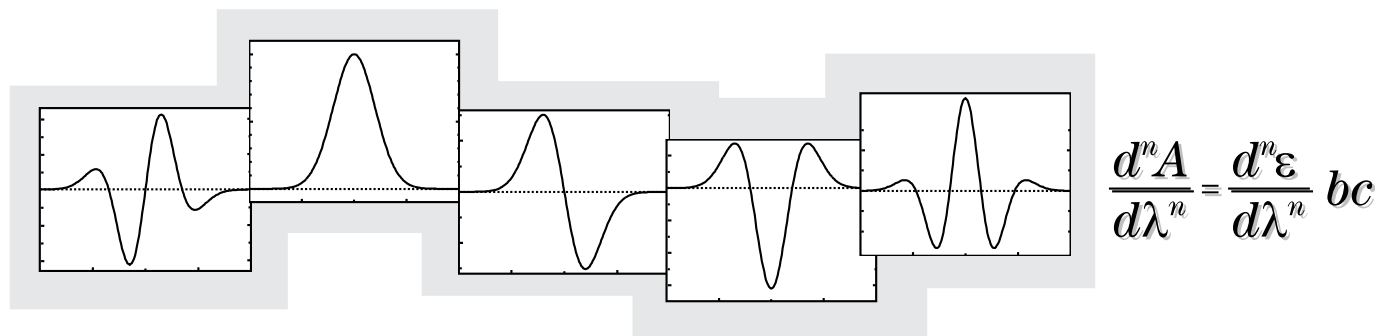


Uses of Derivative Spectroscopy

Application Note

UV-Visible Spectroscopy

Anthony J. Owen



Derivative spectroscopy uses first or higher derivatives of absorbance with respect to wavelength for qualitative analysis and for quantification. The concept of derivatizing spectral data was first introduced in the 1950s, when it was shown to have many advantages. However, the technique received little attention primarily because of the complexity of generating derivative spectra using early UV-Visible spectrophotometers. The introduction of microcomputers in the late 1970s made it generally practicable to use mathematical methods to generate derivative spectra quickly, easily and reproducibly. This significantly increased the use of the derivative technique.

In this application note we review briefly the mathematics and generation methods of derivative spectroscopy. We illustrate the features and applications using computer-generated examples.



Introduction

If a spectrum is expressed as absorbance, A , as a function of wavelength, λ , the derivative spectra are:

Zero order

$$A = f(\lambda)$$

First order

$$\frac{dA}{d\lambda} = f'(\lambda)$$

Second order

$$\frac{d^2A}{d\lambda^2} = f''(\lambda)$$

Figure 1 shows a computer simulation of the effects of derivatization on the appearance of a simple Gaussian absorbance band. Derivative spectra are always more complex than zero-order spectra.

A first-order derivative is the rate of change of absorbance with respect to wavelength. A first-order derivative starts and finishes at zero. It also passes through zero at the same wavelength as λ_{\max} of the absorbance band. Either side of this point are positive and negative bands with maximum and minimum at the same wavelengths as the inflection points in the absorbance band. This bipolar function is characteristic of all odd-order derivatives.

