Applications Of UV-Visible
Derivative Spectrophotometry

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Part I  A review of areas of application and the basic principles of the derivative technique.

Introduction

Since the practical introduction of the concept of derivatization of spectral data over thirty years ago (1-8), the derivative technique had received, until fairly recently, only sporadic attention from analysts working with UV-Visible spectrophotometers (9-37). This was hardly surprising, in view of the complexity of generating derivative spectral data from early UV-Visible instruments - in most cases via the use of the wavelength modulation principle.

However, the rapid developments in electronic and particularly in microcomputer technologies in the last ten years have led to a parallel growth of interest in the use of UV-Visible derivative spectrophotometry as an analytical tool for:

- The enhancement of the resolution of overlapping peaks, and
- The elimination or reduction of background or matrix absorption.

Today, the first and second derivative modes are a standard feature of most microprocessor UV-Visible spectrophotometers, and some instruments offer the third, fourth, and even up to the seventh order derivative modes (38). Furthermore, commercially available computer software offers off-line calculation capabilities on UV-Visible spectral data up to the ninth order derivative (39, 40). Nevertheless, the number of analysts using UV-Visible derivative spectroscopic techniques routinely appears to be still relatively small, despite the enthusiastic pioneering work of A.F. Fell, G. Talsky and others.

In order to highlight the versatility and power of derivative techniques for a wider audience of UV-Visible instrument users, Part I of this work lists some of the more recent areas of application and presents, in a simplified form, the basic features of derivative spectroscopy. Aspects of the technique are discussed under the following headings:

Recent Areas of Application
- Clinical-Pharmaceutical-Biochemical (Life Sciences)
- Inorganic
- Miscellaneous

Basic Features of Derivative Spectrophotometry
- Derivatization
- Gaussian Peaks
- Lorentzian Peaks
- Noise
- Quantitative Analysis
- Conclusion

In Part II practical examples will be presented which will further illustrate the potential usefulness of the derivative technique in UV-Visible spectroscopic measurements.
Recent Areas of Application

In this, by no means exhaustive, overview of the usefulness of derivative techniques in UV-Visible spectroscopic analyses, a cross-section of references to papers published in the last decade is presented.

**Clinical-Pharmaceutical-Biochemical (Life Sciences)**

Measurement of urine porphyrins and porphyrinogens [41]

Second derivative spectrophotometry as an effective tool for examining phenylalanine residues in proteins. [42]

Second derivative quantitative determination of total urinary porphyrins. [43]

Diode array spectrometer for simultaneous determination of hemoglobins in whole blood using signal averaging for low noise and first derivative spectra. [44]

First derivative quantitative determination of theobromine and sodium salicylate mixtures. [45]

Fourth and eighth derivative calculations on the spectra of bacteriochlorophyll - protein at 5-300°K. [46]

Second derivative quantitative assay of drugs in their dosage forms and potential areas of application of derivative techniques. [47]

Second derivative quantitation of urinary porphyrins. [48]

Review of potential areas of application of derivative techniques to clinical analysis. [49]

Various techniques of background matrix absorption correction are reviewed, with specific emphasis on higher derivative techniques. Illustrative examples from fields of amino acid and protein, aromatic amino acid and enzyme analysis are given. [50]

Second derivative spectroscopy of proteins: studies on tyrosyl residues. [51]

Second derivative measurements for the quantitative analysis of vitamin mixtures. [52]

Second derivative assay of active drugs in pharmaceutical preparations. [53]

A rapid method for the emergency analysis of paraquat in plasma by second derivative spectroscopy. [54]

Assay of procyclidine in tablets and injections by second derivative spectrometry. [55]

The determination of protein concentration by second derivative spectrophotometry. [56]

Separation of bilirubin from hemoglobin by recording derivative spectroscopy. [57]

Determination of benzodiazepines by derivative spectroscopy. [58]

Identification of amphetamine, phenethylamine, phentermine, ephedrine and meperidine by second derivative spectroscopy. [59]

Assay of ephedrine or pseudoephedrine in pharmaceutical preparations by second and fourth derivative spectrophotometry. [60]

