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LINEARITY CONSIDERATIONS IN QUANTITATIVE THIN-LAYER CHROMATOGRAPHY

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SUMMARY

The relationship between the densitometric response of thin-layer chromatography (TLC) and the quantity of separated substance contained in a particular spot is discussed. Attention is focused upon remittance measurements. The relationship between the optical response signal and the concentration of the substance is highly non-linear. The quantity of substance contained in a particular spot is obtained by integration of the linearized spot signal over the area of the spot, an operation which requires a linear relation between optical signal and concentration. Transform equations which achieve approximate linearity over a wide range of concentrations are developed with modified solutions to the Kubelka and Munk equations as basis. The design possibilities for (flying) point scanners are briefly discussed. The need to obtain a baseline (surface) by algorithmic processing and the remaining plate noise are discussed as the ultimate limitation to the performance of the point scanning devices. Dual-wavelength scanning is able to reduce substantially both problems. The principles of quantitation are then discussed and the possibility of substituting the reading of peak values for integration. A comparative analysis of slit scanning *versus* point scanning is made.

INTRODUCTION

Densitometric (and fluorometric) methods are today the most frequently used techniques for quantitative thin-layer chromatography (TLC). The fluorometric response of a chromatogram (apart from fluorescence quenching) is a closely linear function of the spatial concentration of the substance and there is, therefore, no need to include it in this study. Densitometry in the wider sense involves both transmission and (diffuse) reflection measurements. The wavelengths used in chromatographic densitometry extend from the visible far into the UV. The transmittance of most chromatographic plates at these wavelengths is rather poor and generally too low for quantitation with adequate accuracy. The considerations which follow concentrate, therefore, explicitly upon measurements in the remission mode. Much of the reasoning is, however, equally valid for transmittance measurements, *e.g.*, for gel electrophoresis. Minor modifications may sometimes be necessary in some situations where direct application is not feasible, but this should rarely pose major problems.

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The beam reflected by a chromatogram consists in principle of several components. One of these is returned by specular reflection in the surface layers of the separating medium; it carries little or no information about the amount of separated substance in the illuminated region of the chromatogram. The coefficient of specular reflection is a random variable which changes from surface element to surface element. It contributes, therefore, to the baseline noise, but does not convey any useful information. The optical path of high-performance instruments ought, therefore, to be designed so as to minimize the amount of specularly reflected light which may reach the photodetector. Reduction of this component to zero is obviously not possible.

The disturbing effect of any residual specular reflection can be further reduced by suitable baseline processing to values which, provided the optical pathway has been appropriately configured, are well below those of other sources of noise*.

In optically thin media, part of the illuminating beam may escape without appreciable scatter through the remote surface. The effect of this directly transmitted light is in almost all regards similar to that of specularly reflected light. An exception are transmittance measurements on nearly transparent media, where it represents the principal useful component.

In all other cases the information which is the purpose of the measurement is carried by light scattered inside the active layer. Part of this light is "back scattered" and is utilized for remission measurements. In media of moderate optical thickness some "forward scattered" light may exit through the remote surface and can be used for measurements in the transmission mode. The scattered light is ideally completely diffuse and its energy distribution follows Lambert's cosine law. The amount by which the intensity of the scattered radiation changes in response to the concentration of substance in the viewed area of the chromatogram is with good approximation described by the equations of Kubelka and Munk[1]. The dependence can be assumed to be linear only in the case of low concentrations. At higher concentrations or, more precisely, at larger absorbances, the non-linearity becomes too strong to be disregarded without serious error. The discussion which follows assumes in all cases an ideally turbid medium which means that specularly reflected and directly transmitted light are discounted.

Non-linearity considerations

In quantitative chromatography the parameter of interest is the amount, C_A, of the analyzed substance A contained in a separated spot or zone. If the volume of the applied sample is constant, C_A is proportional to the concentration, c_A, of A in the sample liquid. The concentration c_A defined in this way must be distinguished from the spatial concentration distribution, $\xi(x,y)_A$, on the chromatogram after separation. Here y and x are the spatial coordinates of an area element, $dxdy$, of the chromatogram in the direction of flow and perpendicular to it. The spatial concentration $\xi(x,y)_A$ produces an optical response, $\Delta\eta(x,y)_A$, *i.e.*, the decrement in the optical response, $\eta_0(x,y)$, of the blank medium when the scanned area element of the chromatogram contains a separated substance with concentration $\xi(x,y)_A$:

$$\Delta\eta(x,y)_A = \eta_0(x,y) - \eta(x,y) \tag{1}$$

* A polarizing filter can be helpful; its use is, however, rarely warranted.

