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## Note

# Linear relationship between concentration and optical response of thin-layer chromatograms

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Photometric methods are today probably the most popular approach to the quantitative determination of the amount of separated substance contained in a particular zone of a thin-layer chromatogram. Highly sophisticated instruments have been developed for this purpose. Many features of the design of these devices depend strongly upon an understanding of the optical response of the chromatogram. The same applies also to the general use of photometric techniques outside the field of thin-layer chromatography (TLC) for the purpose of determining the amount of dispersed substance in a turbid gray support matrix. The term "turbid" applies to media, which exhibit not only absorption, but also internal scattering. The term "grey" here indicates a near uniform optical response over the whole spectral range of concern, which may extend beyond the visible well into the ultraviolet. Most of the media used in TLC belong to this class.

An exact mathematical description of the optical properties of a turbid medium is all but impossible. Fortunately, a simplified theory had been developed in the thirties by the physicists Kubelka and Munk[1], which yields results which are perfectly adequate for many practical applications. The Kubelka and Munk theory is based upon several simplifying assumptions. The two most important ones of these are the following: inside the turbid matrix light propagates only in either the forward or the backward direction, both of which are perpendicular to the surface of incidence. The medium itself is regarded as a thin homogeneous sheet with infinite extension.

Another assumption, which is frequently made only tacitly, is that the medium has sufficient scattering power to abolish any existing collimation of the illuminating beam before it leaves the medium. Alternatively, it can be assumed that the collimation of the incident beam has been abolished already before it reaches the medium, *e.g.* by a diffusing plate placed immediately in front of the entrance aperture.

If neither assumption is justified deviations from the predictions of the Kubelka and Munk theory will appear, which have to be taken into account. Corrections can be carried out by relatively simple procedures. However, this case is not part of the considerations of this paper.

With these assumptions the originally three-dimensional case is reduced to a single dimension. Each surface element of the sheet acts with respect to the light leaving the medium as a Lambert source with cosine intensity distribution. This distribution has to be taken into account, when determining the optical response of the layer with the help of instruments, the entrance aperture of which is not in immediate contact with the surface of the medium.

The Kubelka and Munk theory results in a relatively simple pair of first order differential equations for the forwards and backwards travelling intensities with the distance $x$ from the entrance surface as variable and with two intrinsic parameters: the coefficient of scatter, $S_0$ and the coefficient of absorption, $K_0$. The equations can, without difficulties, be solved in closed form. The resulting expressions are however cumbersome and not very transparent: they are thus not very suitable for routine use. Drawing upon techniques borrowed from electrical transmission line technology simpler expressions can be derived[2], but even these are too complicated for every day purposes.

Easier to use are graphical solutions of the Kubelka and Munk equations. They are mostly displayed as families of curves with $S$ as parameter and $K$ as continuous variable[3,4]. The drawback of the graphical approach is limited accuracy. The curves are highly non-linear and, therefore, difficult to implement by electrical circuits in instrument design. When using graphical methods it should be noted, that the total scatter $S$ and the total absorption $K$ of the medium have to be entered into the graph. Both are proportional to the thickness $X$ of the turbid layer:

$$S = S_0 \cdot X \tag{1}$$

$$K = K_0 \cdot X \tag{2}$$

The proportion of light energy, which leaves the medium at the far side at $x = X$ is called the "transmittance" $A_T$; and that which is returned at the surface near the source is called the "reflectance" $A_R$. Coefficient of back scatter might be a less ambiguous designation for the latter. Light, which is specularly reflected from the illuminated surface has to be discounted from the illuminating intensity.

## INVERSION OF THE KUBELKA AND MUNK SOLUTIONS

Many practical applications of the Kubelka and Munk theory are concerned with the determination of the amount of a selectively absorbing substance dispersed in a turbid gray support matrix. In most cases it is assumed that the distribution of the measured material in the matrix is uniform with respect to depth $x$. In chromatographic applications this assumption is usually fairly close to reality. Considerable errors can however, be incurred, if the concentration distribution $c(x)$ is strongly non-uniform[5].

In most cases of practical interest it can also be assumed, that the presence of the investigated substance alters only the coefficient of absorption $K$, but does not affect significantly the value of $S$. Concentration is here defined as the amount of dispersed material contained in a parallel epiped of medium material with unit surface area.

For low to medium concentrations $c$ the absorbance $K$ changes by an amount $\Delta K$ which is proportional to $c$.

$$\Delta K = \alpha c \tag{3}$$

The essence of the problem is to determine $c$ from a measured change $\Delta A$ in optical response from that of the blank medium $A_0$. The symbol $A$ without index letter is intended to designate either transmittance $A_T$ or reflectance $A_R$ as needed. Determining $c$ from $\Delta A$ amounts to the task of inverting the function $A\,(K, S)$ and to bring it into the form:

$$\Delta K = \varphi\,[\Delta A, A_0, S] \tag{4}$$

Inversion of the rigid solution of the Kubelka and Munk equations in closed form is not feasible. To facilitate the problem a number of approximate solutions has been developed by different authors[6,7] but none of them proved fully satisfactory.

