NON-LINEARITY EFFECTS IN OPTICAL SCANNING OF CHROMATOGRAMS

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SUMMARY

The present paper is concerned with the error due to a finite spatial or spectral width of the illuminating scanning window in photodensitometers and reflectometers. The optical transfer characteristic of the medium is approximated by an exponential dependence upon the increment in absorbance, which produces the largest error. The results obtained are therefore worst case estimates. It is shown, that the absolute error in this case is with good approximation proportional to the mean square deviation of the absorbance from its mean value taken over the window. The more important percentage errors increase with increasing relative non-uniformity, that is with increasing ratio of variance to mean value. The results obtained are illustrated in three fairly typical numerical examples; the last of these showing that contrary to general practice relatively wide spectral windows may be tolerated without excessive error.

INTRODUCTION

In the optical scanning of chromatograms it is common practice to use an illuminated window covering the full active width of the medium. The scanning action is produced by moving the medium with a relative speed v perpendicularly to the slit (see Fig. 1).

The light transmitted or reflected by the illuminated area of the medium is collected by an optical system and concentrated upon a photodetector. The output signal of the photodetector is proportional to $I_0 \bar{A}$, where I_0 is the illuminating light flux and $\bar{A}$ the general transfer function (transmittance or reflectance) of the medium. The bar over the symbol signifies that the mean value over the illuminated area is to be considered.

When an area of the medium containing an absorbing substance with a local
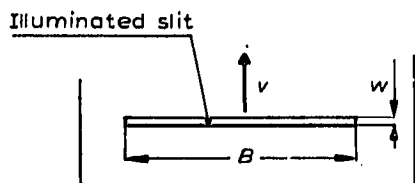
Illuminated slit

Fig. 1. Diagram of a slit scanning operation.

concentration $c(b)$ is reached, the absorbance increases by an amount $\Delta K(b)$ proportional to $c(b)$. We obtain therefore:

$$\Delta \bar{K} = \frac{1}{B} \int_B \Delta K(b) \, db = \frac{f}{B} \cdot Q \tag{1}$$

where Q is the amount of absorbing substance within the slit area and f is a proportionality constant, which is characteristic for the substance involved. ΔK causes a decrease ΔA of the transfer function A. The decrease in output of the photodetector is proportional to $\Delta \bar{A}$, that is to the mean value of ΔA over the slit. Unfortunately, however, $\Delta A(\Delta K)$ is in the general case a non-linear function of ΔK. As a result we obtain:

$$\frac{1}{B} \int_B [\Delta A(\Delta K(b)) - \Delta A(\Delta \bar{K})] \, db = \Delta \bar{A} - A(\Delta \bar{K}) = \varepsilon \neq 0$$

$$\Delta \bar{A} \neq A(\Delta \bar{K}) \tag{2}$$

There are only two cases where the unequality sign in eqn. 2 is not valid, viz. (1) if $\Delta K(b)$ is a constant, so that

$$\Delta K(b) - \Delta \bar{K} \equiv 0$$

and (2) if ΔA is a linear function of ΔK, that is if

$$\frac{\delta \Delta A(\Delta K)}{\delta \Delta K} = \text{const.} \tag{3}$$

If the unequality sign holds, the value of $\Delta \bar{A}$ obtained for a certain medium at a given Q is dependent upon the distribution $c(b)$ of investigated substance over the illuminated area. The purpose of all measurements of the type considered here is to determine $\Delta \bar{K}$ and from that Q as a function of the measured value $\Delta \bar{A}$. In the general case there is, however, no unique relationship between these two values. Since, in general, the distribution $c(b)$ is not known, it is common practice to neglect the non-linearity and to consider $\Delta \bar{A}(\Delta \bar{K})$ as equal to $A(\Delta \bar{K})$. Under this assumption a value for ΔK and from that a value for Q can be determined from the measured value $\Delta \bar{A}$ using a suitable calibration curve. This procedure introduces, however, an error ε_K the magnitude of which is determined by the left-hand side of eqn. 2. The purpose of this paper is to analyse the value of ε_K to be expected under some typical conditions.

Non-linearity errors due to a non-uniform distribution of investigated substance over the illuminated part of the medium may be reduced if the fixed slit illuminating optics are replaced by a flying spot scanning system. In this case, however, it becomes necessary to have an output signal, which is a linear function of concentration, if non-linearity errors in integrating the output of the individual spots over the whole zone

area are to be avoided. Flying spot scanning offers the advantage that in many cases it is possible to linearise the output signal either by analogue circuitry or by digital calculation, before summing is performed to determine the total amount of substance present. Flying spot scanning can therefore be made virtually independent of zone geometry; this is not the case when slit-shaped illumination is used, because part of the summing is performed in the photodetector itself with no possibility of subsequent linearisation.