The quantity C_A is obviously equal to the integral of $\xi(x,y)_A$ over the area, S, of the separated spot. Due to the non-linearity of the relationship between $\xi(x,y)$ and $\Delta\eta(x,y)$, the integral of $\Delta\eta(x,y)$ is generally not proportional to that of $\xi(x,y)$:

$$C = \iint_S \xi(x,y)\mathrm{d}x\mathrm{d}y \neq k \iint_S \Delta\eta(x,y)\mathrm{d}x\mathrm{d}y \tag{1a}$$

In this expression the subscript A denoting substance A has been omitted for simplicity. The same will be done in the following unless such omission would cause confusion. Other things being equal, $\Delta\eta$ is an unique function of ξ. To recover $\xi(x,y)$ from the measured values of $\eta(x,y)$, the latter must be subjected to the inversion:

$$\alpha\xi(x,y) = k[\eta_0^{-1}(x,y) - \eta^{-1}(x,y)] \tag{2}$$

The exponent -1 which designates the inversion operator must not be confused with the same symbol indicating a reciprocal value. The symbol k (capital or lower case) is used throughout to designate a constant, not necessarily the same in different expressions, and α is the substance's coefficient of extinction.

The term linearization will be used for any operation which establishes a closely linear relationship between a function of $\Delta\eta$ and ξ or an integral thereof. A linearizing function can, therefore, be regarded as an approximate realization of the inversion operation[2]. The term "linearization" will also sometimes be used loosely to designate the conversion of densitometric readings into concentration values.

Point scanning and slit scanning

Commercial densitometers for use in TLC (or one-dimensional electrophoresis) can by and large be divided into "slit" scanning and "point" scanning devices. Slit-scanning instruments are easier to build and most often used; they employ an illuminated area in the shape of a narrow transversal rectangle ("slit") covering the width, X, of one track. During operation the slit moves, usually in a stepping motion, along the track. The terms "point scanning", "flying spot" or "meander" scanning are used to designate instruments where a small circular or quadratic spot is employed to scan the chromatogram point by point, in a way similar to the raster scan employed in TV and in general image analysis. Point scanning is in various respects superior to slit scanning, but the instrumentation needed is more complex and requires more sophisticated data processing. For this reason, point scanning has become a viable alternative to slit scanning only since the advent of the computer as an integral part of densitometric instruments.

The size of the scanning spot in a point scanner must be chosen small enough so that $\xi(x,y)$ within the area of the spot can be regarded as approximately constant. The design problems with instruments of this kind are very much alleviated by new developments in the field of opto-electronic sensors. The most noteworthy of these are semiconductor diode arrays especially those of the self-scanning type. Devices of this kind have been known for many years, but have only recently become available with high sensitivity, good amplitude resolution, spectral characteristics which extend well into the UV and, most important, at affordable cost. Picture tubes of the vidicon

or related type have also been perfected in recent years and may provide a cost effective alternative to solid-state devices especially for low-to-medium performance instruments.

With these and other technological advances it can be expected that densitometers of the near future will provide more and more sophisticated processing options for the chromatographic signal without an excessive increase in cost. Moreover, advances in the field of processing may permit the extraction of additional information from the raw data and thereby increase the performance of the method well beyond today's standard.

The development of new and improved photodetectors is not the only technological advance which affects the performance of densitometers for quantitative chromatography. New light sources have appeared with higher efficiency and a wider spectral range. The largest impact, however, has been that of the digital computer. Since the early proposals concerning the use of computer processing in quantitative chromatography[3,4] the computer has become an integral part of modern densitometers and the efficacy of the software is equally decisive for the performance of the instruments as their mechanical and opto-electrical design. Computers have also become more powerful at dramatically lower cost. The amount of preprocessing of the raw acquired data, *e.g.*, for point scanning is, therefore, no longer a serious economic consideration.

