Electronic Signal Derivation to Improve the Performance of Kinetic Methods of Analysis

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Kinetic methods of analysis based on the electronic derivation of absorbance kinetic curves with respect to time were developed. The bromate oxidation of pyridine-2-aldehyde 2-pyridylhydrazone (PAH) and the hydrogen peroxide oxidation of iodide, both showing a Landolt effect, were used as indicator reactions. The analytical performance of the electronic derivation approach was evaluated in the determination of iodide, bromide, iron(II), molybdenum and tungsten. Improvements in precision, accuracy and limit of detection are the main advantages of the approach over conventional rate and induction period methods. The selectivity of the kinetic-based analytical measurements is not degraded by electronic derivation and the inherent simplicity of the analysis is maintained. Electronic derivation of the original kinetic curve provides additional analytical information which can be used with advantage for multi-component analysis.

Keywords: Kinetic method; spectrophotometry; electronic derivation

Since Lord Rutherford first suggested that the signal of the first derivative was more sensitive than that of the primitive spectrum for the determination of gas potentials by mass spectrometry, the derivatives of signals have been used in many other analytical techniques, such as chromatography, potentiometry, densitometry and several kinds of spectroscopy.

In this work, the coupling of an electronic derivation unit to a visible spectrophotometer allowed derivatives of kinetic curves to be obtained in a similar way to the derivatives of other types of signals. The unit stores the recorded signal for subsequent calculation of the derivative and thus the sample can be measured using two or more different types of signal, which is a great advantage over other kinetic techniques. It also permits the development of new methods for quantitative determinations. The fact that this new kinetic approach is simpler and more precise confers considerable advantages over the usual velocity measurement methods.

In the chemical systems studied here, the kinetic curves generated by one of three techniques: numerical differentiation, modulation or direct electronic derivation. Direct electronic derivation offers the most advantages but, above all, it is simple and sensitive.

Much effort has been expended in recent years in developing new methodology which might reduce the imprecision of existing analytical methods. Computerised measurement of reaction velocity in particular has considerably reduced the relative error and standard deviation previously associated with these methods, but the results of kinetic analysis are still greatly affected by the instrumental technique used to follow the reaction and the choice of parameters to be measured. Holler and co-workers recently proposed a temporally optimised, fixed-time ratemeter to calculate velocity and time within an interval in which the kinetic curve may be considered to be linear. This technique is tolerant of instrument noise and temporally optimises the precision of the velocity measurement. The second derivative of the analytical signal has been used to detect the linearity limit of the kinetic curve. Thus, the analytical concentration has been determined in the linear range by means of the reaction rate and its first derivative. It is simple and sensitive.

In this work, the coupling of an electronic derivation unit to an ultraviolet-visible spectrophotometer allowed derivatives of kinetic curves to be obtained in a similar way to the derivatives of other types of signals. The unit stores the recorded signal for subsequent calculation of the derivative and thus the sample can be measured using two or more different types of signal, which is a great advantage over other kinetic techniques. It also permits the development of new methods for quantitative determinations. The fact that this new kinetic approach is simpler and more precise confers considerable advantages over the usual velocity measurement methods.

At present, the technique is restricted to the analysis of chemical systems that exhibit the Landolt effect. However, the improved precision and lower detection limits of this simple and inexpensive instrumentation could greatly improve the analysis of other chemical systems. Moreover, because the existing instrumentation can provide up to four multiple signals, it could be useful for the analysis of multi-component systems.

Theory

Ultraviolet-visible spectrophotometers with electronic derivation compute the spectral derivatives as a function of time, and these are subsequently transformed into derivatives related to wavelength according to the following expression:

\[ \frac{d^nA}{dt^n} = \left( \frac{dA}{dt} \right)^n = \left( \frac{dA}{dt} \right)^n \]  (1)

in which there is a linear relationship between the nth-order derivative of the absorbance and time (dA/dt) and wavelength (dA/dλ); dλ/dt is the scan speed. The optional derivation unit OPI-2 employed in this work for the UV-visible spectrophotometer, in addition to calculating the spectral derivatives up to the fourth order with respect to wavelength, also records the derivatives of absorbance with time. The derivatives may be computed in real time or from absorbance data stored at the time when the kinetic curve was recorded.