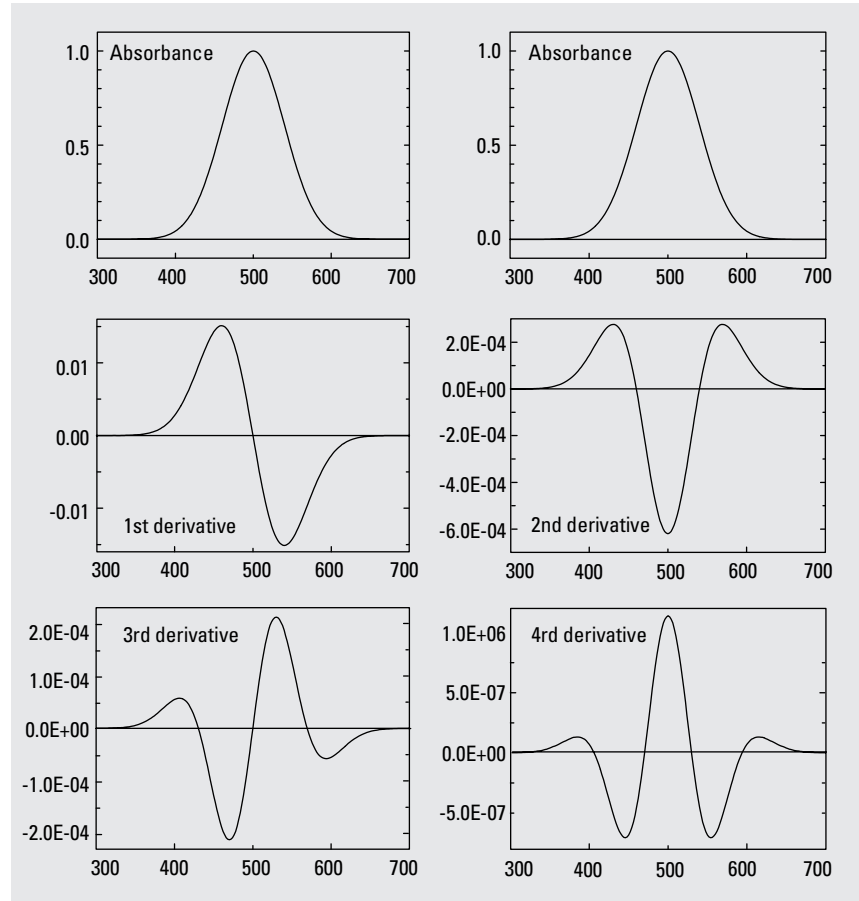


Figure 1
Absorbance and derivative spectra of a Gaussian band

The most characteristic feature of a second-order derivative is a negative band with minimum at the same wavelength as the maximum on the zero-order band. It also shows two additional positive *satellite* bands either side of the main band. A fourth-order derivative shows a positive band.

A strong negative or positive band with minimum or maximum at the same wavelength as λ_{\max} of the absorbance band is characteristic of the even-order derivatives.

Note that the number of bands observed is equal to the derivative order plus one.

Quantification

If we assume that the zero-order spectrum obeys Beer's law, there is a similar linear relationship between concentration and amplitude for all orders of derivative:

Zero order

$$A = \epsilon b c$$

First order

$$\frac{dA}{d\lambda} = \frac{d\epsilon}{d\lambda} bc$$

nth order

$$\frac{d^n A}{d\lambda^n} = \frac{d^n \epsilon}{d\lambda^n} bc$$

λ wavelength
 A absorbance
 ϵ extinction coefficient
 b sample path length
 c sample concentration

For single component quantification the selection of wavelengths for derivative spectra is not as simple as for absorbance spectra because there are both positive and negative peaks. For the even-order derivatives there is a peak maximum or minimum at the same λ_{\max} as the absorbance spectrum but for the odd-order derivatives this wavelength is a *zero crossing* point.

Taking the difference between the highest maximum and the lowest minimum gives the best signal-to-noise ratio but may lead to increased sensitivity to interference from other components.

Obtaining derivative spectra

Derivative spectra can be obtained by optical, electronic, or mathematical methods. Optical and electronic techniques were used on early UV-Visible spectrophotometers but have largely been superseded by mathematical techniques. The advantages of the mathematical techniques are that derivative spectra may be easily calculated and recalculated with different parameters, and smoothing techniques may be used to improve the signal-to-noise ratio.

Optical and electronic techniques

The main optical technique is wavelength modulation, where the wavelength of incident light is rapidly modulated over a narrow wavelength range by an electromechanical device. The first and second derivatives may be generated using this technique. It is popular for dedicated spectrophotometer designs used in, for example, environmental monitoring. First-derivative spectra may also be generated by a dual wavelength spectrophotometer. The derivative spectrum is generated by scanning with each monochromator separated by a small constant wavelength difference.

First and higher-order derivatives can be generated using analog resistance capacitance devices. These generate the derivative as a function of time as the spectrum is scanned at constant speed ($dA/dt=S$). For the first derivative:

$$\frac{dA}{d\lambda} = \frac{1}{S} \frac{dA}{dt}$$

Higher-order derivatives are obtained by using successive derivators. The electronic method suffers from the disadvantage that

the amplitude and wavelength shift of the derivatives varies with scan speed, slit width, and resistance-capacitance gain factor.