The advantages of dual-wavelength scanning[5] have also been recognized for many years. They are discussed in a later paragraph. As with point scanning, the introduction of this principle into practice was impeded by the costs involved. The new devices lend themselves easily to multi-wavelength scanning up to the complete spectral analysis of the sample. The future will show to what extent these highly sophisticated methods find their place in instruments for bread and butter applications, with their demands upon cost effectiveness, rapid and easy operation, etc.

Linearization of point values

In quantitative chromatography the principal aim is the determination of the amount of investigated substance contained in a particular spot. The straightforward answer to this problem is the integration of $\xi(x,y)$ over the area of the spot. The practical implementation of this seemingly trivial task is, however, fraught with a number of problems. The most serious of these are determination of the baseline, delineation of the boundaries of the spot and the non-linearity of the densitometric response in terms of spatial concentration. Only the last problem will be discussed in this paper.

Linearization of the densitometric point response at low concentration

The amount of separated substance contained in a particular spot is obviously determined by the integral of the spatial concentration density, $\xi(x,y)$, over all area elements $dxdy$ within the confines of the spot boundary. In practice these area elements have finite size, $\Delta x \cdot \Delta y$, and the integral becomes a finite sum. Immediately available from densitometric measurements is, however, only the optical response signal, $\eta(x,y)$, which is generally a non-linear function of $\xi(x,y)$. For correct integration it is, therefore, first necessary to transform $\eta(\xi)$ in such a way that the transformed signal is a linear function of ξ (see also eqn. 1).

The mathematically most general approach to linearization is the inversion of $\eta(\xi)$ as described by eqn. 2. Analytically, $\eta(\xi)$ can be derived from the Kubelka and Munk (K&M) equations. The inversion can then be performed on the same basis. A simple and convenient approximate solution to this problem was derived by the author some years ago[6–8]. Other approximations, many derived purely heuristically, are in widespread practical use[9–12]. At this point of the discussion it is assumed that the data to be operated upon are obtained by point scanning. The handling of data supplied by a densitometer with slit scanning will be discussed in the final paragraphs of this paper.

At low substance concentration a completely general approach can be employed with does not require any specific assumptions about the relationship between the concentration and the optical response. Developing the function $\eta(\alpha\xi)$ into a Maclaurin series yields:

$$\eta(\xi=0)=\eta_0$$

$$\eta(\alpha \cdot \xi) = k\left[\eta_0 - \eta_0' \cdot \alpha\xi + \eta_0'' \cdot \frac{(\alpha\xi)^2}{2}, \text{etc.}\right] \tag{3}$$

Truncating the series 3 after the first-order term yields

$$|\Delta\eta[\alpha \cdot \xi(x,y)]| \approx \eta_0'(x,y) \cdot \alpha \cdot \xi(x,y) \tag{3a}$$

which is an adequate simplification if everywhere on the track:

$$|\eta_0'/\eta_0''| \ll \alpha \cdot \xi \tag{3b}$$

When condition 3b is met, $\Delta\eta(\alpha\xi)$ is a closely linear function of $\alpha\xi$, which can be integrated immediately without requiring inversion. Condition 3b can be relaxed to the less restrictive form:

$$|\eta_0'/\eta_0''| \leqslant \alpha\xi_{\max} \tag{3c}$$

The reason is that the concentration profile in a spotted zone drops generally quite rapidly to both sides of its peak value.

Linearization at higher concentration

At concentrations which exceed the limit posed by conditions 3c or 3b, the expansion of expression 3 can still be used, but more terms of the series have to be maintained. The result is a non-linear polynomial approximation. Truncation of the series after the second- or third-order term is usually adequate. Linearization can be performed by solving the polynomial for $\alpha\xi$. It is, however, generally preferable to apply to $\eta(x,y)$ a suitable analytical transform. The transform equations used here are simplified forms of rigid solutions of the K&M equations. They seem to provide a better approximation over a wider range of concentrations than the commonly used empirical expressions.

The transforms below were obtained from a transmission-line equivalent of a

turbid medium[13]. Their identity with the standard form of K&M solution using hyperbolic functions may, therefore, not be obvious.

Though the main interest of this paper lies in measurements in the remission mode, the corresponding expressions for measurements in the transmission mode are briefly discussed as well. Parameters pertaining to transmission are identified by the subscript T, those for remission by R. The subscript R will later be omitted, where the discussion concentrates only upon remission values.