The first derivative may be simply expressed by the equation

\[ \frac{dA}{dt} = \frac{\Delta A}{\Delta t} = \frac{A_{i+k} - A_i}{t_{i+k} - t_i} \]  (2)

where \( A_{i+k} \) and \( A_i \) are the absorbances which correspond to the times \( t_{i+k} \) and \( t_i \), and \( k \) is the number of points stored in the differential interval \( \Delta t \). Analogously, the second derivative can be expressed as

\[ \frac{d^2A}{dt^2} = \frac{\Delta A}{\Delta t} = \frac{\Delta A(\Delta t)_{i+2k} - (\Delta A(\Delta t)_{i+k}}{\Delta t^2} \]  (3)

where \( (\Delta A(\Delta t)_{i+2k} \) and \( (\Delta A(\Delta t)_{i+k} \) are values of the first derivative corresponding to the times \( t_{i+2k} \) and \( t_{i+k} \), respectively. In the chemical systems studied here, the kinetic curves stored in the memory are of the Landolt type and obey a mathematical function with the form

\[ f(t) = A_0 \text{ for } t_0 \leq t \leq t_0 \quad (4) \]

where \( A_0 \) is the constant absorbance with time for values \( t_0 \), i.e., the instant in which there is a sharp change of slope; \( g(t) \) is a function that approximates a straight line of the type

\[ A = at + A_0 \]  (5)

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at least when it is close to the inflection point, \( t_0 \). The slope of this line depends on the change in absorbance (concentration) of the species involved in the reaction. Fig. 1(a) shows the original zero-order derivative kinetic curves with negative slopes when the absorbance decreases (1) and positive when it increases (2).

Fig. 1(b) and (c) represent \( \Delta A/\Delta t \) and \( \Delta^2 A/\Delta t^2 \), respectively, versus time. It should be noted that differentiation of equation (4) provides a discontinuous step function. Similarly, the second derivative of equation (4) is merely a straight line with one undefined point. In both instances, however, the recorder prints a continuous function (Fig. 1), which may be treated by graphical methods. The first curves in each instance correspond to a reaction in which the absorbance is decreasing with time and second curves represent reactions in which absorbance is increasing with time. Kinetically, the first derivative is related to the speed of disappearance of a reactant, or to the appearance of a reaction product. Consequently, taking the derivative of the Beer-Lambert law, one can write

\[
\frac{dA}{dt} = ebv \quad \ldots \quad \ldots \quad (6)
\]

where \( \varepsilon \) is the molar absorptivity of the analyte substance, \( b \) is the optical pathway and \( v \) is the velocity, i.e., the change of concentration with time.

The second derivative will be given by

\[
\frac{d^2 A}{dt^2} = eb \left( \frac{dv}{dt} \right) \quad \ldots \quad \ldots \quad (7)
\]

where \( dv/dt \) is the change in the rate at which the substance appears. In Landolt reactions, the signal of the second derivative is produced by the effect of the retardant agent; however, in those reactions where the change in slope is not due to the retardant, it can be considered as being due to a change in reaction velocity.

**Experimental**

**Instrumentation**

Spectrophotometric data were obtained with a Shimadzu 240 S Graphicord spectrophotometer fitted with a temperature-controlled cell holder. A Shimadzu Model OPI-2 derivation module was coupled to the spectrophotometer. Derivatives can be obtained in real time or from stored absorbance data. The absorbance data are printed at intervals of 0.1 nm on the chart paper.

**Procedure**

An aliquot of sample was introduced into a 10-ml calibrated flask; the reagents were added and the mixture was diluted to volume with de-mineralised water. The absorbance was measured at the absorbance maximum of the reagent against water, beginning 30 s after the start of the reaction. Tangents were measured in the zone where the absorbance began to change. The kinetic curve was recorded and simultaneously entered in the data memory by program 44 of the OPI-2 derivation module. Temperature was maintained constant at 25 °C.