Mathematical techniques

To use mathematical techniques the spectrum is first digitized with a sampling interval of $\Delta\lambda$. The size of $\Delta\lambda$ depends on the natural bandwidth (NBW) of the bands being processed and of the bandwidth of the instrument used to generate the data. Typically, for UV-Visible spectra, the NBW is in the range 10 to 50 nm. First-derivative spectra may be calculated simply by taking the difference in absorbance between two closely spaced wavelengths for all wavelengths :

$$D_{\lambda+\Delta\lambda/2} = \frac{(A_{\lambda+\Delta\lambda} - A_{\lambda})}{\Delta\lambda}$$

Where the derivative amplitude, D_{λ} , is calculated for a wavelength intermediate between the two absorbance wavelengths.

For the second-derivative determination three closely-spaced wavelength values are used:

$$D_{\lambda} = \frac{(A_{\lambda-\Delta\lambda} - 2A_{\lambda} + A_{\lambda+\Delta\lambda})}{\Delta\lambda^2}$$

Higher-order derivatives can be calculated from similar expressions.

This method involves simple linear interpolation between adjacent wavelengths. A better method is that proposed by Savitzky and Golay. To calculate the derivative at a particular wavelength, λ , a *window* of $\pm n$ data points is selected and a polynomial is fitted using the least squares method:

$$A_{\lambda} = a_0 + a_1\lambda + K + a_l\lambda^l$$

Features and applications

An advantage of this method is that it can be used to smooth the data. If the polynomial order, l , is less than the number of data points ($2n+1$) in the window, the polynomial generally cannot go through all data points and thus the least squares fit gives a smoothed approximation to the original data points. This feature can be used to counteract the degradation of signal-to-noise that is inherent in the derivatization process (see below). The coefficients $a_0 \dots a_l$ at each wavelength multiplied by the factorial of the order are the derivative values: a_1 is the first derivative, $2!a_2$ the second derivative, $6!a_3$ the third derivative, and so on.

Savitzky and Golay developed a very efficient method to perform the calculations and this is the basis of the derivatization algorithm in most commercial instruments. Other techniques for calculating derivatives, for example, using Fourier Transforms, are available but are not commercially popular.

One consequence of these mathematical methods for the calculation of derivatives is that data points at the beginning and end of the wavelength range are lost. If three data points are used for the process then one data point will be lost at each end of the range for each derivative order. If five points are used then two points will be lost and so on.

It should be clearly understood that, although transformation of a UV-Visible spectrum to its first or higher derivative usually yields a more complex profile than the zero-order spectrum (see below), the intrinsic information content is not increased. In fact, it is decreased by the loss of lower-order data such as constant *offset* factors.

For clarity the points made in the following discussion are illustrated using computer-generated examples. In the figures, dotted lines show the baseline, dashed lines show component spectra, and solid lines show the *analyte* spectrum made up from the component spectra.

Graphics

As shown in figure 1 there is an increase in the number of bands as higher orders of derivative are calculated. This increase in the complexity of the derivative spectra can be very useful in qualitative analysis, either for characterizing materials or for identification. Spectra that are very similar in absorbance mode may reveal significant differences in the derivative mode.

For example, the absorbance spectrum of the steroid testosterone has a single, broad, featureless band centered at about 330 nm but the second derivative has six quite distinctive peaks.

Resolution

As figure 1 shows, the derivative centroid bandwidth of the even-order derivatives decreases with increasing order. Relative to the zero-order spectrum the derivative centroid bandwidth for a Gaussian band is observed to decrease to 53 %, 41 %, and 34 % of the original bandwidth in the second, fourth, and sixth orders respectively.

This feature may be used in qualitative analysis to identify the presence of two analytes with very similar λ_{\max} values that are not resolved in the absorbance spectrum. Figure 2 shows a computer simulation.