The absorbance of the blank medium, *i.e.*, in the absence of separated material, will be designated by Z_0, which is assumed to be a constant, independent of x and y, equal to the local mean value of the absorbance of the blank medium. In its native form Z_0 does not have these properties. Instead it contains a random and a semi-deterministic component. Here it is assumed that the latter component is removed by suitable preprocessing. The remaining random component combines with other sources of optical (plate) noise which have to be dealt with by appropriate processing steps.

The symbol SC designates the coefficient of scatter of the medium. It can generally be assumed that SC is not affected by the presence of substance and there is, therefore, no need to distinguish between the values of SC for blank and for spotted areas of the medium. Otherwise, SC exhibits a variability of similar character to that of Z_0, which must largely be compensated by the preprocessing algorithm.

The solution of the K&M equations for transmittance obtained from the transmission-line model is[8]:

$$Z(x,y) = Z_0 + \alpha\xi(x,y)$$

$$\eta(x,y)_T \approx K \cdot \exp - [Z^2(x,y) + Z(x,y) \cdot 2SC]^{1/2} \tag{4}$$

$$k \ln \eta(x,y)_T \approx - Z(x,y)\left[1 + \frac{2SC}{Z(x,y)}\right]^{1/2}$$

$$\approx - Z(x,y)\left[1 + \frac{SC}{Z(x,y)}\right] \tag{4a}$$

The term in square brackets can be disregarded if the fraction in the brackets is small:

$$k \cdot \ln \eta(x,y)_T \approx - Z(x,y); \qquad SC/Z_0 \ll 1 \tag{5}$$

In other words the logarithmic transform of the optical transmission signal can be used for efficient linearization over a wide range provided the separating medium has relatively little scatter and a basic absorbance, Z_0, of reasonable magnitude.

The counterpart of expression 4 for remission is[8]:

$$\eta(x,y)_R = K \cdot \frac{1 - \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}}{1 + \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}} \tag{6}$$

For measurements in the remission mode a medium with fairly large scatter is desirable. For good sensitivity the absorbance, Z_0, of the blank medium ought to be small. The simplified form of eqn. 5 is, therefore, not applicable. Instead, the following transform can be used:

$$\eta(x,y)_R + \eta^{-1}(x,y)_R = K \cdot \frac{1 - \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}}{1 + \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}} + \frac{1 + \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}}{1 - \left[\frac{2SC}{Z(x,y)} + 1\right]^{1/2}} \cdot \frac{1}{K}$$

It is now convenient to introduce a normalizing scale transform to make the coefficient (disregarded in 4) K equal to unity. Then we obtain:

$$\eta(x,y)_R + \eta^{-1}(x,y)_R = 4 \cdot \frac{1 + \frac{SC}{Z(x,y)}}{\frac{2SC}{Z(x,y)}} = 2\left[1 + \frac{Z(x,y)}{SC}\right]; \quad K = 1 \qquad (7)$$

The baseline

The linear dependence between a function $\varphi[\eta(x,y)]$ of the optical response signal and the absorbance $Z(x,y)$ as defined by eqns. 4 or 7 is in practice determined with the help of two calibration quantities, C_1 and C_2. One of these quantities can conceivably be zero. The procedure yields both the slope of the calibration line, the scale reading, $\alpha\xi(x,y)$, and the origin $Z_0(x,y)$, of the concentration scale. A third calibration point, C_3, helps to normalize K in eqn. 7. It has already been remarked that the base response, $\eta_0(x,y)$, is not constant, but exhibits semi-deterministic, spatially slow changes superimposed by smaller, spatially fast, random fluctuations. The former can be largely eliminated by an efficient preprocessing system. The fast fluctuations, however, remain and give rise to much of the "optical" or "plate" noise content of the acquired signal. Plate noise can partly be reduced by low pass filtering or integration. The remaining component is, however, the main limit to the performance of densitometric methods at low concentrations.

Direct measurement of $\eta_0(x,y)$ with conventional densitometers is feasible only at a few points. For all other ones it is necessary to find the reference value, $\bar{\eta}_0(x,y)$ or $\bar{Z}_0(x,y)$, from the available measured values of $\eta_0(x,y)$ by use of suitable algorithms. Several approaches have been utilized[14], but a fully satisfactory method remains to be found. The extrapolations necessary always entail a loss of accuracy. The values of $\bar{Z}_0(x,y)$ obtained by these algorithms define what is commonly called the "baseline" or, in the case of two-dimensional separations, the "base surface" of the recording.