**Reagents**

Analytical-reagent grade pyridine-2-aldehyde 2-pyridylhydrazone (PAPH), purchased from Ega-Chemie, was used as received. Solutions were prepared in 0.1 M hydrochloric acid and were stable for at least 2 weeks. Potassium bromate was recrystallised twice from water. A stock solution (0.1 M) was prepared by dissolving 16.70 g in 1 l of water.

The other reagents and solutions were prepared with analytical-reagent grade chemicals. All working solutions were maintained at 25 °C in a water-bath.

**Results and Discussion**

From the derivative calculated from the data stored while recording the kinetic curve, it can be seen that the derivative amplitude is greatest when the change in slope is maximum [Fig. 2(a)]. This leads to the selection of kinetic curves which reveal a sharp change in slope.

The following three types of curve fit this condition: (a) curves produced by the Landolt effect; (b) curves showing a sharp change in slope at a determined instant, as occurs, for example, in the reaction between thiocyanate and chloramine T at pH 5.521; (c) curves showing a steep slope phase followed by a sharp change in slope at the end of the phase when the end of the reaction is close. Type (b) reactions are uncommon and very special and those of type (c) have the inconvenience that the investigator has to wait for a long time until the reaction has finished. Preliminary laboratory experiments showed that these two types of reaction did not have very high sensitivity and so this work was restricted to the study of Landolt-type
reactions, for which measurement of the length of the induction period is the usual method of characterisation, but measurements of the initial tangent to the kinetic curves are also frequently used. Both values are related to the concentration of the catalyst and can be used for quantitative determinations.

Oxidation of PAPH by bromate in an acidic medium in the presence of potassium iodide has an induction period which increases with increasing iodide concentration. Spectrophotometric monitoring of the reaction by measuring the decrease in the absorbance of PAPH at 372 nm gives kinetic curves similar to those in Fig. 1(a) (1) in which there is a sharp change in slope at the end of the induction period, which is most useful for the proposed technique. The minimum of the second-derivative signal is easier to measure than the first-derivative maximum. However, the signals of the third and fourth derivatives are poor with a great deal of noise from instrument circuitry.

The signal peak heights are proportional to the degree of change in slope of the kinetic curve; the greater the change, the higher are the peaks. This is well illustrated in Fig. 2(a), which also reveals the relationship between the second-derivative signal amplitude and the concentration of a species present in the reaction.

The kinetic curves fit the following mechanism, for which the proposed reaction is:

\[ I^- + BrO_3^- \xrightarrow{\text{slow}} I_2 + Br^- \quad \ldots \quad (8)\]

because on obtaining UV-visible spectra for different moments during the oxidation by bromate of PAPH, an absorption maximum corresponding to the formation of iodine appeared early at 536 nm and increased with time; the iodide acted as a retardant. This phase would correspond to the part of the curve for \( t < t_0 \) in Fig. 1(a). The reaction is slow and the reactant is not oxidised until the iodide is completely consumed.

\[ BrO_3^- + PAPH + H^+ \xrightarrow{\text{very slow}} Br^- + \text{products} \quad (9)\]

This second reaction corresponds to the part of the curve for \( t > t_0 \).

Curves similar to those illustrated in Fig. 1(a) (2) were obtained by studying another system, the oxidation of iodide by hydrogen peroxide, in which a retarding agent such as citric or ascorbic acid was added. In this instance, the signal of the second derivative, like that of the previous system mentioned above, showed maxima whose amplitudes increased with an increase in the degree of change in slope of the kinetic curve [Fig. 2(b)]. The change in slope is linear with the concentration of the reaction catalyst. This relationship has been exploited to determine molybdenum(VI) and tungsten(VI) by electronic derivation using the catalysis of the oxidation of iodide by hydrogen peroxide by these two ions.