Determination of serum methernalbumin by second derivative spectroscopy. [61]

Second derivative spectroscopic analysis of diphenhydramine hydrochloride. [62]

The effect of temperature on the second and fourth derivative amplitudes of benzenoid drugs. [63]

The determination of salicylic acid in aspirin powder by second derivative spectrometry. [64]
The determination of methaemoglobin in the presence of light scattering clay solutions as a model to evaluate 0, 1st, 2nd derivatives for problems in clinical chemistry [65]

The assay of tropane derivatives in formulations by second derivative spectroscopy. [66]

Analysis of heroin-morphine mixtures by zero order and second derivative ultraviolet spectrometry. [67]

The determination of carboxyhemoglobin by second derivative spectroscopy. [68]

Determination of chlorpromazine and its sulphoxide in pharmaceutical dosage forms by third-order derivative ultraviolet spectroscopy. [69]

Analysis of 9-tetrahydrocannabinol-cannabinol mixtures by second derivative ultraviolet spectrometry. [70]

Spectrophotometric determination of ascorbic acid and thiamine hydrochloride in pharmaceutical products using derivative spectrophotometry. [71]

**Inorganic**

First derivative spectra of iodine vapour, aqueous praseodymium solution from a vidicon based spectrometer. [72]

Use of higher derivatives for accurate location of peak maxima in solvent polarity studies. [73]

The use of second and fourth derivative spectra in the structural elucidation of mercury (11) mixed halides. [74]

Measurement of copper in drinking water via the second derivative of a copper-complex Soret band. [75]

Determination of micro amounts of samarium and europium by analogue derivative spectrophotometry. [76]

Determination of bismuth in the presence of copper by both first and second derivative measurements. [77]

Determination of nickel at ppb levels by second derivative spectroscopic measurements. [78]

Second derivative spectrophotometric determination of micro amounts of iron. [79]

Determination of iron and copper by first and second derivative spectrophotometry. [80]

Determination of ruthenium and palladium in mixtures by derivative spectrophotometry. [81]

Derivative spectrophotometric determination of p.p.b. levels of cobalt. [82]

Determination of nitrate and nitrite via the use of a Cd-Cu converter and second derivative spectroscopy. [83]

Simultaneous determination of copper and cobalt with EDTA using derivative spectrophotometry. [84]

**Miscellaneous**

A description of the development of electronic analogue differentiation which enables the generation of on-line spectra up to the ninth derivative - illustrated with a wide variety of examples and an introduction to the theoretical and instrumental aspects of derivative spectrophotometry. [85]

First derivative measurements on 2,5- dichlorophenol in the presence of the 2,4- and 3,4- isomers. [86]

A short review of derivative spectroscopy. [87]
Vapour phase and liquid solution measurements of polynuclear aromatic compounds with a wavelength modulated second derivative spectrometer. [88]

Second derivative measurements on plastic antioxidants. [89]

Determination of uric acid in water and wastewater. [90]

Portable derivative UV-spectrometer for monitoring various gases, aromatic organic vapours at sub-p.p.m. concentrations. [91]

Review of the development of derivative techniques and the theoretical aspects. [92]

Application of derivative spectroscopy to the determination of uric acid in municipal wastewaters and nitrite/nitrate in natural waters. [93]

Computed derivative and self-deconvoluted spectra using Fourier transforms. [94]

Application of high resolution, higher order derivative spectrophotometry in micro-analysis. [95]

Some aspects of the scope and limitations of derivative spectroscopy, specifically the second and fourth derivatives, are discussed. [96]

Summary of higher derivative methods. [97]

Discussion of some trading rules for the optimization of derivative spectra. [98]

Discussion of the applications of derivative spectrophotometry. [99]

A review of 'why is higher-order derivative spectrometry useful?' [100]

Determination of phenolic antioxidants in polypropylene by second derivative spectroscopy. [101]

Identification of ketones by second derivative spectrometry. [102]

Continuous determination of urea, based on an urease enzyme reactor producing ammonia gas which is determined by second derivative spectrophotometry. [103]

Quantitative determination of azorubine and naphthol red using higher order derivatives obtained with Savitzky-Golay based algorithms and Fourier transform method. [104]

