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OPTICAL NOISE IN PHOTOMETRIC SCANNING OF THIN MEDIA CHROMATOGRAMS

I. GENERAL THEORY AND ITS APPLICATION TO SINGLE-BEAM TRANSMISSION MEASUREMENTS

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SUMMARY

An attempt has been made to apply the concepts of "signal" and "noise" so important in communication theory to the analysis of the performance of photodensitometers used for the quantitative assessment of thin media chromatograms. Very low concentrations of separated substances are considered thus permitting the linearization of the relationships involved. It has been shown that for the best performance under these conditions a highly stabilized light source is essential and that the spectral width of the scanning beam should be the same, or somewhat less than that of the absorbing zone of interest. At low concentrations flying-spot scanning as opposed to fixed-slit scanning is of no real advantage. The double-beam system described in the following paper is vastly superior to any single-beam arrangement.

INTRODUCTION

There exist numerous devices for the scanning of thin media chromatograms (see recent review[1]); nearly all of them are single-beam devices. Though they are rather inferior in their performance to the double-beam arrangements as described by SALGANICOFF *et al.*[2], based on the time-sharing dual wavelength spectrophotometer of CHANCE[3,4] they still represent the vast majority of all instruments in practical use.

In this paper an effort has been made to analyse the performance of photoelectric densitometers from a theoretical point of view and to determine those factors which affect their sensitivity, stability and reproducibility. The concepts and analytical procedures used are borrowed to some extent from the field of communication theory. In this paper the general theory will be discussed and applied to the analysis

of single-beam devices operating in the transmission mode. Double-beam devices are considered separately in the following paper.

The basic purpose of any physical measuring procedure is to supply information about the quantity to be measured. Regardless of the physical nature of the output signal obtained from the measuring assembly, it is frequently convenient to express it in terms of electrical parameters. The information content of any datum obtained from the measurement is thus determined by the ratio of the amplitude of the useful signal obtained to that of the sum of all undesirable and disturbing signals of any kind, which tend to obliterate the desired signal. These unwanted signals can be divided into two groups: the first, and the one which will be our main concern here, is of a random nature and may comprehensively be called "noise". The other, which will be dealt with in a subsequent paper, is deterministic in nature and essentially the result of non-linearities in the transfer function of the system involved. Neglecting the latter (as is done in this analysis) is tantamount to assuming a strictly linear transfer function of the system. In practical terms this means that we shall here restrict ourselves to the analysis of very small signals (this being the case for very low concentrations of absorbing substances), because in these cases virtually any transfer characteristic can be approximated by a straight line.

Regardless of this restriction it may be shown that the lower the ratio of useful signal amplitude to noise amplitude, the less is the amount of information that may be extracted from the individual measured result. The noise amplitudes determine the lowest signal level that may be detected and the resolving power of the method, in other words the smallest absolute difference in useful signal value that may be reliably distinguished. The signal to noise ratio, therefore, decides the obtainable accuracy.

The general rules mentioned above apply, of course, also to photometric methods of quantitative evaluation of chromatograms. In order to determine the inherent limitations upon the sensitivity, accuracy and resolving power of the evaluation a detailed study of the noise encountered is required. The output signal is the amplified output of the photoelectric detector unit. We have to consider, therefore, noise contributions from both the optical and the electrical parts of the instrument. The study, the results of which are presented here, was undertaken with the aim of analysing the sources of noise encountered, to assess it in a quantitative way and to determine possible approaches to reduce its detrimental influence. In these, the first two papers of a series, we are concerned only with the noise arising in the optical part of the system. An analysis of the electrical noise will be given in a later publication.

Our principal aim in this study was to investigate the feasibility of considerably reducing the levels of detection and quantitative evaluation of absorbing zones separated on various chromatographic media. It is, of course, in these regions of very low concentrations where noise considerations are of primary importance, whilst non-linearity effects may in first approximation be disregarded.

THEORETICAL

Let the absorbance of the chromatogram at a certain wavelength be α_c. Assuming that Beer's law is valid the resulting transmission is:

$$A_c = A_0 e^{-\alpha_c} = A_0 \left(1 - \frac{\alpha_c}{1!} + \frac{\alpha_c^2}{2!} - + \cdots\right) \quad (1)$$

The series expansion for $e^{-\alpha}$ shown in eqn. 1 possesses alternating signs and converges uniformly. It can be shown that the error involved by terminating the series after the nth term is less than the absolute value of the $(n + 1)$th term.