Working on the development of a high-performance photometer[8] especially for use in quantitative chromatography it was empirically found, that a logarithmic plot of $\Delta A\,(c)$ over $c$ produced a straight line for a wide range of media with different degrees of scatter and basic absorption. $\Delta A\,(c)$ designates here the change in transmittance of the medium due to the presence of separated material in concentration $c$. Treiber[9] and Goodall[10] arrived, also empirically, at very similar conclusions.

More elusive was the search for a relationship with similar properties, which would be applicable to measurements in the reflexion mode. Here some theoretical mathematical ground work had to be done to guide the empirical approach. But finally it was found that the simple reciprocal of reflectance yielded a linear characteristic, equal to or even better than the logarithm of transmittance[11]. For analytical purposes it is of course desirable, that the instruments employed have a characteristic, which is linear in terms of the measured quantity. Both linearizing transforms mentioned offer the big advantage, that they can be easily implemented by standard analog or digital circuitry.

## A LINEAR APPROXIMATION TO THE KUBELKA AND MUNK EQUATIONS

On the basis of the findings reported above both transmittance and reflectance can be represented by families of straight lines with the absorption $K$ as continuous variable and scatter $S$ as parameter.

$$\ln |\,A_T\,(K, S)\,| = \alpha\,(S)_T \cdot K + \beta\,(S)_T \tag{5}$$

$$1/A_R\,(K, S) = \alpha\,(S)_R \cdot K + \beta\,(S)_R \tag{6}$$

Eqn. (5) can alternatively be written in exponential form.

$$A_T\,(K, S) = \exp\,[\beta\,(S)_T] \cdot \exp\,[\alpha\,(S)_T \cdot K] = B\,(S)_T \exp\,[\alpha\,(S)_T \cdot K] \tag{7}$$

In this form the equation resembles the law of Beer and Lambert for non-turbid media and can, in a sense, be regarded as a generalization of the latter. Both coefficients $B\,(S)_T$ and $\alpha\,(S)_T$ are functions of $S$, and both converge for media without scatter (the domain of the Beer-Lambert law) to unity.

For practical application the parameters $\alpha\,(S)$ and $\beta\,(S)$ from eqns. 5 and 6 were determined by computing the best (in the least mean square sense) linear approximation to the sequence of values obtained from an exact solution of the Kubelka and Munk equations. The calculations were carried out for the range:

$$0.25 \leqslant K \leqslant 5 \tag{8}$$

$$0.25 \leqslant S \leqslant 5 \tag{9}$$

The results obtained are listed in Tables I and II and plotted in Figs. 1 and 2. Shown in the tables are not only the values of slope $\alpha(S)$ and intercept $\beta(S)$ for the linear approximation, but also the (absolute) mean of the error and the magnitude of the error for $K = 0.5$. In a few cases the error value is given for other values of $K$, which are then shown in the footnotes to the Tables. In all cases the error values are shown in percents of the correct value. It can be seen that the linear approximation for transmittance is the best for media with little scattering power. For reflectance the opposite holds: here the approximation improves for strongly scattering media.

TABLE I

CALCULATED SLOPES AND INTERCEPTS OF LEAST SQUARE LINEAR APPROXIMATION (TRANSMITTANCE)

| *Parameter* | *Value* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| $S$ | 0.0 | 0.25 | 0.5 | 0.75 | 1.0 | 1.25 | 1.5 | 1.75 |
| $\alpha(S)_T$ | −1.00 | −1.0033 | −1.0114 | −1.0227 | −1.0361 | −1.0514 | −1.0670 | −1.0837 |
| $\beta(S)_T$ | 0.00 | −0.2307 | −0.4305 | −0.6076 | −0.7671 | −0.9128 | −1.0470 | −1.1719 |
| $\lvert\varepsilon\rvert_{max.}$ (%) | 0.00 | 0.98* | 2.33* | 3.57* | 2.00 | 2.47 | 2.89 | 3.27 |
| $S$ | 2.0 | 2.25 | 2.5 | 2.75 | 3.0 | 3.25 | 3.50 | 3.75 |
| $\alpha(S)_T$ | −1.1009 | −1.1185 | −1.1363 | −1.1543 | −1.1723 | −1.19036 | −1.2084 | −1.2265 |
| $\beta(S)_T$ | −1.2888 | −1.3989 | −1.5030 | −1.6020 | −1.6964 | −1.7867 | −1.8733 | −1.9566 |
| $\lvert\varepsilon\rvert_{max.}$ (%) | 3.61 | 3.92 | 4.21 | 4.47 | 4.71 | 4.93 | 5.14 | 5.34 |
| $S$ | 4.0 | 4.25 | 4.5 | 4.75 | 5.0 | | | |
| $\alpha(S)_T$ | −1.2444 | −1.2623 | −1.2801 | −1.2978 | −1.3154 | | | |
| $\beta(S)_T$ | −2.0369 | −2.1145 | −2.1896 | −2.26239 | −2.333 | | | |
| $\lvert\varepsilon\rvert_{max.}$ (%) | 5.51 | 5.68 | 5.68 | 5.99 | 6.10 | | | |

