SPECTRAL-BANDWIDTH EFFECTS OF VARIABLE-WAVELENGTH ABSORPTION DETECTORS IN LIQUID CHROMATOGRAPHY

JAMES E. STEWART

Spectra-Physics, 2905 Stender Way, Santa Clara, Calif. 95051 (U.S.A.)

(First received October 17th, 1978; revised manuscript received February 5th, 1979)

SUMMARY

A theoretical discussion is given of the sensitivity and linearity of variable-wavelength absorption detectors used in liquid chromatography. The influence of the effective bandwidth of the monochromator relative to the width of the sample absorption band is considered, as well as the effect of making absorption measurements on the side of an absorption band instead of the peak. Some results of experimental measurements on biphenyl solutions are discussed.

INTRODUCTION

The influence of the shape and width of monochromator-slit functions on the shape and peak height of recorded absorption bands has been investigated many times[1], but not under conditions appropriate for the use of variable-wavelength detectors in liquid chromatography (LC). For example, it is usually assumed that quantitative analyses are made with the monochromator adjusted to place its central wavelength at the maximum of the absorption band. In LC, this is not generally the case, because the spectrometer is set in advance at a selected wavelength for which only a few (if any) of the components in the sample have absorption maxima. Also, the absorption maximum of a component is often not accessible because of wavelength limitations or solvent interference, and measurements must be confined to the side of the absorption band.

In this paper, I shall consider the effect of the monochromator bandwidth and wavelength setting on sensitivity and linearity in the analysis of static samples. The conclusions drawn from this study should be useful to chromatographers, although any complications introduced by flowing samples are not discussed at this time. In order to work with mathematically convenient forms, the monochromator and the absorbing sample are characterized by simple models.

THEORETICAL DISCUSSION

Absorption coefficient, slit function, and apparent absorbance

There are two convenient and widely used models for sample absorption. One

model assumes an absorption coefficient with a Lorentzian wavelength distribution:

$$a(\lambda) = \frac{a_0 s^2}{s^2 + 4(\lambda - \lambda_0)^2}$$

The other model has the form of a Gaussian distribution:

$$a(\lambda) = a_0 \, e^{-4 \ln 2 \, (\lambda - \lambda_0)^2/s^2}$$

In both cases, a_0 is the peak absorption coefficient, λ_0 is the peak wavelength, and s is the absorption bandwidth (full width at half maximum absorption coefficient). A Lorentzian band has stronger absorption in its wings than a Gaussian band of equal width. Real absorption bands very often cannot be represented by one of these simple models because they are skewed or distorted, and, frequently, an observed spectrum is made up of a summation of overlapping, unresolved bands. These cases will not be considered in this study, nor will spectra consisting of overlapping bands from different chemical compounds.

Triangular slit functions are expected for monochromators when entrance and exit slit widths are equal and wide enough to neglect the effects of optical aberrations and diffraction. The equation for a normalized triangular slit function is:

$$\sigma(\lambda_m - \lambda) = \frac{1}{m}\left(1 - \frac{|\lambda_m - \lambda|}{m}\right), \quad |\lambda_m - \lambda| < m$$
$$0 \qquad\qquad\qquad\qquad , \quad |\lambda_m - \lambda| \geq m$$

Where λ_m is the monochromator wavelength setting measured from λ_0 and m is the effective bandwidth (full width at half maximum).

Variable-wavelength detectors for LC sometimes use the sample cell itself in place of a monochromator exit slit. The slit functions of these instruments are not expected to be triangular, but effective bandwidths can still be defined, and the results of measurements made with these detectors will be qualitatively similar to those from instruments with real exit slits.

The apparent spectral transmittance of a sample is the convolution of the absorption band and the slit function,

$$T_{app} = \int_{\lambda_m - m}^{\lambda_m + m} e^{-a(\lambda)} \, \sigma(\lambda_m - \lambda) \, d\lambda \tag{1}$$

where $\alpha = a(\lambda)bc$ includes the path length b and the concentration c. The apparent absorbance is given by the usual relationship:

$$A_{app} = -\log T_{app}$$

In general, eqn. 1 is integrated numerically; for convenience in the following discussion, we take $\lambda_0 = 0$.