As we are concerned with low concentrations and, therefore, low values of incremental absorption α_c in an absorbing zone, the case where α_c is small is of special interest. In this case we may terminate the series in eqn. 1 after the second term.

$$\Delta A = A_0 - A_c = A_0 \left(1 - \frac{A_c}{A_0}\right) \approx A_0 a_c \qquad (a_c \ll 1) \tag{2}$$

The error committed will be less than $(A_0 \alpha_c^2)/2$. It can be shown that this approximation is also valid for all other laws of transmission, deviating from Beer's law, provided $\alpha_c \ll 1$. As a matter of fact it can be shown that (see later papers in this series), other conditions being equal, the error committed in these cases is always less than that committed with a purely exponential dependence.

The adoption of eqn. 2 results in a linearization of the relationship between the decrement in transmission and concentration and consequently it becomes permissible to use average (mean) values of concentration (absorbance) to obtain the average value of transmission or other characteristic optical parameters. Inversely, measured average optical values may be used to determine the average values of absorbance and concentration. The implications and limitations of this assumption will be explained in more detail in a subsequent paper of this series.

Furthermore, let us assume that the spectral density ε of the illuminating beam is constant over a certain spectral width $\Delta\lambda$; this results in an illuminating flux of $\varepsilon\Delta\lambda$. If the spectral density in this region is not a constant ε has to be taken equal to its average value over this region. $\varepsilon\Delta\lambda$ is also assumed to be constant over the spatial cross section of the beam. In practical use this condition may be difficult to achieve specially with long slits.

The background transmission A_0 of the material to be scanned may vary widely from a value of the order of 10^{-3} for paper chromatograms up to close to 1.0 for some films. In a given medium the value of transmittance may vary spatially from one part of the medium to another (see Fig. 1). A_0 is, however, also a function of the spectral wavelength and for paper chromatographic strips a steady increase in transmission occurs with increasing λ over which there may be superimposed random fluctuations. A similar situation is obtained with other types of support material.

When a coloured zone is encountered the transmission of the chromatogram decreases selectively over a certain region of the spectrum the width of which is determined by the effective width of the principal absorption band of the absorbing substance. In most cases this band is fairly wide, of the order of 50 mμ or more.

The absorbance of the investigated substance, however, is not constant throughout this region, but a function of the spectral position inside the absorbing band. The result of this is that the loss in transmission encountered is also a function of wavelength. To avoid the difficulties arising from this condition it is customary to employ

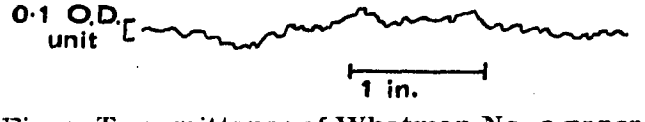

Fig. 1. Transmittance of Whatman No. 2 paper (scanning spot size 1 mm × 1 mm).

a very narrow spectral width of the illuminating beam. With low concentrations, however, where the linear approximation shown in eqn. 2 is reasonably valid, it becomes possible to use a fairly broad spectral bandwidth $\Delta\lambda_c$ and to operate with the average value of transmission over this band. Using a broader spectral width of the scanning beam offers a higher light output to the photoelectric receiver and therefore, a better electrical signal to noise ratio; an additional advantage is a slight decrease in optical background noise; this will be discussed later.

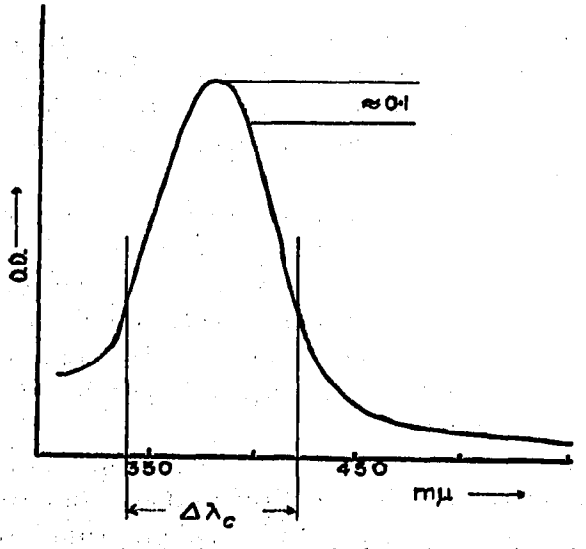

Fig. 2. Typical shape of the absorption band of a chromogen. (4-Hydroxy-4'-nitroazobenzene separated on Whatman No. 2 during 4 h in the solvent system light petroleum–toluene–acetic acid–water (133:66:170:30).)