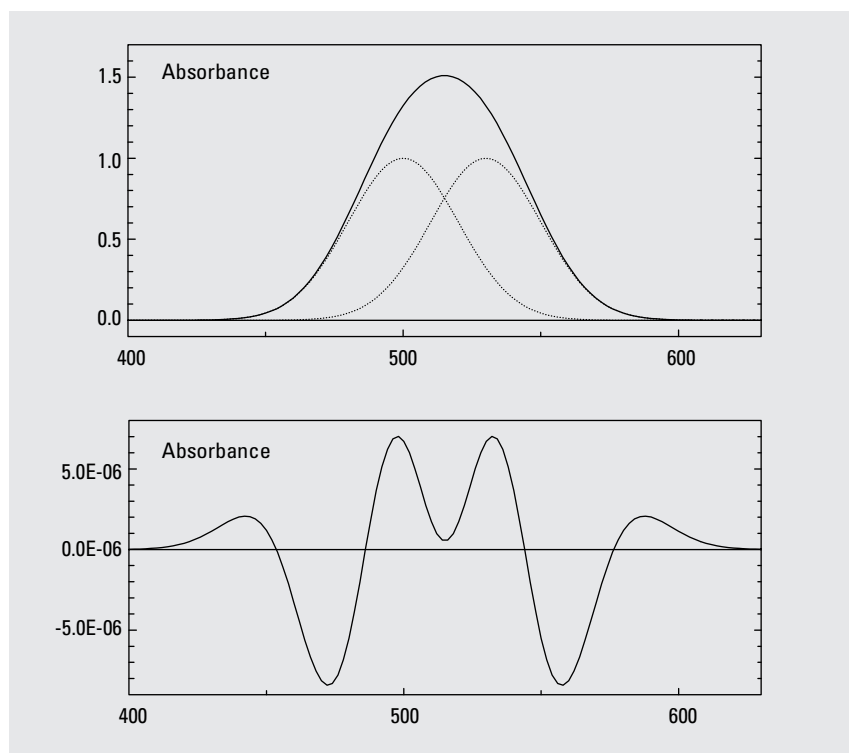


Figure 2
Resolution enhancement

In absorbance mode, when two Gaussian bands with 40 nm NBW and separated by 30 nm, are added the result is a single band with a maximum midway between the two component bands. The two components are not resolved. In the fourth derivative the presence of these two bands is clearly visible with maxima centered close to the λ_{max} of the component bands.

Although the bands have been resolved there is no indication of whether these arise from two chromophores in a single compound or in two different compounds. It is often claimed that this increased resolution and the increased differentiation between spectra in the derivative mode allows multicomponent analysis of mixtures of components with similar spectra that cannot be resolved in the absorbance mode.

However, as noted above the information content of derivative spectra is, in fact, less than the absorbance spectra and it can easily be shown that the improvements in quantitative accuracy are the result of other effects as described below.

Background elimination

A common, unwanted effect in spectroscopy is baseline shift. This may arise either from instrument (lamp or detector instabilities) or sample handling (cuvette repositioning) effects. Because the first derivative of a constant absorbance offset is zero, using the first-derivative spectra always eliminates such baseline shifts and improves the accuracy of quantification. This is illustrated in figure 3 where a 0.1 A offset, that would cause a 10 % quantitative error for the analyte, is completely eliminated by the first derivative.

Other background effects that are directly proportional to higher orders of wavelength with the general form:

$$A = a_0 + a_1\lambda^1 \dots + a_n\lambda^n$$

can be eliminated by using higher orders of derivative but such spectral features with exactly this form are very uncommon so this effect has little practical use.

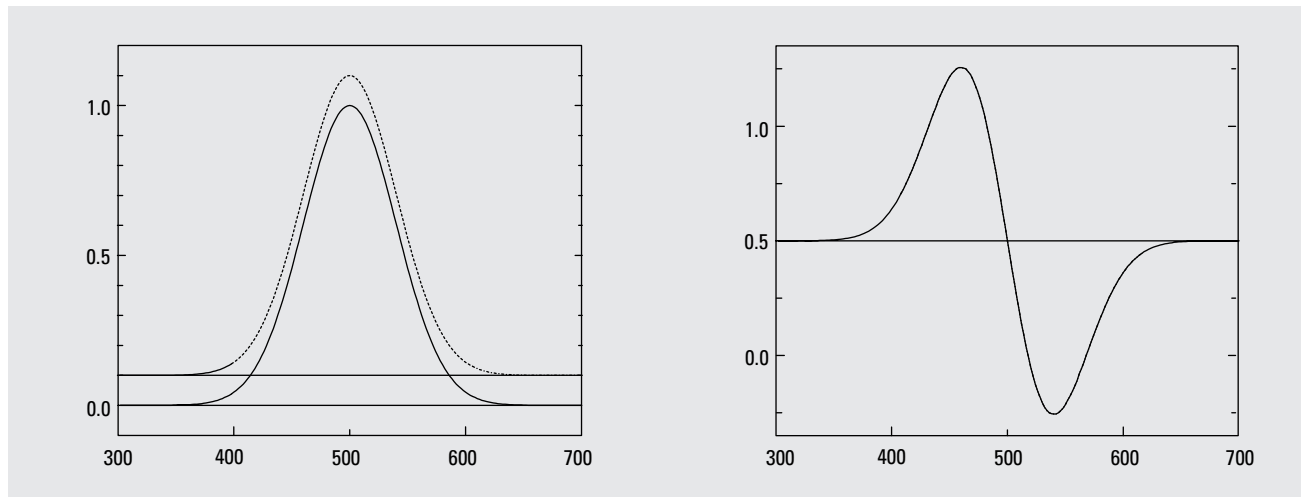


Figure 3
Background elimination

Discrimination

Probably the most important effect of the derivative process is that broad bands are suppressed relative to sharp bands and this suppression increases with increasing derivative order.