The ratio of effective bandwidth to absorption bandwidth is defined by the bandwidth ratio:

$$\beta = m/s$$

The monochromator setting may be given in terms of the absorption band width by

$$\gamma = |\lambda_m/s|$$

The rest of this paper treats the dependence of A_{app} on the parameters α, β and γ.

Band-shape distortion

Fig. 1 shows how Gaussian and Lorentzian bands are distorted by triangular slit functions ranging from 0 to 5 times wider than the absorption bands. Figures resembling this are well-known. They illustrate the rule-of-thumb used by spectroscopists that the effective bandwidth of the monochromator should be no greater than 10 to 20% of the absorption bandwidth for tolerable distortion and peak-height loss. Distortion becomes excessive if much wider slits are used. On the other hand, because the irradiance of the sensor is proportional to the square of the slit width, signal-to-noise ratio is sacrificed unnecessarily with narrower slits.
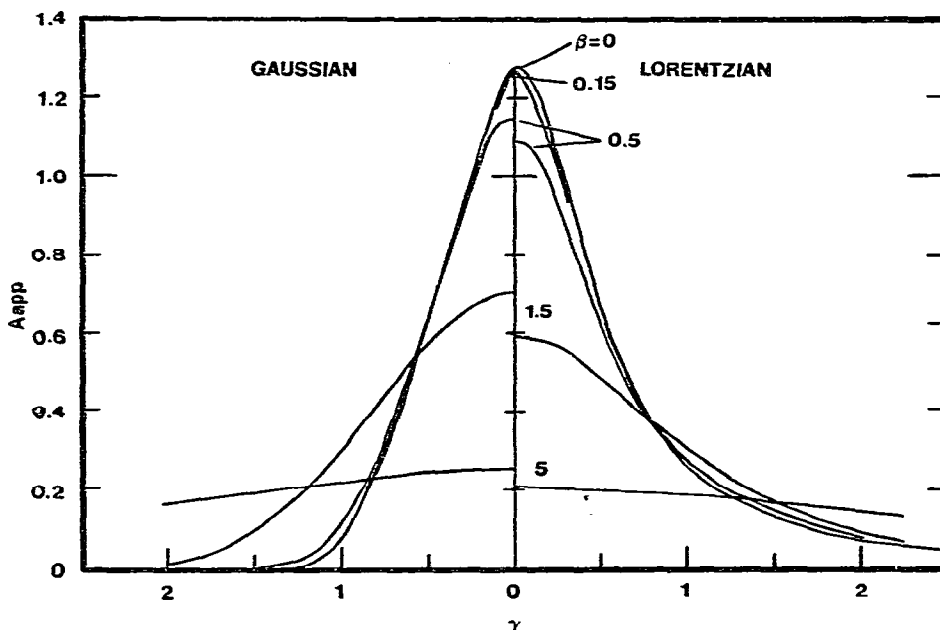

Fig. 1. Influence of the effective bandwidth of a monochromator with a triangular slit function on Gaussian- and Lorentzian-shaped absorption bands. The parameter β is the ratio of effective bandwidth to absorption bandwidth, and γ is the number of absorption bandwidths from the absorption maximum to the monochromator setting.

Many organic molecules in solution have absorption bands around 25 nm wide in the UV region. Therefore, for measurements made near the absorption maxima of such bands, the effective bandwidth should be 2.5 to 5 nm. However, the situation is different when, for whatever reason, measurements are made far from the band maximum $(0.5 < \gamma < 2)$. Indeed, the apparent absorbance may even be increased by using a relatively large effective bandwidth.