The concentration of the absorbing substance varies over the coloured zone. In order to obtain meaningful data from current scanning devices, therefore, efforts are made in the practical methodology of chromatography (deposition of the solutes in bands, equilibration, constant temperature, etc.; see ref. 5 for further details) to ensure that the distribution of concentration within the illuminated scanning slit remains as constant as possible. With low concentrations where again eqn. 2 may be assumed to hold with reasonable accuracy, it is possible to relate the average concentration over the illuminated slit area linearly to the average decrease in transmission. This means that to a large extent the geometry of the zone and the distribution of concentration within the zone can be disregarded.

The transmission A_0 of the medium, as already mentioned, is spatially not constant but may vary from one point of the illuminated area to another in a random fashion. These fluctuations are caused partly by variations in the thickness of the medium, partly by local inhomogeneities. Variations in thickness produce transmission fluctuations, which are virtually independent of the wavelength of the scanning beam, whilst irregularities in composition may have effects, which are strongly dependent upon wavelength.

Let the mean value of A_0 over a very large (infinite) area of a particular type of chromatogram (paper, film, etc.) and at a given wavelength λ_0 of the scanning

beam be $\bar{A}$ and the standard (r.m.s.) deviation from this value be $\bar{\bar{A}}$. Since $\bar{\bar{A}}$ is usually quite small, we can again apply the linear approximation as in eqn. 2.

$$A \approx \bar{A}e^{\alpha} \simeq \bar{A}(1 + \alpha) \qquad \alpha \ll 1$$

$$\bar{\bar{A}} \approx \bar{A}(1 + \bar{\bar{\alpha}}) - \bar{A} = \bar{A}\bar{\bar{\alpha}} \tag{2a}$$

Let the mean value of transmission over a given slit of area F be A_s. $F = b\Delta W$ as illustrated in Fig. 3.

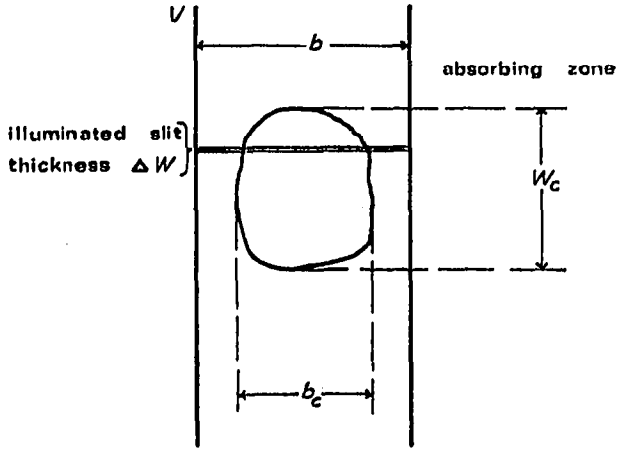

Fig. 3. Usual arrangement for fixed-slit scanning of chromatogram strips.

Since the area F is finite, A_s will vary. The mean value of A_s over many areas of the same size will, however, be $\bar{A}$. In accordance with the central limiting theorem of probability theory, A_s will approach a normal (Gaussian) distribution around $\bar{A}$ with a standard deviation $\bar{\bar{A}}_s$.