This arises from the fact that the amplitude, D^n , of a Gaussian band in the n^{th} derivative is inversely proportional to the original bandwidth, W , raised to the n^{th} degree:

$$D^n = \frac{1}{W^n}$$

Thus for two coincident bands of equal intensity but different bandwidth in the zero order, the n^{th} derivative amplitude of the sharper band, X , is greater than that of the broader band, Y , by a factor that is dependent on the relative bandwidth and the derivative order:

$$\frac{D_X^n}{D_Y^n} = \frac{W_Y^n}{W_X^n}$$

Figure 4 shows the effect of taking derivatives of two bands, one with 160 nm NBW and one with 40 nm NBW. In absorbance mode they have equal amplitude, in first derivative the narrower band has four times greater amplitude and in the second derivative it has sixteen times the amplitude.

This property is used to improve the accuracy of quantification of a narrow band component in the presence of a broad band component and to reduce error caused by scattering.

Scattering is a common problem in biological analyses resulting from the measurement of small particulates present in the sample.

Scattering is inversely proportional to the fourth (Rayleigh, small particles) or second (Tyndall, larger particles) power of the wavelength. Because the relationship is inverse, the use of derivatives will not eliminate the scattering component from the spectrum as has been claimed in some publications. However, because the scattering component resembles a very broad absorbance, using derivatives discriminates against it and reduces its effect on quantification.