Sensitivity at small absorbance

For small values of a, eqn. 1 can be simplified by approximating the exponential factor with the first two terms of a power series. The integration can then be performed, and an expression for the sensitivity of A_{app} to small changes in concentration can be written for a Gaussian band

$$\frac{1}{0.4343a_0b}\left(\frac{dA_{app}}{dc}\right) = \frac{1}{2\beta}\left\{\frac{1}{4\beta\ln 2}\left(e^{-4\ln 2(\gamma-\beta)^2} + e^{-4\ln 2(\gamma+\beta)^2} - 2e^{-4\ln 2\gamma^2}\right)\right.$$

$$+ \sqrt{\frac{\pi}{4\ln 2}}\left[\left(1 - \frac{\gamma}{\beta}\right)\right.$$

$$\times\left(\mathrm{erf}\ \sqrt{4\ln 2}\gamma - \mathrm{erf}\ \sqrt{4\ln 2}(\gamma - \beta)\right)$$

$$\left.\left. - \left(1 + \frac{\gamma}{\beta}\right)\left(\mathrm{erf}\ \sqrt{4\ln 2}\gamma - \mathrm{erf}\ \sqrt{4\ln 2}(\gamma + \beta)\right)\right]\right\}$$

$$\tag{2a}$$

and for a Lorentzian band

$$\frac{1}{0.4343a_0b}\left(\frac{dA_{app}}{dc}\right) = \frac{1}{4\beta^2}\ln\left(\frac{4\gamma^2 + 1}{\{1 + 4(\gamma - \beta)^2\}^{1/2}\{1 + 4(\gamma + \beta)^2\}^{1/2}}\right)$$

$$- \frac{\gamma}{\beta^2}\tan^{-1}2\gamma - \frac{1}{2\beta}\left(1 - \frac{\gamma}{\beta}\right)\tan^{-1}2(\gamma - \beta)$$

$$+ \frac{1}{2\beta}\left(1 + \frac{\gamma}{\beta}\right)\tan^{-1}2(\gamma + \beta) \tag{2b}$$

These equations are plotted in Fig. 2 as functions of β for a variety of values of γ. When measurements are made close to the absorption band centre ($\gamma \leq 0.25$), sensitivity is reduced if the effective bandwidth exceeds about 0.2 absorption bandwidth. However, signal-to-noise ratio improves with increased effective bandwidth, compensating somewhat for the lowered sensitivity. When measurements are made farther from the absorption band center ($\gamma > 0.5$), sensitivity may be increased by increasing the effective bandwidth up to several times the absorption bandwidth. The magnitude of the effect of β and γ on sensitivity is strongly dependent on the shape of the absorption band. The effect is less pronounced for a Lorentzian band than for a Gaussian because of the stronger wings of the former.

Absorbance linearity

Plots of A_{app} *versus* concentration, calculated from eqn. 1, show noticeable departure from linearity at bandwidth ratios greater than *ca.* 0.5, especially when the spectrometer is set on the side of the absorption band.

Let us define a linearity error parameter

$$E(c) = \left(c \cdot \frac{dA_{app}}{dc} - A_{app}(c)\right)\bigg/c \cdot \frac{dA_{app}}{dc}$$

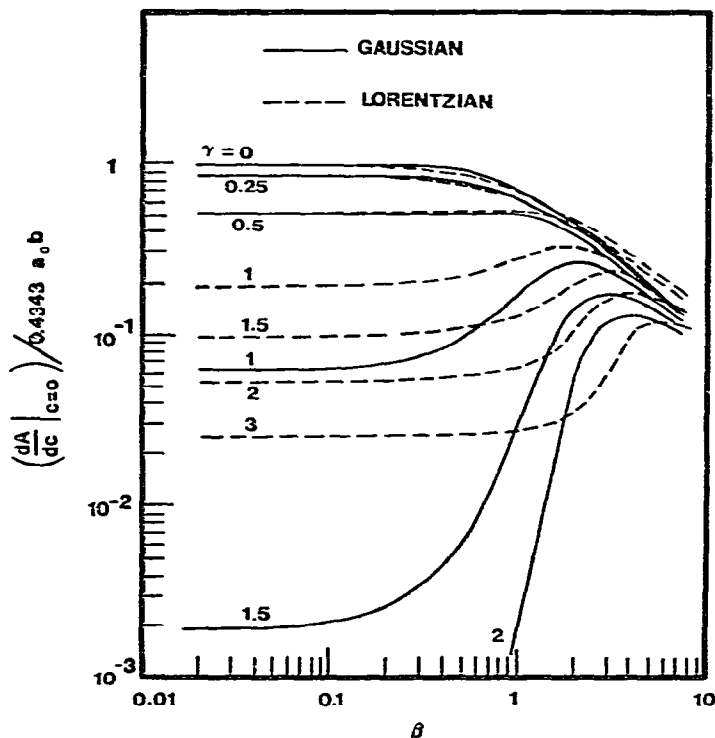which is the relative difference between the absorbance that would be predicted by

Fig. 2. The sensitivity of absorbance to concentration change at very small concentration for Gaussian- and Lorentzian-shaped bands as a function of bandwidth ratio for a series of monochromator settings.

extrapolation of the sensitivity at vanishing concentration (eqn. 2) and the apparent absorbance at concentration c.

