter, grating and similar instruments, but not in Fourier transform (FT) instruments. While the stray light level in most contemporary grating spectrometers is rarely much greater than 0.1%, there are other cases where the sample can cause an equivalent effect to stray light. The most common of these is in diffuse reflection spectrometry, where front surface (specular) reflectance from the sample can be as high as 4%. In addition, the effect of a non-linear detector response is to give rise to an effective stray light level in FT-IR spectra that can be as high as 10%.[1–5] All these contributes to an "effective stray light" and are from now on termed "stray light".

The topic of optimal *S/N* has been discussed in several books[6,7] and articles.[8–12] Mark and Workman also previously presented a thorough discussion about noise in a series of seven articles in *Spectroscopy*; in this context part III[13] of the series is of most interest.

Contrary to other authors, Isaksson and Griffiths[8] and Mark and coworkers[13,14] in addition to Cole[9] present a theory in which the effect of noise from the reference spectrum is taken into account. Including this contribution to the noise makes a small numerical difference compared to the case where it is ignored, but it is expected to give a better approximation of the errors and an improvement of the theory.

The aim of the present paper is firstly to present the theory to calculate the absorbance signal-to-noise ratio, when detector noise predominates and in the presence of stray light. A simple, general theoretical method (i.e. error propagation) to analyse noise and *S/N* and their effect on the optimum absorbance in absorption spectroscopy, such as diffuse reflection and transmission spectra measured in the near infrared (NIR), mid-infrared and ultraviolet/visible regions using dispersive spectrometers, will be presented. The second aim is to calculate the effect of these results presented as concentration signal-to-noise ratio, including Beer–Lamberts law. The present treatment will contribute to a better pedagogical understanding and an improvement of the theory of these subjects.

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Stray light

Absorbance (A_t, subscript t for "true") in transmission and reflectance spectroscopy is defined as the logarithmic ratio of the intensity (or energy) of radiation prior to the sample (I_o), also called reference intensity, and the intensity of radiation after the sample (I):

$$A_t = \log\frac{I_0}{I} \tag{1}$$

When stray light is included, background intensity or surface reflection (K_o and K) adds to the light intensities, here denoted I_o' and I'. Measured absorbance, A_m (subscript m for "measured") can then be written as:

$$A_m = \log\frac{I_0'}{I'} = \log\frac{I_0 + K_0}{I + K} = \log e \times \ln(I_0 + K_0) - \log e \times \ln(I + K) \tag{2}$$

Assume, that K_o and K are small parts of I_o, $K_o = k_oI_o$ and $K = kI_o$, where k_o and k are small numbers (for example, 0.1 % of I_o giving $k_o = 0.001$). Substituting Equation (1) into Equation (2), then gives:

$$A_m = \log\frac{1 + k_0}{10^{-A_t} + k} \tag{3}$$

Equation (3) is illustrated in Figure 1, showing that when stray light is present, measured absorbance will only be a portion of the theoretical absorbance.

Absorbance signal-to-noise ratio (S/N)

The instrument and the detector measure radiation intensity I_o' and I', together with some noise; the noise may be expressed as standard deviations, $s_{I_0'}$ and $s_{I'}$, respectively. Assume, for simplicity, that the noise $s_{I_0'}$ and $s_{I'}$ are normally distributed and constant over the intensity range of interest. Assume also that the stray light (K_o and K) is constant over the region in interest, and that there is no correlation between the noise $s_{I_0'}$ and $s_{I'}$. Under these assumptions, the following approximate estimate of the standard deviation of the measured absorbance (s_{A_m}) is expected due to the well-known theory of error propagation:

$$s_{A_m}^2 \approx \left(\frac{\delta A_m}{\delta I_0'} s_{I_0'}\right)^2 + \left(\frac{\delta A_m}{\delta I'} s_{I'}\right)^2 = \left(\frac{\log e}{I_0 + K_0} s_{I_0'}\right)^2 + \left(\frac{\log e}{I + K} s_{I'}\right)^2 \tag{4}$$