Mathematical examination of the suitability of using various derivative orders. [105]

3rd-6th order derivative determination of aniline and phenol in waste water and pentachlorophenol in polluted potable waters. [106]

Application of UV-derivative spectrophotometric detection in gas chromatography. [107]

Quantitative determination of solvent Red 24 in hydrocarbon oils by derivative spectroscopy. [108]

Novel digital methods for the qualitative characterization of some acid dyes applied to wool and nylon. [109]
Basic Features of Derivative Spectrophotometry
Many gases and vapours, and some solids, frequently exhibit characteristically sharp, well-resolved bands in their UV-Visible absorption spectra. For example, iodine vapour, when scanned with a high resolution, double dispersion spectrophotometer, exhibits more than 70 sharp peaks in a 130 nm wavelength region (Figure 1).
However, many liquids and solutions, even when scanned with a high-resolution instrument, tend to give often rather featureless absorption bands with broad maxima and unresolved, unresolvable 'shoulders'. A typical example, a solution of potassium permanganate shows almost identical spectra, when scanned either with medium resolution - 1.0 nm S.B.W. (spectral band width) - monochromator slits (Figure 2a) or with very high resolution - 0.07 nm S.B.W. - (Figure 2b).
CARY 2300 SPECTROPHOTOMETER

Sample: Potassium Permanganate Solution
Cell Pathlength: 10 nm
Chart Display: 10 nm/cm
Spectral Bandwidth: 0.07 nm
Pen Period: 1 s
Scan Speed: 0.1 cm/s

Figure 2b
These broad bands and shoulders arise from the overlap of adjacent peaks, which cannot be fully resolved even with the highest performance spectrophotometers. Thus, in many instances the UV-Visible absorption spectroscopic technique has not been specific enough to characterize adequately individual molecular compounds in solution, particularly in the presence of other absorbing molecular species and scattering particles.

Nevertheless, despite these shortcomings, the UV-Visible absorption spectroscopic technique remains the universal method for the quantitation of molecular species in solution. Although, undoubtedly many of the published methods of quantitation are based on suspect assumptions of the applicability of the Beer-Lambert-Bouger law, i.e. the linear relationship between absorbance and concentration.

The type and degree of peak overlap cannot be usually ascertained from a casual or often even a detailed inspection of a spectrum. Several examples of overlap are illustrated in the following computer generated 'spectral' plots (Figures 3a-3i).
Derivatization
The derivatization of spectra can lead to more accurate determination of the wavelengths of broad peak maxima, of peaks which appear only as shoulders, as well as the isolation of small peaks from an interfering large background absorption. This is illustrated in the following second order derivation scan of a potassium permanganate solution (Figure 4).
In derivatizing spectral data, the process taking place is that of differentiating a curve, or more correctly, the mathematical representation or function of that curve, i.e. simply determining the slope or gradient of the whole absorption envelope (Figure 5).

\[
\frac{dA}{d\lambda} = \frac{A_2 - A_1}{\lambda_2 - \lambda_1}
\]
The determined individual gradient values, $dA/d\lambda$, are plotted against the wavelength values, $\lambda$, to give the 1st order derivative plot.

This 1st order derivative plot can in turn be subjected to a similar slope determining treatment to yield values of $d^2A/d\lambda^2$, which, when plotted against the wavelength values gives a 2nd order derivative plot.

An iteration of this process results in increasingly higher order, $n$, derivative plots, i.e. plots of $d^nA/d\lambda^n$ versus $\lambda$.

Such an iterative process, from the zero to the 4th order derivative, is illustrated graphically, using a rather simple triangular peak envelope function (Figure 6).
It can be readily seen that in the 1st order derivative plot, dy/dx versus x (where y = absorbance, A, and x = wavelength, \( \lambda \)), the trace passes through zero at \( x = 0 \), i.e. at the same wavelength as that of the original peak maximum in the zero order (absorbance versus wavelength) plot. The 2nd order derivative plot has a minimum at \( x = 0 \), whereas the 3rd order derivative plot, like the 1st, once more passes through zero at \( x = 0 \), and the 4th order derivative plot is again similar to the 2nd except that it exhibits a maximum instead of minimum at \( x = 0 \).