Fig. 3 contains plots of E at $c = 1$ *versus* γ over a range of values of β for Lorentzian and Gaussian bands. The product $a_0 b$ is arbitrarily chosen to be 2.947, to give a maximum absorbance of 1.28 at $c = 1$. This is consistent with the maximum absorbance attainable by many LC detectors. It is evident that, for any bandwidth ratio, the magnitude of the linearity error first increases as the measuring wavelength is moved away from the absorption band center, then it decreases. The effects of β and γ on linearity are also strongly dependent on band shape.

EXPERIMENTAL MEASUREMENTS

A few experimental measurements were made in order to test the theoretical predictions. I used a versatile spectrometer designed in this laboratory; it has an off-axis Ebert monochromator configuration with a telescope mirror of focal length 0.25 m, and is capable of 0.1-nm resolution when a fine diffraction grating is installed, but, for the present purpose, a coarse grating (258 lines per mm) was used. Biphenyl was selected as a test compound. The spectrum of a methanolic solution (8.5 mg/l) in a 1-cm cell was measured with a spectrometer effective bandwidth of 2.4 nm. The absorbance spectrum could be fitted closely to a Gaussian function with 1.03 peak
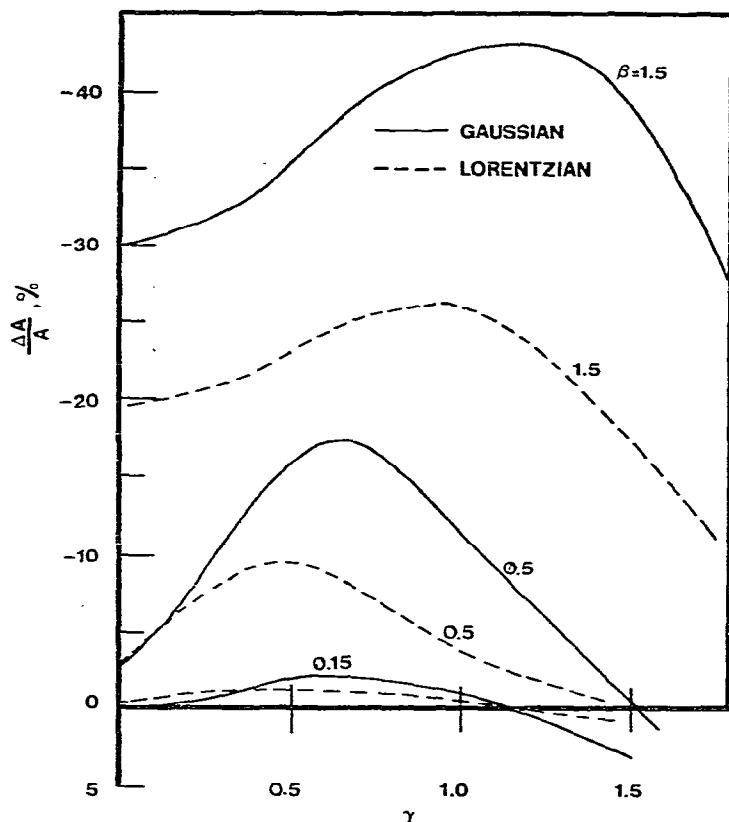
Fig. 3. Absorbance non-linearity for Gaussian and Lorentzian bands as a function of monochromator setting for several values of bandwidth ratio. $\Delta A/A$ is the relative departure of the absorbance from the small concentration value extrapolated to 1.28 absorbance units.

absorbance at 247.5 nm and a half bandwidth (corrected for the spectrometer effective bandwidth) of 33.9 nm.

