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Note

Effect of non-uniform concentration distribution with depth upon quantitative optical analysis of chromatograms

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Optical methods of quantitative assessment of the amount of separated substance on thin-layer chromatograms are essentially based upon a comparison of the optical response of the chromatogram in the presence of separated substance and without it. The amount of material present is determined from the difference between the two observations. Theoretical relationships can in most cases provide only a qualitative understanding. For quantitative results it is almost always necessary to correlate the measured values with an empirically determined calibration curve. The concentration c will here be defined as the amount of separated substance contained in the volume element of the medium under an (infinitely small) area element Δz of the surface divided by the size of this element. The quantity of separated material Q in a zone with area Z becomes then:

$$Q = \Sigma c(z)\Delta z \rightarrow \int_Z c(z)\,\mathrm{d}z \tag{1}$$

The thickness X of the chromatogram does not appear in this expression; c is therefore not a volume concentration in the usual sense. Most theoretical relationships between concentration and the resulting change of the optical response ΔA of the chromatogram are based upon the assumption that $c(z)$ is not a function of the distance x from the illuminated surface. In words, it is assumed that the analyzed material is uniformly distributed throughout the depth of the medium. But this assumption may not always be true. Empirical calibration methods can cope with arbitrary distributions $c(z,x)$, but require that these are the same in the measured chromatogram and in that used for calibration. If this is not the case, rather serious errors may result.

THE FOUR MODES OF OPTICAL ANALYSIS

There are four modes of photometric analysis, which are commonly used for quantitative determinations. Two of them, densitometric transmittance and reflectance measurements, work directly at the wave length of the illuminating radiation. The two other ones use excited fluorescence of the separated material, which has a wavelength different from that of the exciting radiation. The measurements may be carried out at the near (illuminated) side or at the far (not illuminated) surface of the medium.

A fifth method, fluorescence quenching, is not very suitable for precise quantitative determinations and shall, therefore, not be further considered here.

The four modes mentioned above respond quite differently to possible variations of the concentration profile with depth and have, therefore, to be considered separately. It turns out that densitometric transmittance and fluorescence measured from the far side have many features in common and the same holds for direct reflectance and fluorescence measured at the near surface. This provides justification, for calling the fluorescent response measured from the far and near side fluorescence transmittance and fluorescence reflectance, respectively. A mathematically rigorous determination of the change ΔA of the optical response with variations of the concentration profile $c(x)$ would be very difficult. Instead, two extreme distributions will be considered with the standard uniform density profile about halfway between them. The latter will serve as comparison standard for the other two. As extreme case it will be assumed that the separated material is concentrated in an (infinitely) thin layer either at the near or at the far surface. In either case the bulk of the medium is thought to be free of separated solute.

ERROR ESTIMATE

Consider first the case where the separated material is concentrated at the far side of the chromatogram (Case I). Let the concentration in the layer be c. The response can then be assumed to follow Beer's law and the transmittance of the layer is consequently $\exp(-\alpha c)$, where α is the extinction coefficient of the analyzed solute. No reflectance can occur in the layer. The presence of separated substance causes, therefore, the transmittance of the blank medium to change by a factor $\exp(-\alpha c)$ but the reflectance does not change at all.

$$\Delta A_T(c)_I = A_T(0)\cdot(1 - \exp(-\alpha c)) \tag{2}$$

$$\Delta A_R(c)_I = 0 \tag{3}$$

Things are similar with fluorescence. The intensity of the excited fluorescence is proportional to $F\cdot c$, where F is the coefficient of fluorescence of the material in question. The exciting radiation has to traverse the whole bulk of the medium and is therefore, attenuated in proportion to the transmittance $A_{TE}(0)$ for the exciting radiation of the blank medium. Half of the excited fluorescence appears now without attenuation at the far surface. The other half appearing at the near surface is attenuated in proportion to the transmittance $A_{Tf}(0)$ of the blank medium, but now for the wavelength of the excited fluorescence. $A_{Tf}(0)$ will in general be different from the transmittance $A_{TE}(0)$ for the exciting radiation. Part of the radiation propagating towards the near surface is scattered back in proportion to the reflectance $A_{Rf}(0)$ of the medium. It combines with the former component at the far surface. The layer of fluorescent solute is assumed to have negligible absorption at the wavelength of fluorescence. Thus