It then follows that $\bar{\bar{A}}_s$ will be approximately inversely proportional to the square root of the area of the illuminated slit.

$$\bar{\bar{A}}_s = \bar{\bar{A}} \sqrt{\frac{b^*\Delta W^*}{b\Delta W}} = \bar{\bar{A}} \sqrt{F} \tag{3}$$

$b^*\Delta W^*$ is the area of the smallest slit which may be used to define $\bar{\bar{A}}$. A decrease below this value does not increase the measured value of $\bar{\bar{A}}_s$. The values b^* and ΔW^* correspond approximately to the average dimensions of the irregularities in the density of the medium. These may of course vary considerably from one type of medium to another. They may also be interpreted as the fundamental spatial frequency of the noise caused by background irregularities. It is convenient if all the areas and cross sections are measured in multiples of $b^*\Delta W^*$; this means that on all subsequent occasions $b^*\Delta W^*$ is considered equal to unity. Since there is no reason to prefer one of the directions in the medium (provided it is reasonably homogeneous and isotropic), we may postulate that $b^* = \Delta W^* = 1$. Eqn. 3 is based on the assumption that

the variations in transmission A from one part of the chromatogram strip to another are random and uncorrelated provided their mutual distance is larger than one unit. Since A_s varies, the intensity I_s of the light transmitted by the strip will also vary from one strip to another. Let the mean value of I_s be $\bar{I}_s$ and its standard deviation $\bar{\bar{I}}_s$; let the light intensity entering the medium be I_0; due to a certain amount of surface reflection I_0 is smaller than the illuminating light intensity; the amount of light lost in this way is expressed in the following equation by the coefficient ψ. We thus obtain:

$$\varepsilon_0 = \varepsilon \cdot b^* \Delta W^* \cdot \psi$$

$$I_0 = \varepsilon_0 \Delta\lambda \cdot b \Delta W = \varepsilon_0 \Delta\lambda \cdot F$$

$$\bar{I}_s = I_0 \bar{A}_s(\Delta\lambda)$$

$$\bar{\bar{I}}_s = I_0 \bar{\bar{A}}_s(\Delta\lambda) = I_0 \bar{\bar{A}}(\Delta\lambda) \cdot 1/\sqrt{F} \qquad (4)$$

F denotes the illuminated slit area, measured in multiples of the area unit $b^* \Delta W^*$.

$\bar{\bar{I}}_s$ is the r.m.s. value of the light fluctuations at the input of the photoelectric receiver; it represents, therefore, the effective (r.m.s.) amplitude of the optical noise signal.

As already mentioned none of the media used in chromatography is ideally "grey"; this means, therefore, that the transmission A will, to a certain extent, be dependent upon the wavelength of the scanning beam. This dependence may be expressed as follows:

$$A(\lambda) = A(\lambda_0)\,[1 + g(\lambda - \lambda_0)] \qquad (5)$$

Over a reasonably small spectral distance we may approximate $g(\lambda - \lambda_0)$ by a linear trend component (g_0) superimposed by a random term (γ). The latter term as previously mentioned is a consequence of the slight inhomogeneities in the medium thus resulting in random changes in the transmission for different wavelengths in different parts of the medium.

$$g(\lambda - \lambda_0) \simeq g_0(\lambda - \lambda_0) + \gamma(\lambda) \qquad (6)$$

From the way $\gamma(\lambda)$ is defined it becomes evident that the spatial average of $\gamma(\lambda)$ obtained over a slit of infinite area is equal to zero. By a reasoning, which is completely analogous to that used in eqn. 3, it may be shown that the standard deviation $\bar{\bar{\gamma}}_s(\lambda)$ of $\gamma_s(\lambda)$, as measured over slits of finite area F, is proportional to $1/\sqrt{F}$. By analogy it may also be shown that spectral averaging of $\gamma_s(\lambda)$ over a spectral window of finite width $\Delta\lambda$ decreases the r.m.s. value $\bar{\bar{\gamma}}_s(\lambda,\Delta\lambda)$ approximately in proportion to $\sqrt{(1/\Delta\lambda)}$. Since $\bar{A}_s$ contains a component due to $\gamma_s(\lambda)$, it follows that an increase in spectral width of the scanning beam should decrease $\bar{A}_s$ and therefore improve the optical noise conditions. The improvement to be expected will depend upon the degree of inhomogeneity of the medium.