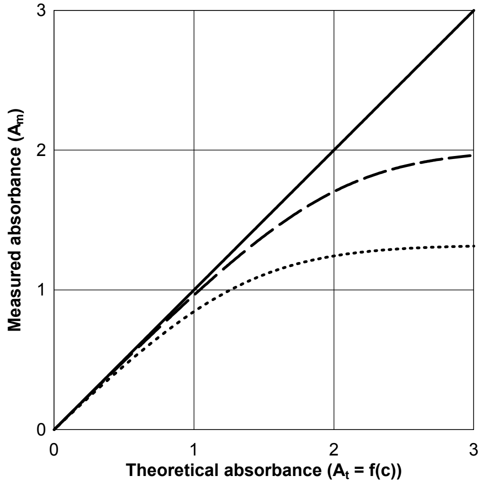

Figure 1. Measured absorbance as a function of theoretical absorbance (according to equation (3)) with a stray light of 0 (_______), 1 (----------) and 5 (………) % when (k = k_o). As an example: if an analyte with a given concentration (and pathlengths) theoretically should give an absorbance of 3.0, the measured absorbance is 1.96 and 1.31 when it is 1 and 5 % stray light, respectively.

Assume also that the noise levels for both light intensities are equal ($s_{I_0'} = s_{I'} = s$). This assumption could be debated but should be reasonable, when using the same detector and a narrow intensity range. Equation (4) then becomes:

$$s_{A_m}^2 \approx (\log e)^2 s^2 \left[\frac{1}{(I_0 + k_0 I_0)^2} + \frac{1}{(I + kI_0)^2} \right] = \frac{(\log e)^2 s^2}{I_0^2} \times \frac{1 + 10^{2A_m}}{(1 + k_0)^2} \tag{5}$$

The signal-to-noise ratio in absorbance is defined as $S/N = A_m / s_{A_m}$. Thus from equation (5) above, the signal-to-noise ratio can be expressed as:

$$\frac{A_m}{s_{A_m}} \approx \frac{I_0}{s \times \log e} \times \frac{A_m (1 + k_0)}{(1 + 10^{2A_m})^{1/2}} \tag{6}$$

The absorbance signal-to-noise ratio [Equation (6)] forms a maximum measured absorbance (Figure 2). To find the maximum of A_m/s_{A_m}, differentiation of Equation (6) with respect to A_m gives:

$$\frac{d\left(\frac{A_m}{s_{A_m}}\right)}{dA_m} = \frac{I_0}{s \times \log e} \times \frac{(1 + k_0)(1 + 10^{2A_m} - A_m \times \ln 10 \times 10^{2A_m})}{(1 + 10^{2A_m})^{3/2}} \tag{7}$$

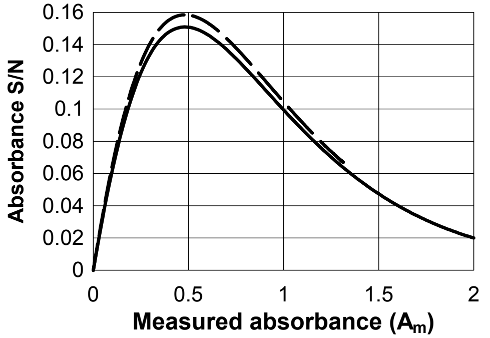

Figure 2. Absorbance signal-to-noise ratio as a function of measured absorbance, according to Equation (6). Lower curve (____) represents 0 % (k_o = 0) and upper curve (------) represents 5 % (k_o = 0.05) stray light.

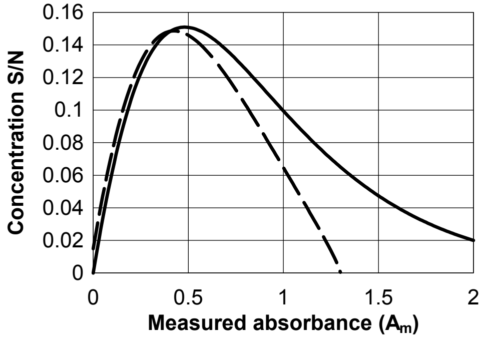

Figure 3. Concentration signal-to-noise a ratio as a function of measured absorbance according to equation (17). Upper curve (____) represents 0 % (k_o = 0) and lower curve (------) represents 5 % (k_o = 0.05) stray light.