$$\Delta A_{TF}(c)_I = A_{TE}(0)(1 + A_{Rf}(0))\cdot\frac{cF}{2} \tag{4}$$

$$\Delta A_{RF}(c)_I = A_{TE}(0) \cdot A_{Tf}(0) \cdot \frac{cF}{2} \tag{5}$$

Assume now that the layer of separated substance is concentrated at the near side of the chromatogram (case II). The transmittance of the blank medium is reduced by the same factor as above. Light diffusely reflected in the bulk of the medium has to pass the surface layer before leaving the medium and is therefore attenuated by exp $(-ac)$. This yields

$$\Delta A_T(c)_{II} = A_T(0) \cdot (1 - \exp(-ac)) \tag{6}$$

$$\Delta A_R(c)_{II} = A_R(0) \cdot (1 - \exp(-2ac)) \tag{7}$$

In the fluorescence mode the intensity observed at the near side is proportional to $cF/2$. That leaving the medium at the far side is attenuated in proportion with the transmittance $A_{Tf}(0)$. As before part of it is back scattered and adds to the component leaving the medium at the near surface. The radiation which excites the emitted fluorescence is not attenuated at all. Thus

$$\Delta A_{TF}(c)_{II} = A_{Tf}(0) \cdot \frac{cF}{2} \tag{8}$$

$$\Delta A_{RF}(c)_{II} = \frac{cF}{2}[1 + A_{Rf}(0)] \tag{9}$$

The theoretical expressions for the response functions for the four modes considered here, but based upon a uniform density profile, can be found, *e.g.*, in refs. 1 and 2. These are in first-order approximation:

$$\Delta A_T(c) \approx A_T(0) \cdot (1 - \exp(-ac)) \tag{10}$$

$$p \cdot a\, c \approx \left[\frac{1}{A_R(0)} - \frac{1}{A_R(0) + \Delta A_R(c)}\right]$$

$$\Delta A_R(c) \approx p \cdot A_R^2(0) \cdot a\, c \tag{11}$$

The value of the scale factor p depends upon the scattering power of the medium; it is in most cases not too far from unity. For fluorescence with uniform density profile the corresponding expressions are

$$\Delta A_{TF}(c) \approx A_{Tf}(0) \cdot \frac{cF}{2} \tag{12}$$

$$\Delta A_{RF}(c) \approx \frac{1}{\log A_{Tf}(0) + \log A_{TE}(0) + a\, c} \cdot \frac{cF}{2} \tag{13}$$

For easy comparison the results above are displayed combined in the Table I.

TABLE I

COMPARISON OF THE EFFECTS OF A VARYING CONCENTRATION PROFILE WITH DIFFERENT MODES OF OPERATION

	Extremum I	*Uniform*	*Extremum II*
$\Delta A_T(c)$	$A_T(0)\cdot[1-e^{-\alpha c}]$	$A_T(0)\cdot[1-e^{-\alpha c}]$	$A_T(0)\cdot[1-e^{-\alpha c}]$
$\Delta A_R(c)$	0	$p\cdot A_R^2(0)\cdot\alpha c$	$A_R(0)\cdot[1-e^{-2\alpha c}]$
$\Delta A_{TF}(c)$	$A_{TE}(0)\,(1+A_{Rf}(0))\cdot\frac{cF}{2}$	$A_{Tf}(0)\cdot\frac{cF}{2}$	$A_{Tf}(0)\cdot\frac{cF}{2}$
$\Delta A_{RF}(c)$	$A_{TE}(0)\cdot A_{Tf}(0)\cdot\frac{cF}{2}$	$\frac{1}{\log(A_{Tf}\cdot A_{TE})+\alpha c}\cdot\frac{Fc}{2}$	$[1+A_{Rf}(0)]\cdot\frac{cF}{2}$

CONCLUSION

Densitometric transmittance measurements and fluorescence determinations from the far (non-illuminated) side yield results which are near independent from the distribution of the analyzed material with depth. On the contrary, densitometric reflectance and fluorescence measurements from the near side are strongly dependent upon that distribution. Thus, whever there are reasons to suspect a variable distribution of concentration or a changing coefficient of fluorescence of the separated material in the depth of the chromatogram, transmittance or fluorescence transmittance methods are to be preferred.

Inversely, by comparing the results obtained by both transmittance and reflectance type measurements against a well defined calibration standard, it can be verified, whether the distribution of the separated material with depth is reasonably uniform, and if not, conclusions can be drawn upon the character of the concentration profile.

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