In a recent scanning device of more sophisticated design a double-beam arrangement is used[2]. In this device the two beams are arranged to have different spectral positions. It is the fluctuation of the difference in transmission between both

beams which is here of key importance. From the arguments developed above it is evident that this difference is made up from two terms: the first being variations in transmission, affecting both beams proportionally, *e.g.* random variations in thickness of the medium, and the second being local inhomogeneities affecting both beams in a randomly different way. Both terms may be considered as statistically independent. The standard deviation of the difference can, therefore, be calculated as the sum of the squared terms:

$$\overline{\overline{A_s(\lambda_0) - A_s(\lambda)}} = \frac{1}{\sqrt{F}} [g_0^2(\lambda - \lambda_0)\bar{\bar{A}}^2(\Delta\lambda) + \bar{\bar{\gamma}}^2(\lambda_0,\Delta\lambda)\bar{A}^2(\lambda_0) + \bar{\bar{\gamma}}^2(\lambda,\Delta\lambda)\bar{A}^2(\lambda_0)]^{1/2}$$

$$= \frac{1}{\sqrt{F}} [g_0^2(\lambda - \lambda_0)\bar{\bar{A}}^2(\Delta\lambda) + 2\bar{\bar{\gamma}}^2(\lambda,\Delta\lambda)\bar{A}^2(\lambda_0)]^{1/2} \quad (7)$$

In most cases one of the two terms in this expression will prevail; because of the quadratic law of addition, the other term may then be neglected especially if the spectral width $\Delta\lambda$ of the scanning beams is not too small. This will usually apply to the second term.

From the reasoning given above it appears that for the investigation of absorbents in low concentrations as separated on chromatograms a relatively large spectral width of the scanning beam is desirable. This reduces the amount of optical noise produced by possible inhomogeneities of the chromatograms with a non-grey absorbance characteristic. That the increased light intensity at the photodetector, which is obtained in this way, will decrease the relative weight of the electrical noise, originating in the photodetector and the associated amplifying equipment, has already been mentioned.

For high concentrations of absorbent, where consequently strong optical signals are encountered, both optical and electrical noise become less important. Here the linear approximation of eqn. 2 may entail large errors. To avoid these the spectral width of the scanning beam should then be confined to a region where the absorbance of the substances investigated is reasonably constant. In general a compromise between these conflicting requirements has to be made. More details on this will be presented in a subsequent paper.

In the next section and the following paper the performance of the two basic transmission design alternatives for chromatogram scanning devices, the single-beam and the double-beam arrangement, will be discussed with regard to the obtainable optical noise performance.

SINGLE-BEAM ARRANGEMENT

First let us consider a single-beam arrangement in which the light source is illuminating a slit of length ΔW and extending across the full width b of the chromatogram strip as shown in Fig. 3.

The basic arrangement of a scanning device of this type is illustrated in Fig. 4.

In the receiving photoelectric device (Ph) the transmitted light intensity is converted to a proportional electrical signal. The random component of I_s produces fluctuations in the electrical output which are for all practical purposes equivalent

to the noise in a communication system; they superimpose upon the electrical noise generated here.

There is, however, one important difference to be noted. In an electrical communication system most of the noise originates independently of the useful signal and therefore merely adds to it. The optical noise considered here, however, is multiplicative in nature (see eqn. 8).

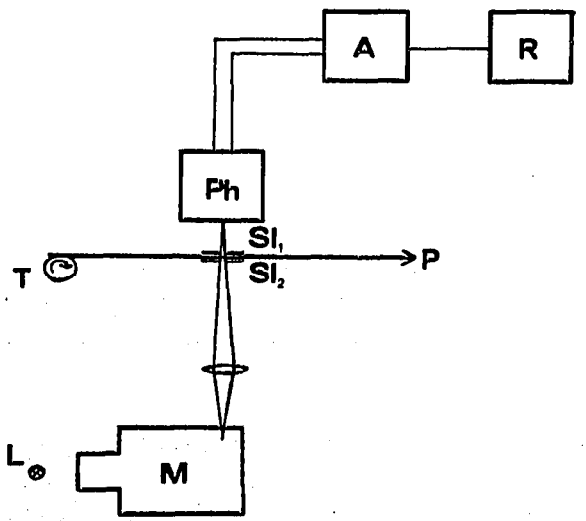

Fig. 4. Schematic representation of a single-beam chromatogram scanning device. L = light source; M = monochromator or filter; P = thin medium chromatogram; T = chromatogram transport mechanism; Sl_1 and Sl_2 = entry and exit slits respectively; Ph = photoelectric device; A = amplifier; R = analogue recorder.