* Error value for $K = 0.25$; in all other cases the error given refers to $K = 0.5$. For values $0.50 \leqslant K \leqslant 5$ and $0.75 \leqslant K \leqslant 5$, respectively, substantially smaller errors are obtained.

In almost all cases the error of the approximation increases when $K$ becomes very small. For this reason the error for $K = 0.25$ is almost always substantially larger than for $K = 0.5$, whilst for $K \geqslant 0.75$ error values are obtained, which are typically 40% and less of the value shown as maximum error.

The Kubelka and Munk equations have over the years proved their usefulness for many technical applications involving optical measurements on turbid media in general and on TL-chromatograms in particular. This despite the fact, that the form in which the solutions to these equations are commonly presented, is rather cumbersome and difficult to handle. The linearized expressions given in this paper do not suffer from this drawback. They are easy to apply even without any background in mathematics and optics and thus convenient for routine use by the practical chromatographer. Equally important is also the ease, with which they can be incorporated into the design of photo-densitometers for quantitative chromatography. In this way

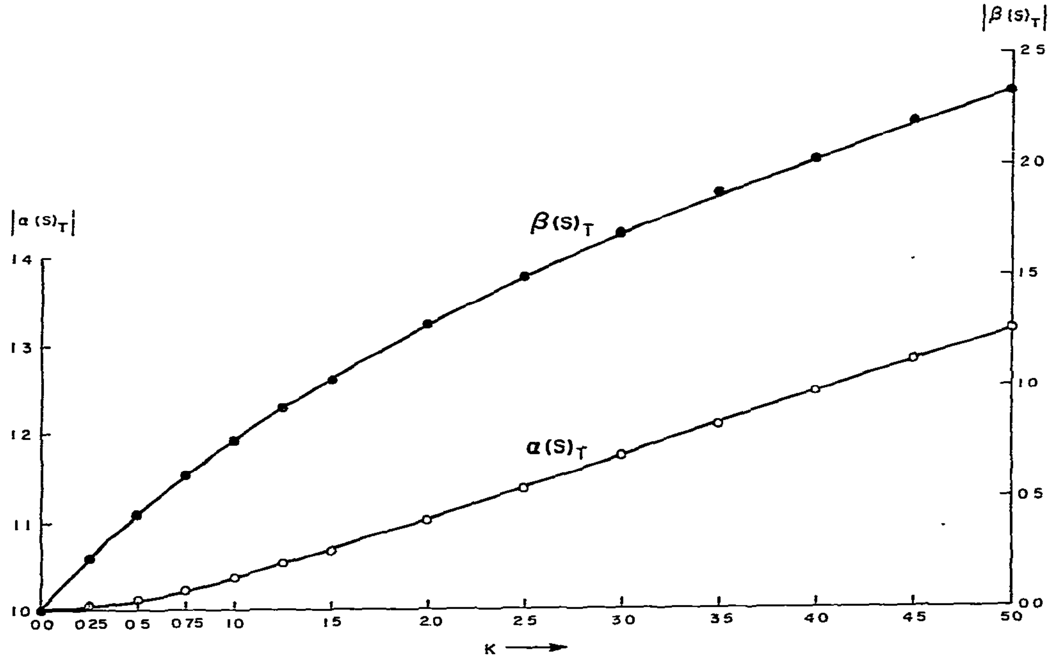


Fig. 1. The absolute values of coefficients $\alpha(S)_T$ and $\beta(S)_T$ of the linear approximation: $\ln |A_T| = K \cdot \alpha(S)_T + \beta(S)_T$, plotted as function of the coefficient of scatter $S$ of the medium. ○, $|\alpha(S)_T|$; ●, $|\beta(S)_T|$.