In densitometry and reflectometry it has been common practice to employ a nearly monochromatic beam of light. Good monochromacity implies, however, a very narrow spectral width of the beam and therefore a considerable loss of light. If high sensitivities are required, the resulting decrease in illuminating intensity may be a severe limitation[1]. Using a beam with a relatively large spectral width may appreciably alleviate the situation and thus decrease the relative value of the electrical noise generated in the photodetector and the associated electronic circuitry. Since, however, the absorbance of a given substance is a function of wavelength, a non-linearity error entirely analogous to that defined in eqn. 2 arises.

$$\frac{1}{\Lambda}\int_{\Lambda} [\Delta A(\Delta K(\lambda)) - \Delta A(\overline{\Delta K(\lambda)})]\ d\lambda \neq 0$$

$$\Delta \overline{A(\lambda)} - A(\overline{\Delta K(\lambda)}) \neq 0 \tag{4}$$

Λ signifies here the spectral width of the scanning beam and $\Delta A(\lambda)$ the transmittance or reflectance as a function of wavelength. $\overline{\Delta K(\lambda)}$ is the mean value of the increment in absorbance $\Delta K(\lambda)$ over the band Λ.

Eqns. 1 and 2 are virtually identical and can, therefore, be treated in a common way. The expressions derived below, which give an estimate of the magnitude of the non-linearity error incurred, apply therefore equally to both situations.

THE CASE OF BEER'S LAW

The optical behaviour of most chromatographic support media may be described with adequate accuracy by the KUBELKA AND MUNK equations. The controlling parameters are the coefficient of scattering S and the absorbance K. In general, it may be assumed that the chromogen does not affect the scattering, so that for the purpose envisaged here the optical transfer of the medium (that is either transmittance or reflectance) can be considered as a function of K only with the value of S as a constant parameter.

The dependence of the transfer function $A(K)$ upon the absorbance K can be shown[1] to vary from a linear law to the exponential dependence, known as Beer's Law describing the transmittance of a medium with negligible scattering. The non-linearity error ε_K is evidently dependent upon the curvature of the function $A(K)$. If the radius of curvature R is large, the error will be small and vice versa. Fig. 2 shows a graph of $R(K)$ for four extreme conditions of scattering. The curves shown there are calculated from the theoretical expressions for the transmittance $A_T(K)$ and reflectance $A_R(K)$ derived in ref. 2 on the basis of the KUBELKA AND MUNK theory. From the figure it may be seen that for practical values of K the strongest curvature occurs with the exponential dependence. With regard to non-linearity effects the exponential
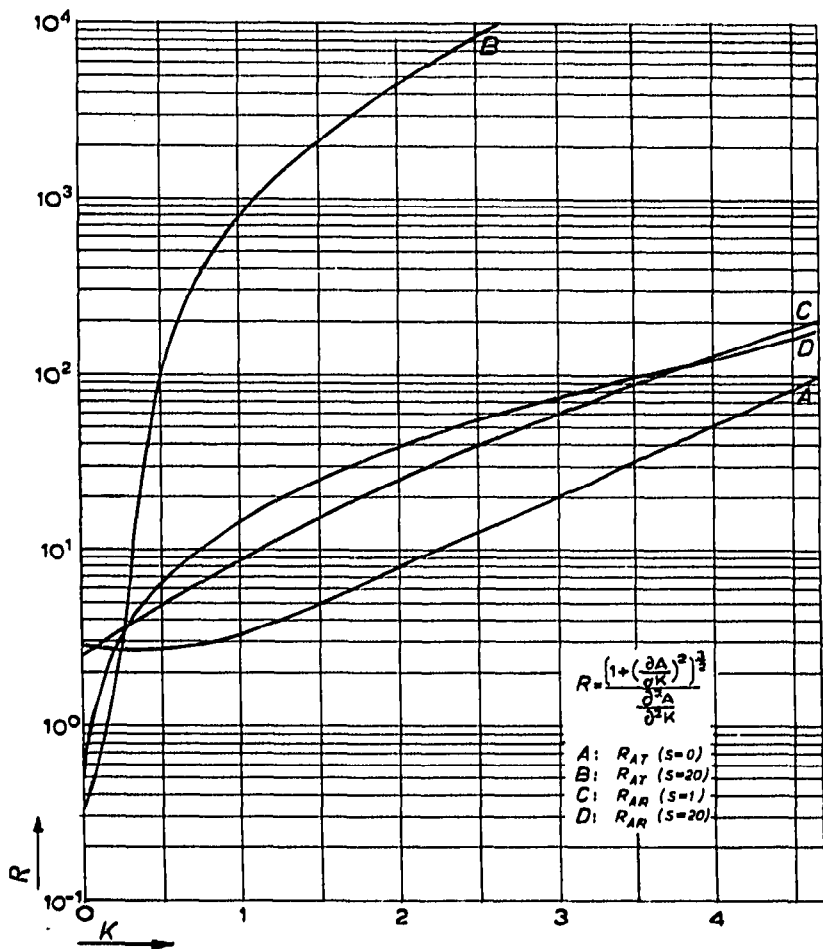
Fig. 2. The radius of curvature of transmittance and reflectance in dependence upon absorbance for extreme values of scattering. (A) R_{AT} ($S = 0$); (B) R_{AT} ($S = 20$); (C) R_{AR} ($S = 1$); (D) R_{AR} ($S = 20$).