It should also be noted that with each iteration the two end data points are lost for subsequent calculations. In practice, of course, UV-Visible spectra do not consist of series of triangular peaks. It is widely accepted that a Gaussian profile (Figure 7) provides a better fit for peaks in UV-Visible spectra, whereas Lorentzian (Cauchy) profiles (Figure 7) are generally regarded as being more appropriate for I.R., Raman and N.M.R. spectra (110, 111). Although French and Prager (112),

**Figure 7**

and Butler and Hopkins (20) have obtained better curve fits using mixtures of Gaussian and Lorentzian band shapes. However, even the most complex mathematical representation of a spectral peak can be at best only an approximation of the 'true' peak profile. As Griffiths et al (96) have pointed out, 'each combination of bands and the extent of their overlap will be unique'.

**Gaussian Peaks**

The standard Gaussian curve function (Figure 8) is:

\[
y = y_0 \exp \left(-\ln 2 \left(\frac{2(x-x_0)}{w}\right)^2\right)
\]

where: 
- \( y_0 \) is the maximum band height at wavelength \( x_0 \)
- \( w \) is the bandwidth at half maximum band height (sometimes referred to as FWHM, i.e. full width at half maximum absorbance)
Before differentiating, this equation may be simplified along the lines used by Morrey (113), or by Griffiths et al.* (96), or by Butler and Hopkins** (20).

Thus, substituting $A$ for $y$, $A^0$ for $y_0$, $\lambda$ for $x - x_0$, $k$ for $4\ln 2$, but leaving $w$ in the equation, we have:

$$A = A^0 \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

zero th order

and differentiating:

$$\frac{dA}{d\lambda} = -2\frac{kA^0}{w^2} \lambda \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

1st order

$$\frac{d^2A}{d\lambda^2} = \frac{2kA^0}{w^2} \left(2k\lambda^2 - 1\right) \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

2nd order

$$\frac{d^3A}{d\lambda^3} = -4\frac{k^2A^0}{w^4} \lambda \left(2k\lambda^2 - 3\right) \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

3rd order

$$\frac{d^4A}{d\lambda^4} = \frac{4k^2A^0}{w^6} \left(4k^2\lambda^4 - 12k\lambda^2 + 3\right) \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

4th order

$$\frac{d^5A}{d\lambda^5} = -8\frac{k^3A^0}{w^8} \lambda \left(4k^2\lambda^4 - 20k\lambda^2 + 15\right) \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

5th order

$$\frac{d^6A}{d\lambda^6} = \frac{8k^3A^0}{w^{10}} \lambda \left(8k^3\lambda^6 - 60k^2\lambda^4 + 90k\lambda^2 - 15\right) \exp\left(-k \frac{\lambda^2}{w^2}\right)$$

6th order

etc.

* In reference (96), probably due to a printing error, the 1st derivative equation is given incorrectly as $\frac{dy}{dx} = 2xy$, instead of $\frac{dy}{dx} = -2xy$.

** In reference (20), unfortunately, a number of errors are present in the mathematical expressions:

For Gaussian bands $A^I$ should equal $2A_0 c \left(2c\lambda^2 - 1 - e^{-c\lambda^2}\right)$, and it should be noted that in both $A^I$ and the $A^{IV}$ expressions 'w' is also contained in 'x'.

For Lorentzian bands $A^I$ should equal $8A_0 \left(12\lambda^2 - 1\right) \left(1 + 4\lambda^2\right)^{-3} \left(1 + 4\lambda^2\right)^{-5}$, and again 'x' in these equations also contains 'w'.

It should be noted that the exponential part, $\exp\left(-k \frac{\lambda^2}{w^2}\right)$ has a maximum value of '1', when $x = x_0$, and values approaching '0', when $x$ is well separated from $x_0$. Thus, at the peak maximum, when $x = x_0$, i.e. $\lambda = 0$, the odd order derivative expressions will equal zero.

Whereas the even order derivative expressions will be proportional to the reciprocal of 'w', the band width at half maximum band height.