Setting equation (7) equal to zero, gives:

$$1+10^{2A_m}-A_m\times\ln 10\times 10^{2A_m}=0 \tag{8}$$

Equation (8) does not have an analytical solution. A numerical calculation, gives the solution and the maximum *S/N* at $A_m \approx 0.4816$. Note, that the transmission, $T = 10^{-A_m}$, gives the maximum *S/N* at $T \approx 0.3299$ which is equal to the result of Cole[9] and Mark and Griffiths.[13] It can also be seen [Equation (8) and Figure 2] that stray light does not influence the optimum *S/N*.

To further compare this theory to earlier treatments, such as that of Ewing[6] by elimination of noise in I_o, and setting $k_o = 0$, the above equation becomes:

$$s_{A_t}\approx\frac{s_I\times\log e\times 10^{A_t}}{I_0} \tag{9}$$

The signal-to-noise ratio is then:

$$\frac{A_t}{s_{A_t}}\approx\frac{I_0}{s_I\times\log e}\times\frac{A_t}{10^{A_t}} \tag{10}$$

and differentiation of Equation (11) gives:

$$\frac{d\left(\dfrac{A_t}{s_{A_t}}\right)}{dA_t}=\frac{I_0}{s_I\times\log e}\times\frac{1-A_t\times\ln 10}{10^{A_t}} \tag{11}$$

Setting the right hand side of Equation (11) equal to zero gives:

$$1 - A_t \times \ln 10 = 0 \Leftrightarrow A_t = \frac{1}{\ln 10} \approx 0.4343 \tag{12}$$

This is equal to the result obtained by eliminating the error in I_o, in which case the maximum *S/N* is found for an absorbance of $A_t = 1/(\ln 10) \approx 0.4343$, or a transmittance of 0.3679.

Concentration signal-to-noise ratio, *S/N*

From a quantitative point of view, the effect of *S/N* in terms of concentration may be the most interesting factor in this context, as can be seen from the following argument. Beer's law can be expressed as:

$$A_t = \varepsilon \times c \times d \Leftrightarrow c = \frac{A_t}{\varepsilon \times d} \tag{13}$$

where c is molar concentration, ε is the molar absorptivity and d is the pathlength. Combining equations (1), (2) and (13), and setting $K_o = k_o I_o$ and $K = kI_o$:

$$c = \frac{\log e}{\varepsilon \times d} \ln \frac{10^{A_m}}{1 + k_0 - k \times 10^{A_m}} \tag{14}$$

If ε is considered to be constant and d is constant in a transmission measurement (or the scattering coefficient is constant in a diffuse reflection measurement), the theory of error propagation can be applied. The noise, s_c, in the concentration is then:

$$s_c \approx \frac{\delta c}{\delta A_m} s_{A_m} = \frac{s_{A_m}}{\varepsilon \times d} \times \frac{1 + k_0}{1 + k_0 - k \times 10^{A_m}} \tag{15}$$

Combining equations (5) and (15):

$$s_c \approx \frac{s \times \log e}{I_0 \times \varepsilon \times d} \times \frac{(1 + 10^{2A_m})^{1/2}}{1 + k_0 - k \times 10^{A_m}} \tag{16}$$

The *S/N* for concentration is then defined as Equation (14) divided by Equation (16):

$$\frac{c}{s_c} \approx \frac{I_0}{s} \times \frac{1 + k_0 - k \times 10^{A_m}}{(1 + 10^{2A_m})^{1/2}} \, ln \frac{10^{A_m}}{1 + k_0 - k \times 10^{A_m}} \tag{17}$$

The optimum value of c/s_c could be found by differentiating Equation (17) and setting the result equal to zero, but this is non-trivial. Figure 3 illustrates the variation of concentration *S/N* [Equation (17)] for different levels of stray light. Numerical calculation gives the maximum concentration *S/N* at different measured absorbances that vary from 0.4816, 0.4246 to 0.3739 when k equal 0, 0.05 and 0.1, respectively ($k_0 = 0$).

From a practical standpoint, these differences in the optimum values are small and insignificant when the stray light is small compared to the intensity of the reference spectrum. However, the range of absorbance values giving a *S/N* that is within a certain fraction of the optimum is significantly affected when $k > 0.01$.

Further details about these theoretical calculations can be found in Isaksson and Griffiths[8] and in the other references listed below.

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