dependence as postulated by Beer's Law represents the worst situation and produces the largest errors. A treatment based upon this law gives us therefore a worst case estimate.

The exponential relation representing Beer's Law can be expanded into a power series

$$e^{-K(x)} = 1 - K(x) + \frac{K(x)^2}{2!} - + \qquad (5)$$

In a qualitative way this may also be seen from Fig. 3 in ref. 3.

Breaking off the series after the nth term produces an error which can be shown to be less than the absolute value of the $(n + 1)$th term and to have the opposite sign as this term.

Let us now assume that in the region of concern the mean value of $K(x)$ is $\overline{K(x)}$, the bar again indicating the operation of averaging. The local value $K(x)$ will have a maximum absolute deviation of K_m from this value. Introducing $k(x)$ as a new variable, we obtain:

$$k(x) = K(x) - \overline{K(x)}$$

$$- K_m \leqslant k(x) \leqslant + K_m$$

Introducing this new variable, we may write:

$$A(K(x)) = e^{-K(x)} = e^{-[\overline{K(x)}+k(x)]} = e^{-\overline{K(x)}}\left[1 - \frac{k(x)}{1!} + \frac{k(x)^2}{2!} - +\right] \qquad (6)$$

Let the absorbance of the blank medium be K_0. The total absorbance of the medium, when an absorbing zone is scanned, is then of course the sum of K_0 and the increment $\Delta K(x)$ due to the absorbing substance. This gives us:

$$\overline{K(x)} = K_0 + \Delta \overline{K(x)} \qquad (7)$$

With a non-uniform distribution of $\Delta K(x)$ over the window space, as specified by the non-equality in eqn. 2, we obtain an output signal from the photodetector which is proportional to $\Delta A(K(x))$. Since the distribution of $\Delta K(x)$ is in general not known, it is common to determine the required amount of investigated substance, Q, by inverting eqn. 6 and introducing the result into eqn. 1. This procedure entails, however, an error ε, equal to:

$$\varepsilon_K = - [\log \overline{A(K(x))} - \log A\overline{(K(x))}] = - \log \frac{\overline{A(K(x))}}{A(\overline{K(x)})}$$

$$= - \log \frac{\Delta \overline{A(\Delta K(x))}}{\Delta A(\overline{\Delta K(x)})} \qquad (8)$$

Introducing the expansion (5) we obtain:

$$\varepsilon_K = - \log \int_0^x \left[1 - \frac{k(x)}{1!} + \frac{k(x)^2}{2!} - +\right] \frac{dx}{X} \qquad (9)$$

X is here the maximum value of the variable x, specifying the width of the optical window. X may stand for any variable upon which the absorbance increment $\Delta K(x)$ may depend. In the cases examined here X may signify the width coordinate b of the sample or the spectral width λ.

The second term of the integral in eqn. 9 is by definition zero. If K_m is not too large, the expansion may be terminated after the second order term resulting in:

$$\varepsilon_K \simeq - \log \left[1 + \frac{\overline{k(x)^2}}{2}\right] \qquad (10)$$

The error in the term under the logarithm, committed in this way, is less than

$$\left| \frac{1}{X} \int_0^x \frac{k(x)^3}{6} \, dx \right|$$

(See remarks following eqn. 5.) Expression (10) can be further expanded, giving

$$\frac{k(x)^2}{2} = u$$

$$- \varepsilon_K = \log(1 + u) \simeq u - \frac{u^2}{2} + \frac{u^3}{3} - \qquad (-1 < u \leqslant 1) \qquad (10a)$$

Terminating after the first term results in the important relation

$$- \varepsilon_K \simeq \frac{\overline{k(x)^2}}{2} \qquad \left(\frac{\overline{k(x)^2}}{2} \leqslant 1 \right) \tag{11}$$

According to the statement concerning the magnitude and the sign of the error incurred, if an alternating uniformly converging series is terminated after a finite number of terms, which was discussed in relation to eqn. 5 the value of ε_K obtained in eqn. 11 is larger than the real value and therefore too unfavourable. If the term $\overline{k(x)^2}$ is kept smaller than unity the error committed by using only one term of the series in eqn. 10 is less than $1/8$, which represents a maximum of approximately 12%.