Hence, for even order derivatives, $n = even$:

$$\frac{d^nA}{dx^n} \propto \frac{1}{w^n}$$

but only if the centroid amplitude, $D_2$, is measured.

This relationship is not strictly correct for peak-satellite amplitudes, due to the extra terms containing $W$ in the derivative equations.
Thus, the observation by Butler and Hopkins (20) that higher derivatives discriminate strongly in favour of narrower bands, i.e. with smaller 'w' values, was certainly of fundamental importance. Their statement that the second derivatives are inversely proportional to the square of the bandwidth and the fourth derivatives are inversely proportional to the fourth power is only quantitatively correct for the centroid amplitude. It is certainly not valid to generalize, without qualification, this derivative amplitude reciprocal bandwidth relationship as has been done in reference (87).

The following computer calculated 0th to 4th order derivative plots (Figure 10a - 10c) illustrate:

- The increasing amplitudes of the derivative peaks with decreasing bandwidth values, w.
- The enhancement of the satellite peak with decreasing bandwidth values.
Lorentzian Peaks
Similarly, the standard Lorentzian (Cauchy) curve function is: $y = y_0 / [1 + 4(x-x_0)^2/w^2]$

Again, this equation may be simplified before differentiation. Thus, substituting $A$ for $y$, $A^o$ for $y_0$, $\lambda$ for $x-x_0$, $c$ for 4, we have:

$$A = A^o / [1+c\lambda^2/w^2]$$

and differentiating:

$$\frac{dA}{d\lambda} = -\frac{2A^o c \lambda}{w^2(1+c\lambda^2/w^2)^2}$$ \hspace{1cm} \text{1st order}

$$\frac{d^2A}{d\lambda^2} = \frac{2A^o c (3c\lambda^2/w^2-1)}{w^4(1+c\lambda^2/w^2)^3}$$ \hspace{1cm} \text{2nd order}

$$\frac{d^3A}{d\lambda^3} = -\frac{24A^o c^2 \lambda (c\lambda^2/w^2-1)}{w^6(1+c\lambda^2/w^2)^4}$$ \hspace{1cm} \text{3rd order}

$$\frac{d^4A}{d\lambda^4} = \frac{24A^o c^2(1-10c\lambda^2/w^2+5c^2\lambda^4/w^4)}{w^8(1+c\lambda^2/w^2)^5}$$ \hspace{1cm} \text{4th order}

$$\frac{d^5A}{d\lambda^5} = -\frac{240A^o c^4 \lambda (3-10c\lambda^2/w^2+3c^2\lambda^4/w^4)}{w^6(1+c\lambda^2/w^2)^6}$$ \hspace{1cm} \text{5th order}

$$\frac{d^6A}{d\lambda^6} = \frac{720A^o c^6 \lambda (7c\lambda^6/w^6-35c^2\lambda^4/w^4+21c^3\lambda^2/w^2-1)}{w^8(1+c\lambda^2/w^2)^7}$$ \hspace{1cm} \text{6th order}

etc.

Again, at the peak maximum, when $x = x_0$, i.e. $\lambda = 0$, the odd order derivative expressions will equal zero, and for even order derivatives, $n = \text{even}$, and:

$$\frac{d^nA}{d\lambda^n} \propto \frac{1}{w^n}$$

It can be seen (Figures 11a, 11b) that the peak amplitudes of Lorentzian curve derivatives are even more sensitive than the Gaussian to the bandwidth values (Table 1).
Table 1
Peak amplitudes at $x = x_0$, i.e. $\lambda = 0$