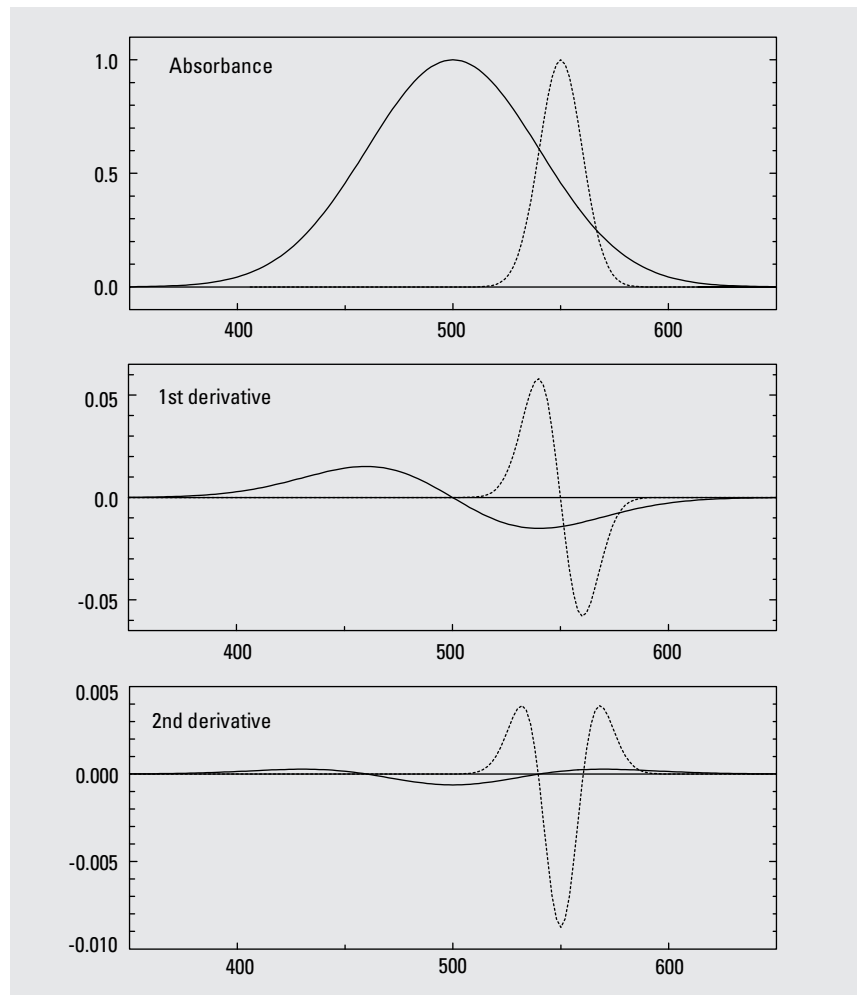


Figure 4
Discrimination against broad bands

Figure 5 shows an absorbance band with 40 nm NBW and the same band in the presence of a scattering background. Without any correction, the amplitude at 500 nm is 1.0920 A instead of 1.0 A because of the scattering contribution. Quantification at this wavelength results in an error of 9.2 %. Using the first derivative the contribution from the scattering component is reduced such that, using peak maximum to minimum, the signal in the presence of scattering is 0.02992 instead of 0.03024, that is a quantification error of only -1.1 %. This ability to discriminate against scattering components is widely used in the analysis of biological fluids that contain a high level of particulates, and in pharmaceutical analyses where particulate excipients in tablets and capsules cause quantification errors.

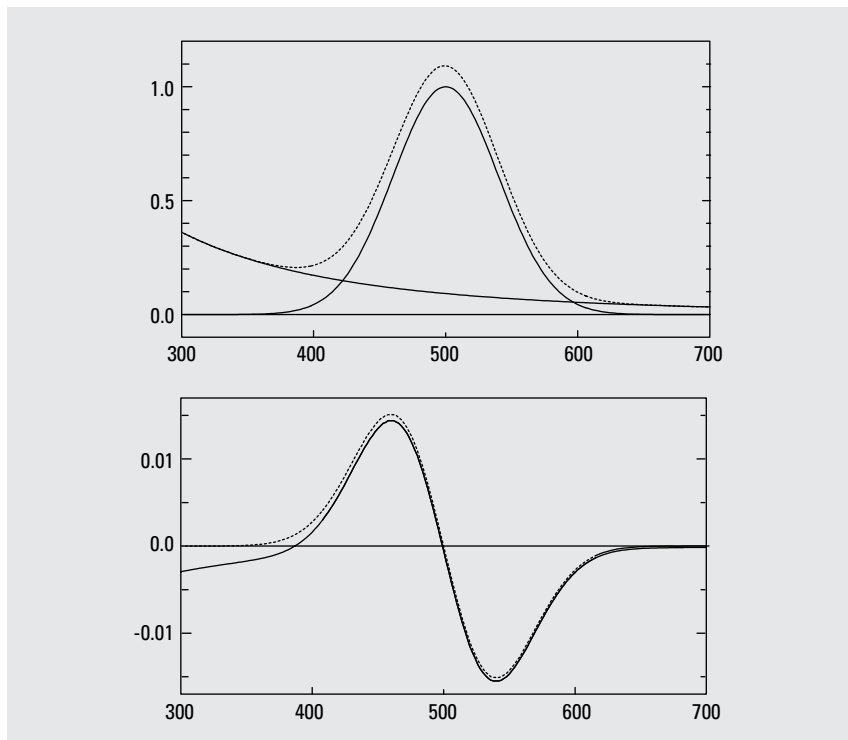


Figure 5
Scatter elimination

Matrix suppression

The analytical problem is often not simply scattering, baseline shift, or unwanted broad absorbing components. It is a combination of two or more of these that results in a broad absorbing background matrix.

In qualitative analyses, derivatization often allows the detection and positive identification of trace levels of a component in the presence of a strongly absorbing matrix. This is illustrated in Figure 6. A trace, 0.01 A, of a 40 nm NBW component with λ_{\max} at 500 nm was added to a synthetic matrix. The matrix comprised offset, second and fourth-order scatter, and 320 nm NBW components with λ_{\max} at 300 and 600 nm. In absorbance mode the presence of this component is virtually undetectable. In second-order derivative mode its presence is obvious.