The error ε_K as determined by eqn. 10 is expressed in natural units of absorbance. To obtain the error in optical density units, ε_K has to be divided by log 10 (*i.e.* 2.3).

The term $\overline{k(x)^2}$ in eqn. 10 is the mean square deviation of $\Delta K(x)$ from its mean value $\overline{\Delta K(x)}$. For practical applications the following relation, which can be proved easily, may be useful:

$$\overline{k(x)^2} = \frac{1}{X} \int_0^X \left[K(x) - \overline{K(x)} \right]^2 \mathrm{d}x = \frac{1}{X} \int_0^X \left[K(x)^2 - \overline{K(x)^2} \right] \mathrm{d}x$$

$$= \frac{1}{X} \int_0^x k(x)^2 \, \mathrm{d}x \tag{12}$$

Frequently it is not so much the absolute value ε_K of the error which is of importance but its relative value with respect to the total mean increase in absorbance $\overline{\Delta K(x)}$ over the absorbance value of the blank medium. Using eqns. 8 and 11 this relative error ε_{KR} turns out to be

$$\varepsilon_{KR}(\%) = 100 \, \frac{\varepsilon_K}{\overline{\Delta K(x)}} \simeq - 100 \, \frac{\overline{k(x)^2}}{2 \left[\log \Delta \overline{A(K(x))} - \dfrac{\overline{k^2(x)}}{2} \right]} \tag{13}$$

The conditions under which the right-hand approximation in eqn. 13 may be used are the same as those quoted in connection with eqns. 9 and 10a.

Once more it should be stressed that eqns. 11 and 13 are based upon Beer's Law and thus represent a worst case estimate; in most cases, and those with which practical photodensitometry or reflectometry are concerned, the curvature of A as a function of K is smaller than with Beer's Law and the error estimates obtained here are too unfavourable.

SOME NUMERICAL EXAMPLES

The implications resulting from eqns. 11 and 13 are probably best illustrated by considering some numerical examples, which approach the real situations frequently encountered in practice.
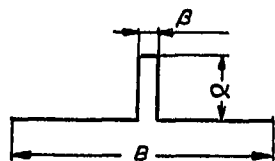
Fig. 3. Idealised absorbance profile of a narrow zone illuminated by a transversal slit.

As a first example we shall consider the extremely unfavourable case of a narrow zone, covering only a small part of the illuminated window. The idealised absorbance profile for this case is shown in Fig. 3.

If the zone is very narrow, that is if $\beta/B \ll 1$, we can put $k(b) \simeq K(b)$ and consequently

$$\overline{k^2(b)} \simeq \alpha^2 \cdot \frac{\beta}{B}$$

$$\Delta\overline{K(b)} = \frac{\alpha \cdot \beta}{B} \ll 1 \tag{14}$$

Introducing these relations into eqn. 13 we have:

$$\varepsilon_{KR} \, (\%) \simeq 100 \, \frac{\alpha^2 \cdot \dfrac{\beta}{B}}{2 \dfrac{\alpha \cdot \beta}{B}} = 100 \, \frac{\alpha}{2} \, (\%) \tag{15}$$

As mentioned before, for the numerator in eqn. 15 to be valid without excessive error α should be $\lesssim 1$. As a test upon the accuracy of the approximations involved, let us consider the exact result for the case where $\alpha = 1$ and $\beta/B = 0.1$. The true value of ΔK is evidently 0.1 and for $\Delta\bar{A}$ we obtain 0.1 $(1 - e^{-1}) \simeq 0.063$. From this we would obtain an apparent value of $\Delta K = \log(1 - 0.063) \simeq 0.063$ resulting in a relative error ε_{KR} $(0.1 - 0.063)/0.1 = 37\%$. The approximate expression (15) gives for the same case an error of 50%, that is a value about 30% too unfavourable. Since, however, the conditions of this particular example are rather extreme, this accuracy may be considered adequate. For less extreme cases the accuracy in determining the error by eqn. 15 is much better.