What is important is not so much the amplitude of the noise signal itself but, as already pointed out in the introduction, the ratio of the amplitude of the useful signal to that of the noise. We, therefore, have first to determine the useful signal amplitude. For this purpose let us consider an illuminated slit of length ΔW and width b, covering part of an absorbing zone with the dimensions b_c and ΔW_c (see Fig. 3). Let the concentration in this area be c (not necessarily homogeneously constant) and the corresponding increase in absorbance α_c. α_c is the mean value of absorbance, measured over the full width $\Delta\lambda_c$ of the spectral absorption characteristic, covered by the illuminating beam. Let the spectral width of the illuminating beam be $\Delta\lambda$ and it is assumed that it comprises the region of absorption of the investigated substance.

For small concentrations the transmission of any point within the absorbing zone may be written in the form shown in eqn. 2. The decrease in transmission produced by the absorbing material is therefore:

$$\Delta A = A_0 \left(1 - \frac{A_c}{A_0}\right) = A_0 \alpha_c \tag{8}$$

The decrease in light intensity at the photodetector is obtained by integrating eqn. 8 over the whole illuminated part of the absorbing zone. It represents the useful optical signal amplitude I_c.

$$b_c \Delta W_c = F_c$$

$$I_c = \varepsilon_0 \Delta\lambda_c \cdot F_c \cdot \bar{A}\bar{\alpha}_c = I_0 \cdot \frac{F_c}{F} \cdot \frac{\Delta\lambda_c}{\Delta\lambda} \cdot \bar{A}\bar{\alpha}_c \tag{9}$$

$\bar{A}\bar{\alpha}_c$ is the mean value of the decrease in transmission over the illuminated part of the zone. The use of $\bar{A}$ instead of A_s implies that I_c represents the mean value of the useful signal, disregarding local changes in transmission A_s from strip to strip.

From Fig. 3 it is apparent that the illuminated slit may also include parts of the medium outside of the absorbing zone. For the mean total light input It to the photodetector we obtain therefore:

$$It = I_s - I_c = I_0 \bar{A} \left(1 - \frac{F_c}{F} \cdot \frac{\Delta\lambda_c}{\Delta\lambda} \cdot \bar{\alpha}_c\right) \tag{10}$$

It will of course fluctuate from one strip to the other, since the transmission A_s of the strips varies around the mean value $\bar{A}$. The optical noise signal produced in this way is designated Iv. Usually the second term in eqn. 10 is small as compared with unity; thus we obtain:

$$Iv \simeq I_0 \bar{A} \cdot \frac{1}{\sqrt{F}} \tag{11}$$

We can now obtain the optical signal to noise ratio σ by forming the ratio of I_c/Iv.

$$\sigma = \frac{I_c}{Iv} = \alpha_c \cdot \frac{\bar{A}}{\bar{\bar{A}}} \cdot \frac{\Delta\lambda_c}{\Delta\lambda} \cdot \frac{F_c}{F} \cdot \sqrt{F}$$

$$= \frac{\alpha_c}{\bar{\bar{\alpha}}} \cdot \frac{\Delta\lambda_c}{\Delta\lambda} \cdot \frac{F_c}{F} \cdot \sqrt{F} \tag{12}$$

$\bar{\alpha}_c/\bar{\bar{\alpha}}$ is the basic densitometric signal to noise ratio of the chromatogram.

For low concentration, where eqn. 2 holds, we can assume that the quantity of investigated substance Q_s in the illuminated part of the zone is proportional to $\bar{\alpha}_c$ (eqn. 2).

$$Q_s = k\bar{\alpha}_c F_c \tag{13}$$

k is a proportionality constant, taking into consideration the absorbance of the investigated substance per unit of concentration. In actual measurements k has to be found by calibration against a known concentration. If the linear approximation in eqn. 2 holds, the value of Q_s obtained will be independent of the distribution of the investigated substance over the slit. For higher concentrations an error will be committed; but this will be discussed in more detail in a later paper in this series.

Introducing eqn. 13 into eqns. 9 and 12 we obtain:

$$I_c = I_0 \cdot \frac{\bar{A} Q_s}{kF} \cdot \frac{\Delta\lambda_c}{\Delta\lambda}$$

$$\sigma = \frac{Q_s}{k\bar{\bar{\alpha}}} \cdot \frac{\Delta\lambda_c}{\Delta\lambda} \cdot \frac{1}{\sqrt{F}} \tag{14}$$

To determine the total amount of absorbing substance present in the zone, we must, of course, integrate over all the slits in contact with this zone.