<table>
<thead>
<tr>
<th>Gaussian Peaks</th>
<th>Lorentzian Peaks</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A = A^\circ$</td>
<td>$A = A^\circ$</td>
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<tr>
<td>$dA = 0$</td>
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<td>$dA = 0$</td>
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<tr>
<td>$d^2A = -\frac{2kA^\circ}{w^2} = -\frac{5.54A^\circ}{w^2}$</td>
<td>$d^2A = -\frac{2A^\circ c}{w^2} = -\frac{8A^\circ}{w^2}$</td>
</tr>
<tr>
<td>$d^3A = 0$</td>
<td>$d^3A = 0$</td>
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<tr>
<td>$d^3A = 0$</td>
<td>$d^3A = 0$</td>
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<tr>
<td>$d^4A = \frac{12k^2A^\circ}{w^4} = \frac{92.23A^\circ}{w^4}$</td>
<td>$d^4A = \frac{24A^\circ c^2}{w^4} = \frac{384A^\circ}{w^4}$</td>
</tr>
<tr>
<td>$d^5A = 0$</td>
<td>$d^5A = 0$</td>
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<tr>
<td>$d^5A = 0$</td>
<td>$d^5A = 0$</td>
</tr>
<tr>
<td>$d^6A = \frac{120k^3A^\circ}{w^6} = \frac{2557A^\circ}{w^6}$</td>
<td>$d^6A = \frac{720A^\circ c^3}{w^6} = \frac{46080A^\circ}{w^6}$</td>
</tr>
</tbody>
</table>

($k = 4\ln2$) ($c = 4$)

It is also readily apparent that for the same bandwidth, $w$, Lorentzian peak amplitudes are greater than Gaussian peak amplitudes, and the magnitude of peak amplitude increase with increasing derivative order is greater for Lorentzian peaks.

For more detailed discussions of the various parameter interactions the works of Giese and French [5], Butler and Hopkins [20], Fell [92], Morrey [113], Allen and McMeeking [114] may be consulted.

A word of caution must be introduced on the subject of overlapping peak profiles and composite curves, since spectral anomalies can be produced [115].

Composite curves can be 'synthesized' very easily with the aid of computers. Such curves can have some unexpected properties. (Figure 12a-12d)
Similar composite spectra can also be obtained for overlapping Lorentzian peaks (Figure 13a-13c) and, of course, for mixtures of Gaussian and Lorentzian profiles.
It is fairly obvious that each combination of bands and the extent of their overlap will be unique. But, unfortunately, neither derivative spectroscopy (Figure 14)

nor spectral deconvolution (unbundling of peaks) necessarily reveal unambiguously the total number and the individual peak maxima of all the bands within a spectral profile. Probably, the lack of a priori knowledge of the relative half-widths of the component peaks is the biggest limitation to quantitative curve fitting and correct assignment of wavelengths to component peak maxima. Also, most importantly, we are not dealing with smooth peak profiles. In practice, there will always be some random noise present in the original spectrum.
Noise
The detectability of absorption peaks via higher derivatives is very much determined by the signal-to-noise ratio, S/N, in the original spectral data. And of fundamental importance to the analyst is the removal or the minimization of the noise, without, at the same time, unduly degrading the underlying information. Thus, there is a need for some appropriate mathematical smoothing either before or during the derivatization process.
A process whose use has been seldom reported in UV-Visible spectrophotometry is the averaging of accumulated spectral runs [44]. However, the availability of the microprocessor has opened up new possibilities in this area. Spectral averaging leads to a reduction in random noise, as can be seen in the following very simple graphical illustration where three sets of data are averaged (Figure 15):
One of the simplest ways to smooth fluctuating data is by a so-called 'moving average', i.e. taking a fixed number of points, adding their ordinate values (absorbances) together and dividing by the total number of points to obtain the average ordinate value at the abscissa centre of the group. Figure 16 shows a simple graphical presentation of a very noisy signal, together with the corresponding increasingly noisy 1st, 2nd and 3rd order derivatives.
Ever, a 4-point "moving average" smooth leads to a significant reduction in noise (Figure 17).
This concept of convolution, i.e. rolling or bunching together of data, can be expressed mathematically:

\[ Y_\ast = \sum_{i=-m}^{i=m} C_i \cdot \frac{Y_j}{N} \]

where: \( c_i \) are the convoluting integers
(e.g. for a moving average each \( c_i = 1 \))
\( j \) represents the running index of the ordinate data \( Y \),
\( N \) is the number of convoluting integers.