From eqn. 15 it follows that in the case considered the integration error is rather severe; to keep it within tolerable limits α has to be very small, of the order of 0.2 (less in natural units). To obtain a reasonable accuracy when narrow zones with higher increments in absorbance are to be scanned, the conventional slit-shaped window has to be replaced by a flying spot system. As already shown (see refs. 4 and 5), a further advantage of flying-spot arrangements is the considerable improvement in optical signal-to-noise ratio. In order to make full use of the advantages of a flying spot system, however, linearisation of the output signal is essential. This can be done by digital processing or by analogue circuitry; the latter procedure will be extensively discussed in ref. 2.

If β/B becomes larger, the situation improves due to an increase in ΔK. Let us assume $\beta/B = \frac{1}{2}$, then

$$\Delta\overline{K(b)} = \frac{\alpha}{2}$$

$$\overline{k^2(x)} = \frac{a^2}{4}$$

$$\varepsilon_{KR}\,(\%) = 100\,\frac{a}{8} \tag{16}$$

and this results in a fourfold improvement against the previous case.

A more favourable situation, which is a good approximation similar to many practical cases, will now be discussed. Let us assume that the distribution of $K(x)$ over the slit is parabolic although this is by no means essential (see Fig. 4). Here we obtain:

$$aK_1 = K_2$$

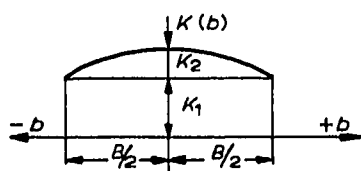$$K(b) = K_1 \left[1 + a \left\{ 1 - \left(\frac{2b}{B}\right)^2 \right\} \right]$$

Fig. 4. Parabolic absorbance profile over the window aperture.

$$\overline{K(b)} = \frac{1}{B} \int_{-B/2}^{+B/2} K(b)\cdot \mathrm{d}b = K_1 \left(1 + \frac{2a}{3}\right)$$

$$k^2(b) = \frac{1}{B} \int_{-B/2}^{+B/2} \left[K(b) - \overline{K(b)}\right]^2 \mathrm{d}b$$

$$= \frac{1}{B} \int_{-B/2}^{+B/2} K_1^2 a^2 \left[\frac{1}{3} - \left(\frac{2b}{B}\right)^2\right]^2 \mathrm{d}b = K_1^2 a^2 \left[\frac{1}{9} - \frac{1}{5}\right] \simeq 0.09\,a^2 K_1^2 \tag{17}$$

Introducing this value into eqn. 13 we obtain:

$$\varepsilon_{KR}\,(\%) = 100\,\frac{0.09 a^2 K_1^2}{2K_1 \left(1 + \dfrac{2a}{3}\right)} \simeq 4.5 a^2 K_1\,(\%) \qquad (a \ll 1) \tag{18}$$

If we assume $a = 0.3$ and $K_1 = 1$, we obtain an error $\varepsilon_{KR} \simeq 0.4\%$, a value which in most cases is negligible.

AN EXAMPLE FOR SPECTRAL NON-UNIFORMITY

Let us now consider a spectral window of finite width $\varLambda$ (see Fig. 5). The absorbance of the chromogen will in general vary over this width. The real curve of
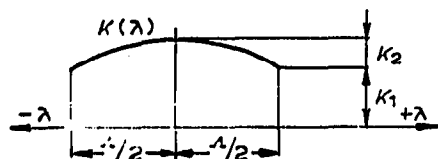
Fig. 5. Approximation of the spectral absorbance curve by a parabolic arc.

absorbance against spectral position within the window will again, as was done in the previous example, be approximated by a parabolic arc, though any other reasonable analytical expression (*e.g.* a $\cos^2$ or Gaussian curve) will lead to very similar results.

The total increment in absorbance is, of course, proportional to the concentration c of the chromogen in the scanned part of the zone. We can therefore apply the results obtained in the previous example, putting:

$$K_1 = \Delta K_{max} \cdot \gamma$$

$$K_2 = aK_1$$

$$a = \frac{1 - \gamma}{\gamma}$$

This gives an error

$$\varepsilon_{KR}\ (\%) \simeq 4.5\ \frac{(1 - \gamma)^2}{\gamma} \cdot \Delta K_{max}\ (\%) \tag{19}$$

Assuming a rather broad spectral window with $\gamma = 0.5$ and the peak absorbance increment $\Delta K_{max} = 2.5$ the error still does not exceed approximately 5%. This illustrates that relatively broad spectral windows may be tolerated without any sizeable deterioration of measurement accuracy.

ACKNOWLEDGEMENT

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