A simple but highly effective way to reduce the residual plate noise is integration. Concentrations determined from integrals of $\alpha \cdot \xi(x,y)$ over the spot area are, therefore, generally more reliable than the method of peak amplitude reading mentioned below. This may not apply to very low concentrations near the limit of detectability. Dual-wavenlength scanning as a means for dealing with the problem of the baseline and baseline noise is discussed in the next paragraph.

Dual-wavelength scanning

Scanning with light of two different wavenlengths, one at the absorption maximum of the compound and one as far as possible outside the absorption band of the latter, is another possibility not yet fully appreciated. The method has the big advantage that the baseline is obtained by direct measurement and not indirectly by algorithmic processing. Another advantage is that residual plate noise is substantially reduced. To achieve that, the design must allow the two beams to illuminate identical area elements of the chromatogram. The method is helpful principally for point scanning; with slit scanning its effectiveness is substantially reduced.

Despite the obvious and well known advantages of dual-wavelength scanning[4], the method is rarely used in contemporary commercial instruments. The reason is likely the higher complexity which is reflected in higher costs.

With computer processing of the acquired data and the gradual transition to multi-detector circuits, the design difficulties and the resulting price disadvantage should slowly disappear and be more than compensated by the higher performance, which can be attained in this way.

Quantitation methods

The purpose of densitometric analysis is in most cases the determination of the amount, C, of substance contained in a separated spot. With point scanning, C is best obtained by integrating the concentration density, $\xi(x,y)$, over all scanning points within the area, S, of the spot:

$$\xi = \Delta Z/\alpha$$

$$C = \iint_S \xi(x,y)\mathrm{d}x\mathrm{d}y \tag{8}$$

Directly obtained from the measurement is of course not ξ, but the response signal, $\Delta\eta$, measured with respect to a baseline $\bar{Z}_0$ or $\eta(\xi=0)$. To take full advantage of the point-scanning approach, linearization should be performed before the integration 8 is carried out (eqn. 1). The value obtained for C is within wide limits independent of the sample volume, V, provided that

$$V \cdot c = C = \text{constant} \tag{9}$$

where c is the concentration in the sample solution which is in general more important than C. It is seen that to obtain c from C, the sample volume, V, must be known precisely. For calibration purposes it may sometimes be advantageous to change C by changing V whilst keeping c constant[15].

The shape of the concentration density distribution, $\xi(x,y)$, is, other things being equal, almost independent of C[14]. It can also be shown that the area, S, of a spot changes, other things begin equal, only slightly with C. Since the fringe elements of the spot area contain little substance, minor changes in S (or errors in the determination of the boundary of S) can be tolerated. It follows that C can also be approximately determined from the ratio of the corresponding values of ξ in the sample

spot and a calibration spot containing a known amount of substance, C_{cal}. Most frequently this is done by correlating the peak values of ξ in both spots, making separate integration redundant. The procedure has the disadvantage that it is generally more sensitive to plate noise and consequently less accurate. It is, however, extremely valuable for the separation of partly overlapping spots. In the latter case the accuracy can be increased by assuming that $\xi(x,y)$ can, regardless of the kind of substance, be regarded as a Gaussian cylinder with elliptical cross-section. This hypothesis is supported by both empirical findings and theoretical considerations[14]. It is, however, by no means essential. The weaker assumption that the concentration distribution, $\xi(x,y)$, has for all spots a closely similar shape is generally adequate.

Calibration

A straight line calibration characteristic is fully defined when two points on the characteristic are known. Two calibration quantities, C_1 and C_2, are, therefore, required for its determination. With some loss of accuracy one of these quantities can be zero. It has already been mentioned that in the general case a third calibration point, C_3, is necessary to eliminate the scale constant, K, in expression 8. It is probably not necessary to emphasize that the determination of $\bar{Z}_0(x,y)$ from the virgin plate is generally not very helpful, because development may produce significant changes in the optical parameters of the plate, hence the need for preprocessing of the finished chromatogram[16].

Calibration is usually performed by comparing the integrals of $\eta(x,y)$ over the area of the spot. Comparing the peak values of $\eta(x,y)$ is also feasible but seldom used. Using more than three calibration quantities may improve the accuracy, however, it is rarely warranted for routine work. Without linearization, however, a large number of calibration points becomes essential.