As pointed out by Savitzky and Golay [116], for many types of data the moving average, where all \( c_i \)'s are equal to 1, is not particularly useful. Thus, for example, the moving average would tend to degrade a sharp peak (Figure 18), since all values in the moving average interval are 'weighted' equally.
Other smoothing functions can be employed, such as, for example, exponential functions, triangular functions, etc., and although they do reduce noise levels, they do not necessarily extract the maximum amount of information present in the original spectral data.

Hence, Savitzky and Golay [116] were prompted to apply the method of least squares to a set of data points in order to filter out noise and to arrive at new convolution functions or so-called 'least-squares digital polynomial smoothing filters'. Probably, the Savitzky-Golay smoothing filters are the most frequently used digital smoothing filters in spectrometry [117], despite the errors in the original convolution array tables, which were corrected only eight years later [118]. Madden [119] has also commented on the fact that the optimum width of a single-pass smoothing array will depend on the criteria set by the user, and that this optimum width will sometimes exceed the 25-points maximum in the original Savitzky-Golay tables.

Bromba and Ziegler [120] have pointed out that there is still some vagueness about the properties of Savitzky-Golay smoothing filters. This has prompted them to investigate the properties of such 'filters' and to present some trading rules. In general, the effect of smoothing will be to reduce both the noise and the signal. The reduction in noise is related to the number of original data points, which are averaged. Whereas, signal attenuation is related more to the width of the convolution function.

In higher derivatives the optimum number of passes through a smoothing filter appears to be \( n + 1 \) passes (where \( n \) = derivative order), and the extent of S/N degradation depends strongly on the so called smoothing ratio, i.e. the width of the smoothing function to the peak half height bandwidth [121].

As Fell and others [92] have pointed out, the smoothing ratio will critically determine the extent of peak distortion, i.e. attenuation of the peak amplitude and broadening of the band width. When resolution enhancement is the primary goal then the smoothing ratio and S/N will have to be balanced against the fidelity of transcription of the derivative spectra.

It is probably true to say that the derivative UV-Visible spectra obtained on instruments from different manufacturers, or even on different models from one manufacturer, are unlikely to be identical in terms of:

- the wavelengths of peak maxima,
- peak amplitudes,
- peak widths,
- peak amplitude-to-width ratios,
- signal-to-noise ratios

This statement may even be further generalized to include off-line computer calculations on collected spectral data. In other words, 'portability' of derivative spectra for identification and comparison purposes is beset with many pitfalls, even where the mathematical basis is sound, and is a consequence of the various approaches used to obtain derivative spectra. Thus, derivative spectra will be influenced by many factors, such as, for example:

- the monochromator slit function,
- overall noise level,
- noise in the analogue-to-digital conversion,
- the number and frequency of data points collected,
- the number of data points taken into the smoothing function,
- the type of smoothing (filtering) employed
- the derivatization method used.
Quantitative Analysis

Of course, for quantitative analytical purposes, only the amplitudes versus concentrations are measured and here spectral distortion is an acceptable sacrifice, as long as the impact of S/N on the quantitative measurement precision is kept to a minimum.

In general, the quantitative analytical problems can be divided into two categories:

(a) Single or multi-component analysis of overlapping absorption peaks.

The quantitative determination of either one or several compounds depends on each compound obeying the Beer-Lambert Law in the zero order spectrum at a specified wavelength, i.e.:

$$\log I_0 = \text{ABSORBANCE}, A = \varepsilon . c . l$$

where:
- $I_0$ and $I$ are the incident and transmitted intensities respectively,
- $\varepsilon$ is the molar absorptivity
- $c$ is the molar concentration
- $l$ is the cell pathlength

Thus, the analyte concentration is linearly related to the absorbance at the specified wavelength. Similarly, the analyte concentration is linearly related to the amplitude of the nth derivative peak at the specified wavelength:

$$\frac{d^n A}{d \lambda^n} = d^n \varepsilon . c . l$$

Furthermore, an important extension of the Beer-Lambert Law is the 'law of additivity', which states that the absorption of radiation by one species will be unaffected by the presence of other materials, whether they absorb or not. Thus, the more general form of the law may be written:

$$A = \sum \varepsilon_i . c_i . l_i$$

where the summation is over all substances, $i$, present.