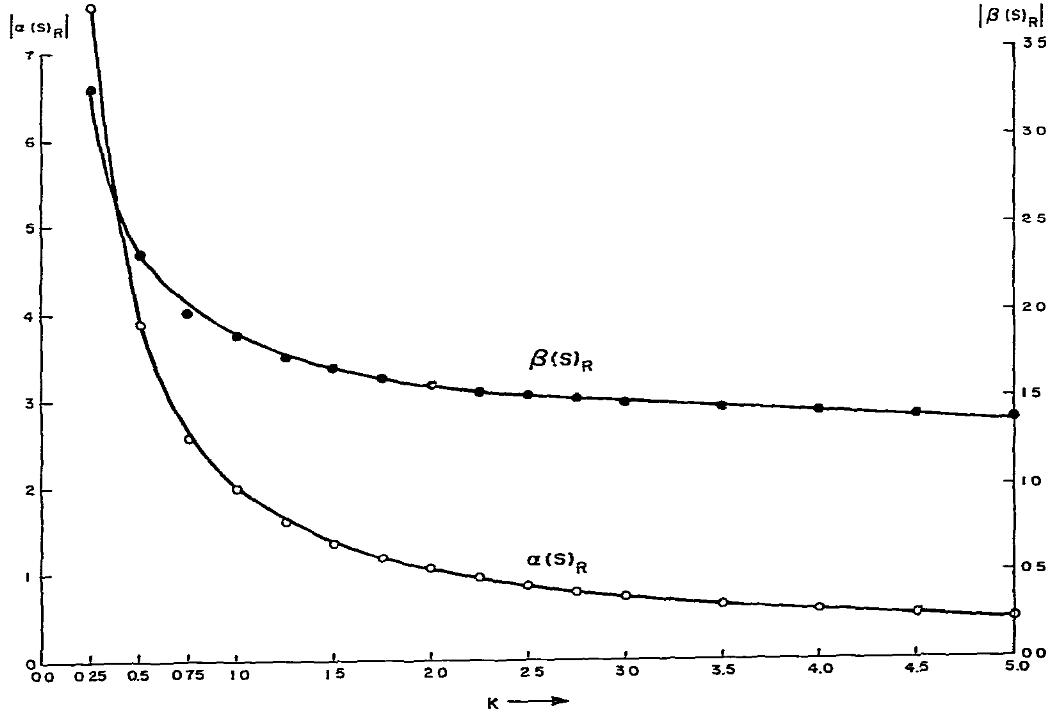


Fig. 2. The absolute values of the coefficients $\alpha(S)_R$ and $\beta(S)_R$ of the linear approximation: $1/A_R = K \cdot \alpha(S)_R + \beta(S)_R$, plotted as functions of the coefficient of scatter $S$ of the medium. ○, $|\alpha(S)_R|$; ●, $|\beta(S)_R|$.

TABLE II

CALCULATED SLOPES AND INTERCEPTS OF LEAST SQUARE LINEAR APPROXIMATION (REFLECTANCE)

With the exception of $S = 1.25$* the error $|\varepsilon|_{max.}$ (%), is given for $K = 0.5$. At all values $0.75 \leqslant K < 5.00$ the error is less than one half the maximum given. In all cases the error is substantially larger for $K = 0.25$. For $S = 1.25$ the maximum error occurs at $K = 1$.

| *Parameter* | *Value* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $S$ | 0.25 | 0.50 | 0.75 | 1 | 1.25 | 1.5 | 1.75 | 2.0 | 2.25 | 2.50 |
| $\alpha(S)_R$ | −7.6587 | −3.8959 | −2.6397 | −2.0104 | −1.6320 | −1.3790 | −1.1978 | −1.0616 | −0.9553 | −0.8700 |
| $\beta(S)_R$ | −3.2570 | −2.3308 | −2.0141 | −1.8506 | −1.7488 | −1.6783 | −1.6258 | −1.5849 | −1.5518 | −1.5242 |
| $\|\varepsilon\|_{max.}$ (%) | 7.31 | 4.42 | 2.44 | 1.04 | 0.33* | 0.76 | 1.3376 | 1.78 | 2.13 | 2.40 |
| $S$ | 2.75 | 3.00 | 3.25 | 3.50 | 3.75 | 4.00 | 4.25 | 4.50 | 4.75 | 5.00 |
| $\alpha(S)_R$ | −0.7999 | −0.7414 | −0.6917 | −0.6490 | −0.6119 | −0.5792 | −0.5504 | −0.52462 | −0.5015 | −0.4806 |
| $\beta(S)_R$ | −1.5008 | −1.4806 | −1.4629 | −1.4471 | −1.4330 | −1.4203 | −1.4087 | −1.3981 | −1.3884 | −1.3794 |
| $\|\varepsilon\|_{max.}$ (%) | 2.61 | 2.78 | 2.91 | 3.02 | 3.10 | 3.17 | 3.22 | 3.26 | 3.29 | 3.31 |

scales which are linear in terms of concentration can easily be obtained and calibration procedures greatly simplified.

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