DISCUSSION

The most important relationship so far derived is that exhibited in eqn. 12, which determines the optical signal to noise ratio obtained. This ratio is the principal factor which limits the sensitivity, accuracy and resolving power of a photodensitometer of the type described. It is apparently dependent upon several factors.

The first and most important one is the relative non-uniformity of the transmission of the chromatogram itself, as expressed by the relation $\bar{A}/\bar{\bar{A}}$; this factor is clearly a characteristic parameter of the medium used, depending in general only to a minor degree upon the spectral width of the scanning beam.

The second factor is the ratio $\Delta\lambda_c/\Delta\lambda$; the largest value obtainable is evidently 1. To obtain it, the spectral width of the illuminating beam $\Delta\lambda$ must be equal to or smaller than the width $\Delta\lambda_c$ of the absorption characteristic of the substance investigated. With the nearly monochromatic light, which is conventionally used for scanning, this condition is always fulfilled. For the best results the spectral position of the illuminating beam should coincide with the reasonably flat part of the absorption characteristic. For low concentrations $\Delta\lambda$ should be made almost equal to $\Delta\lambda_c$, in order to obtain the highest input signal at the photodetector. It can be shown that the best signal to noise ratio is achieved, if the scanning beam is spectrally shaped in such a way that it models the absorption characteristic of the substance involved. This is of course feasible only if linearity errors are of no concern.

For higher concentrations, where the linear approximation of eqn. 2 cannot be applied, $\Delta\lambda$ should be smaller than $\Delta\lambda_c$ and cover that part of the absorption characteristic where $\alpha_c(\lambda)$ does not change too much. The error committed by using a finite value of $\Delta\lambda$ is analysed in detail in another paper to appear later. At higher concentrations and consequently higher values of the useful output signal I_c the electrical noise becomes less important and the decrease in Iv, caused by a smaller value of $\Delta\lambda$ is of less significance.

It appears to be immaterial whether the spectral shaping of the scanning beam is done on the primary side, that is between the light source and the chromatogram, or on the secondary side, that is between the chromatogram and the photoelectric converter. In the case of fluorescence measurements filtering on the secondary side is, of course, mandatory.

The last important factor to be examined is the ratio of the total area illuminated by the scanning beam to that part of it which contains the absorbing zone of interest. For this purpose we may write:

$$F = \mu F_c \qquad (\mu \geqslant 1)$$

$$\frac{F_c}{\sqrt{F}} = \sqrt{\frac{F_c}{\mu}} \tag{15}$$

The highest value is evidently obtained if $\mu = 1$, that is if all the illuminated area belongs to a stained zone. At the same time F_c should be as large as possible. In

practice this condition is met by a slit aperture, extending over the full width of the chromatogram and applying the solution to be chromatographed in bands over the whole width of the medium. At higher concentrations, however, errors are incurred if the distribution of concentration is not exactly uniform over the band. This error can be reduced or avoided if the area of the scanning beam is reduced to virtually a point. This arrangement is usually termed "flying-spot scanning". Subsequent integration of the signals I_c obtained for each spot has to be performed over the whole zone. If Beer's law can be assumed to be valid, the usable range can be extended to higher concentrations by using a logarithmic converter before summing of the individual spot signals. If considerable deviations from Beer's law are to be expected, more complicated procedures than simply forming the logarithms of the spot signals should be used. More details about this will be found in a subsequent paper to be published soon.

The difficulty with spot scanning and subsequent integration is to determine the area over which integration has to be performed. The criterion, therefore, has to be derived from the spot-output signal I_c exceeding a certain preset threshold value. Since the area of the scanning spot is small, the noise content in I_c is high and the decision whether a particular I_c is above or below threshold is affected with a high degree of uncertainty. Some smoothing and curve fitting operations on the set of values I_c obtained may alleviate this problem; they will, however, need processing by a computer.

There are still other sources of error to be mentioned, namely variations in the coefficient of surface reflection ψ (see eqn. 4) and instability of the light source I_0. They are discussed in the following paper. These sources of error are of equal influence in single- and double-beam difference forming instruments; the latter device, however, produces a much better optical signal to noise ratio. Double-beam instruments appear, therefore, to be preferable for all but the most unsophisticated measurements and our further attention will be centred on them.

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