In Fig. 4, absorbance values are plotted for a series of concentrations for effective bandwidths of 2.4 and 32 nm ($\beta = 0.071$ and 0.94, respectively) and for wavelength settings of 248, 264 and 280 nm ($\gamma = 0$, 0.48, and 0.96, respectively). These measurements verify the loss of sensitivity with increasing value of γ predicted in Fig. 2:

β	γ	$A'(\gamma)/A'\ (\gamma = 0)$	
		Calculated	*Experimental*
0.071	0.48	0.50	0.53
	0.96	0.064	0.068
0.94	0.48	0.72	0.76
	0.96	0.25	0.31

The measurements also verify the predicted behaviour of sensitivity with β for given values of γ:

	$A'\,(\beta = 0.94)/A'\,(\beta = 0.071)$	
γ	Calculated	Experimental
0	0.71	0.77
0.48	0.99	1.05
0.96	2.8	3.4

Sensitivity is lost by using the wide slit at $\gamma = 0$, it remains relatively unchanged at $\gamma = 0.48$, and it increases by a factor of 3 at $\gamma = 0.96$. Finally, the experimental curves display the predicted non-linearity in response.
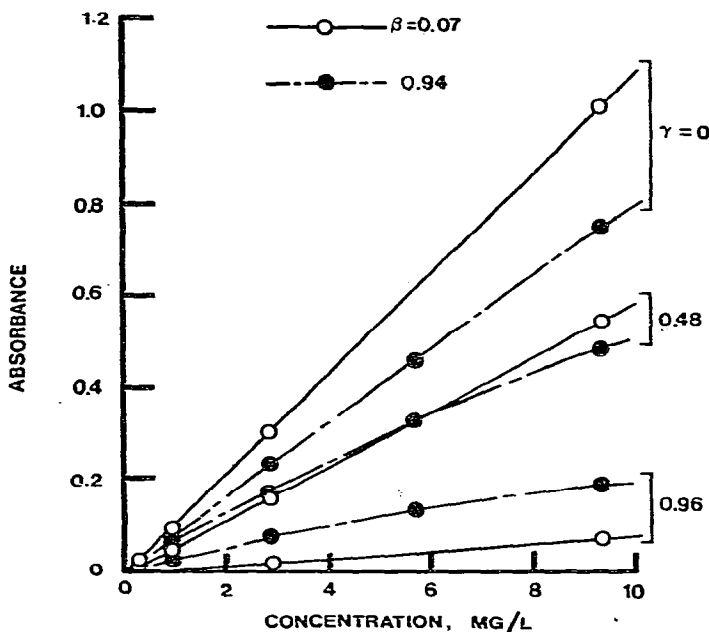

Fig. 4. Measured absorbance of biphenyl in a 1-cm cell as a function of concentration for narrow and wide spectrometer effective bandwidths and for different monochromator wavelength settings. Effective bandwidths are 2.4 and 32 nm, for which $\beta = 0.071$ and 0.94, respectively. Measurements are made at 247.5 nm at the peak of the absorption band and at 264 and 280 nm. Respective values of γ are 0, 0.48, and 0.96.

CONCLUSION

If a variety of components are to be detected in a single chromatogram, and their absorption maxima occur at different wavelengths, it is often better to use a large bandwidth ratio. This sacrifices sensitivity for the components that happen to absorb close to the monochromator setting, but improves sensitivity for others. Linearity is acceptable with a large bandwidth ratio for quantitative analysis at very small absorbance values, for which the enhanced signal-to-noise ratio afforded by the wider spectrometer slits is particularly desirable. If, on the other hand, linearity is required for effective quantitative analysis over a wide range of concentrations, the

bandwidth ratio should not in general exceed *ca.* 0.15. If there is assurance that the monochromator is tuned close to the absorption maximum of the component to be measured, it appears that the bandwidth ratio can be increased to *ca.* 0.3 if a 1% non-linearity is tolerable at 1.28 absorbance units. Rules such as these must be treated with caution because of the dependence of the numerical results on the shape of the absorption band.

REFERENCE

1 J. E. Stewart, *Infrared Spectroscopy: Experimental Methods and Techniques*, Marcel Dekker, New York, 1970, Ch. VII.