Similarly,

$$\frac{d^n A}{d \lambda^n} = \sum \frac{d^n \varepsilon_i}{d \lambda^n} . c_i . l_i$$

i.e. the total nth derivative amplitude will be equal to the sum of the individual nth derivative amplitudes at the specified wavelength.

Since the derivative amplitudes are conventionally measured to a short, $D_s$, or long-wavelength, $D_l$, first satellite [50], the derivative amplitude will depend on the 'law of additivity' being obeyed, since two amplitude measurements at two different wavelengths are made, namely, the 'true' centroid-to-zero amplitude, $D_z$, and the first satellite-to-zero amplitude, $S_z$. (Figure 19).
Thus:

\[ D_L = D_z + S_{z(L)} \]
\[ D_S = D_z + S_{z(s)} \]

Hence, a plot of the derivative amplitudes against a series of standards of known concentrations should give a linear calibration (Figure 20), from which the sample concentration can be determined.

In this example the amplitude \( D_S \) is greater than amplitude \( D_L \), however in other examples the reverse may be the case. Of course, it must be noted that here ideal calibrations are presented. In practice, concentrations may not be exactly linearly proportional to measured absorbances or derivative amplitudes, due to a variety of factors.

Solvation, hydrogen bonding, ion pair formation, and chemical reactions can all cause incorrect calculations of sample concentration in the solvent medium. Reflections and scattering may also lead to non-linear behaviour. Last but not least, instrumental factors such as stray light will also tend to reduce the measured absorbance or derivative amplitudes at higher concentrations, and too wide a monochromator slit width, relative to the absorbing peak width, will also produce non-linear behaviour.
(b) Single or multi-component analysis in the presence of broad, interfering background matrix absorption. Such measurements are of particular importance in clinical, biological, biochemical and food laboratories. In those cases where the background interferences approximates to a linear function of wavelength (Figure 21).
i.e. \( A = m\lambda + k \)

differentiation gives:
\[
\frac{dA}{d\lambda} = m \quad (m = \text{slope of the background interference})
\]

Thus, as can be seen (Figure 21) the amplitude of the 1st derivative at \( \lambda_0 \), i.e. an offset from zero, gives us the slope of the linear background interference.

Further differentiation yields:
\[
\frac{d^2A}{d\lambda^2} = 0
\]

i.e. the background interference has been completely removed (deconvoluted) from the spectrum (Figure 22).

Of course, an interfering background absorption or scattering will not necessarily be linear with wavelength. However, if it can be described by a polynomial:
\[
A = k + m\lambda + p\lambda^2 + q\lambda^3 + \ldots \ldots
\]

then, upon differentiation:
\[
\begin{align*}
\frac{dA}{d\lambda} & = m + 2p\lambda + 3q\lambda^2 + \ldots \ldots \\
\frac{d^2A}{d\lambda^2} & = 2p + 6q\lambda + \ldots \ldots \\
\frac{d^3A}{d\lambda^3} & = 6q + \ldots \ldots
\end{align*}
\]

Thus, for example, the contribution of an interfering background absorption, which can be described by a third order polynomial, is completely removed in the fourth order derivative.

In general, a \( n \)th order polynomial interfering absorption band will be eliminated (deconvoluted) by the \( (n + 1) \)th order derivative.

Of course, in practice it may not always be possible to remove the background matrix interference by going to higher and higher derivatives. In such cases it may be worthwhile to employ the 'method of standard additions' in order to compensate for matrix effects.
Conclusion

It is hoped that this discussion has highlighted the advantages of derivative measurements in UV-Visible spectrophotometry. These advantages can be summarized as follows:

(i) - enhanced resolution of overlapping peaks for the separation of superposed spectra; particularly useful in multi-component analysis,
- quantitative determination of trace components,
- characterization of individual pure compounds; particularly for archiving purposes and for complementing the information obtained from other techniques such as for example IR, NMR, MS.
- purity testing of products.

(ii) - background absorption minimization or elimination for:
- measurements on turbid, scattering solutions and suspensions,
- the analysis of trace components in complex absorbing matrices.
References

[109] T.P. Bridge, R.H. Wardman and A.F. Fell; Analyst, 110, 1307 (19895)