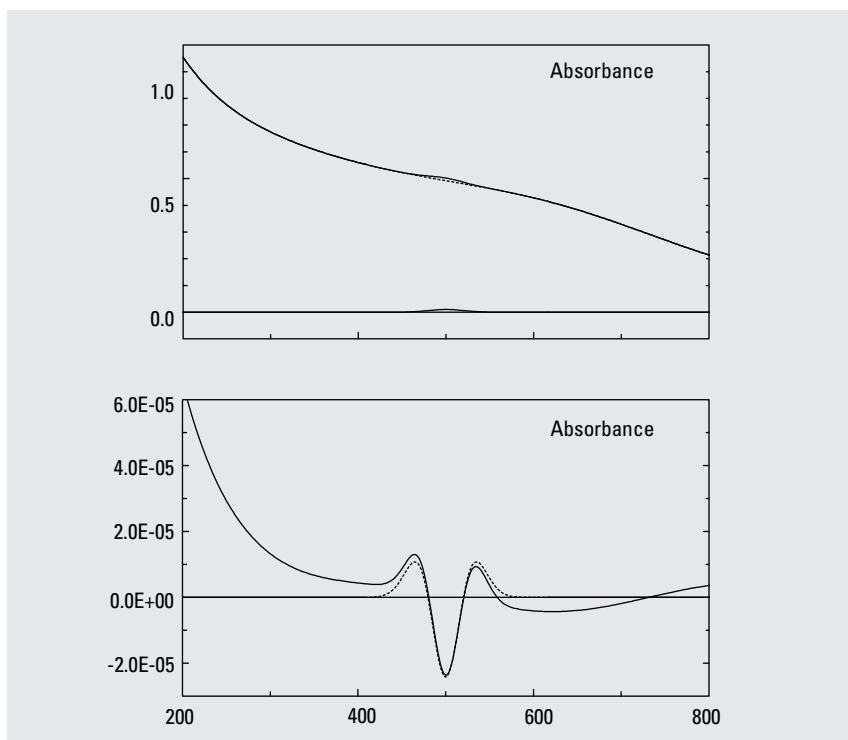


Figure 6
Matrix suppression

In quantitative analyses, derivatization improves the accuracy of quantification in the presence of interference caused by a broad absorbing component, matrix, or scattering. Thus in the example given above, quantification of the analyte in the absorbance mode without any correction results in an error of nearly 5000 % (absorbance of 0.502 A instead of 0.01 A). Using the baseline-to-valley signal of the second-order derivative the error is -2.1% (2.37×10^{-5} instead of $2.42 \times 10^{-5} A/\lambda^2$).

An example of discrimination against a broad absorbing matrix is the quantification of caffeine in soft drinks. Soft drinks generally contain a mixture of natural and synthetic products with added colorants, resulting in a broad featureless absorbance over a wide wavelength range. In absorbance mode, quantification of caffeine is inaccurate because of the matrix effect but good accuracy can often be achieved using the second-derivative spectra.

Instrument considerations

Virtually all current UV-Visible spectrophotometers generate derivative spectra by mathematical means so instrument considerations for generation of derivative spectra by optical and electronic techniques are not discussed. Instrument requirements for derivative spectroscopy are, in general, similar to those for conventional absorbance spectroscopy but wavelength reproducibility and signal-to-noise are of increased importance.

The increased resolution of derivative spectra puts increased demands on the wavelength reproducibility of the spectrophotometer. Small wavelength errors can result in much larger signal errors in the derivative mode than in the absorbance mode.

The negative effect of derivatization on signal-to-noise also puts increased demands on low noise characteristics of the spectrophotometer. It is an advantage in this case, if the spectrophotometer can scan and average multiple spectra before derivatization to improve further the signal-to-noise ratio.

For the derivatization process it is important to be able to control the degree of smoothing that is applied in order to adapt to differing analytical problems. In the case of the Savitzky-Golay method this means being able to vary the order of polynomial and the number of data points used.

Signal-to-noise ratio

An unwanted effect of the derivatization process is that the signal-to-noise ratio decreases as higher orders of derivatives are used. This follows from the discrimination effect and the fact that noise always contains the sharpest features in the spectrum. Thus, if the spectral data used in the derivative calculation is at 2 nm intervals, the noise has a 2 nm bandwidth. If the analyte band has a bandwidth of 20 nm then the signal-to-noise ratio of the first derivative is ten times worse than the zero-order spectrum. The decrease in signal-to-noise ratio can be reduced by using the smoothing properties of the Savitzky-Golay polynomial smoothing technique but great care must be taken as too high a degree of smoothing distorts the derivative spectrum.

Alternative techniques, such as using a reference wavelength or full spectrum multicomponent analysis with a scattering spectrum as standard, may often be used to achieve the same analytical goals but without the reduced signal-to-noise penalty.

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