Determining C after integration

In practice it is quite common to reverse the procedure considered so far, that is to integrate $\eta(x,y)$ first over the spot area and to determine C from the integrated value. The advantage of this approach is that processing of the raw data acquired is simpler. The disadvantages are lower accuracy and loss of most of the advantages of points scanning over slit scanning.

Full equivalence obviously applies at low concentrations where the truncated form of 3 holds. At larger concentrations the error begins to increase and it becomes necessary to look for other more sophisticated techniques.

It has already been pointed out that the shape of the spatial concentration distribution, $\xi(x,y)$, is virtually independent of the sample quantity C:

$$\iint_S \xi(x,y)\mathrm{d}x\mathrm{d}y = KC = \iint_S \{\varphi[\eta(x,y)] - \varphi(\bar{\eta}_0)\}\mathrm{d}x\mathrm{d}y = \psi\{\iint_S [\eta(x,y) - \bar{\eta}_0]\mathrm{d}x\mathrm{d}y\} \quad (10)$$

Only the argument $\iint [\eta(x,y) - \bar{\eta}_0]\mathrm{d}x\mathrm{d}y$ of ψ is directly measurable. To find C it is

first necessary to determine the functional dependence ψ. This can again be done by developing ψ into a Maclaurin series, which is truncated after a suitable number of terms. The approach becomes impractical when the number of terms needed is too large. Point determination of the conversion characteristic by a sufficiently dense sequence of calibration points is then indicated. Analytical determination of ψ, *e.g.*, on the basis of the K&M theory is more difficult and actually feasible only when certain assumptions are met. Verifying that this is the case is impractical in most real situations. The method can, therefore, not be recommended for general use. All in all, it appears that, for point-scanning, linearization before integration ought to be the method of choice, especially since inversion after linearization does not seem to offer any significant simplifications. In the paragraph below it is shown that this finding applies also to slit scanning with line application of the sample solution.

Slit scanning versus point scanning

Modern commercial densitometers utilize mostly "slit scanning", though some of them have provisions for point scanning as well. Since the scanning operation in contemporary designs is implemented by mechanical means, the construction of slit-scanning instruments is simpler and the devices, therefore, are less costly. They are also faster in operation and the data processing is somewhat simpler. These advantages seem to outweigh the higher performance of point-scanning devices for most routine applications. This is, however, likely to change when electronic scanning begins to supersede today's mechanical methods. When maximum performance is required, point-scanning densitometers are decidedly superior.

The optical response signal, $\varepsilon(y)$, of a slit-scanning device is

$$\varepsilon(y) = \int_X \eta(x,y)\mathrm{d}x \tag{11}$$

where X is the length of the slit. It is implied that the width, Δy, of the slit is small enough, so that, over the distance Δy, $\eta(y)$ can be regarded as approximately constant. This condition is analogous to that governing the dimensions of the scanning spot in point-scanning devices.

Consider first the case where the analyzed sample is applied at the origin by a special device so as to form a line of uniform width across the whole width of the track. Assuming that the propagation velocity of the sample substances is uniform over X, the concentration profile of a spotted area is only a function of y:

$$\xi(x,y) = \xi(y) \tag{12}$$

Point scanning and slit scanning are then fully equivalent.

The situation is different when the examined solution is applied as a point at the origin. During migration the sample spreads both in the direction of flow and transversally to that. The useful optical response signal, $\Delta\varepsilon(y)_{\mathrm{Sl}}$, at the slit position y is then:

$$\Delta\varepsilon(y)_{\mathrm{Sl}} = \int_x [\eta(x) - \bar{\eta}_0(y)]\mathrm{d}x; \quad y = \text{constant} \tag{13}$$

The analogy with expression 10 is obvious. In general it cannot be assumed that $\xi(x,y = \text{constant}) \neq 0$ over the full slit length, X. The optical signal-to-noise ratio is, therefore, lower than the one obtained by point scanning with subsequent integration only of values $\Delta\varepsilon(y)_{SI} \neq 0$.

Conversion of the optical slit signal into concentration values can here be performed virtually only empirically using a sufficiently dense sequence of calibration points. Conversion based upon analytical expressions is scarcely feasible because the proportion of X over which $\xi(x,y) \neq 0$ is generally not known. The situation is in this regard equivalent to point scanning with integration of the optical signal without previous linearization.

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