**Selection of Parameters**

Fig. 3(a) shows (A) the kinetic curve for the oxidation of PAPH in hydrochloric acid medium by potassium bromate in the presence of iodide ions, (B) the first derivative and (C) the second derivative. The second derivative is less noisy than the first derivative because of a scale change. There are many parameters that can provide analytical information, but the most important are:

- \( h \) = peak height of the second derivative which corresponds to the inflection point of the kinetic curve;
- \( x \) = horizontal distance between the two peaks of the second derivative;
- \( y \) = peak-to-peak distance in the second derivative;
- \( T \) = distance from the origin of the second derivative to the first peak (this corresponds to the induction period of the kinetic curve).

These parameters were characterised by observing the variation in each with different iodide concentrations during the bromate oxidation of PAPH [Fig. 3(b)]. The parameter \( x \) was easy to measure, but there was neither a direct nor a reciprocal linear dependence on iodide concentration. The measurements of \( T \) were not very satisfactory because they demanded a rigorous measurement of the time elapsed from the initiation of the reaction until the data recording was started. In this particular reaction, there was no linear dependence of \( 1/T \) on the concentration of iodide, but the
parameters \( y \) and \( h \) did show linear relationships with concentration. The parameter \( h \) was chosen owing to its advantage over \( y \) and all the others that it can be measured without waiting for the end of the reaction.

**Instrumental Variables: Effect on Signal**

Two instrumental variables, chart speed and absorbance scale, must be taken into account when working with graphical spectrophotometric kinetic methods based on kinetic curve measurements, because they can affect the quality of analytical methods. Derivative techniques have several more variables in addition to chart speed; the most important are listed, together with their effects on the different parameters, in Table 1.

Derivatives can be obtained either in real time or retrospectively from stored data. Fig. 4 shows that the instrument sensitivity was much higher in the latter instance and this characteristic permits the employment of more instrumental variables which appreciably increase the signal peak amplitude.

As the absorbance scale depends on three factors, the species being investigated, its concentration and the length of the optical path, it is independent of instrument signal amplification. Changes in the absorbance scale only affect the slope of the kinetic curve and the derivative is not affected. Similarly, changes in scale on the ordinate of the derivative signal are confined to the derivative and do not affect the kinetic curve.

Fig. 5(a) and (b) show the recorded signals for the bromate oxidation of PAPH in the presence of bromide and indicate that the chart speed of the kinetic curve recorder \( (V_c) \) distorted both signals. A decrease in speed shortened the abscissa and steepened the curve, and also increased the derivative signal because the number of points that separate the two extremes of the differentiation interval in the data memory was reduced. As the points represent the time intervals along the abscissa, the derivative signal is narrowed and peak amplitude is increased. For this reason, it is advisable to select initially a moderate chart speed of about 5–10 mm min\(^{-1}\). Very low chart speeds give rise to much signal noise and loss of precision. Chart speed is an important consideration because, as Fig. 5 shows, a low chart speed causes an apparent shortening of the length of the induction period. As a graphical method is being dealt with, this artificial shortening of the line representing the induction period caused a lack of linearity at the upper limit of the calibration graph so that, as the concentration of the analyte was increased, the moment arrived when the period ended prematurely. However, this effective decrease in recorded induction time was non-linear, and the analyte concentration conformed to a type of exponential equation\(^{22}\) as follows:

\[
X = a - a^{-[(b/c) - k_2 c t]} \quad (10)
\]

where \( X \) is the concentration at time \( t \) and \( a, b, c, K_1 \) and \( K_2 \) are constants. Consequently, at high analyte concentrations, the decrease in the induction period is not significant and the dynamic range is maintained at reasonable levels.

![Fig. 4. A, Original kinetic curve and second derivative obtained; B, retrospectively from stored data; and C, in real time.](image)

![Fig. 5. Effect of chart speed on 1, kinetic curve; and 2, its second derivative. \( V_c \), Chart speed in the printing of the kinetic curve; \( V_d \), chart speed in the printing of the second derivative. (a) \( V_c = 5 \) and \( V_d = 5 \) mm min\(^{-1}\); (b) \( V_c = 3 \) and \( V_d = 3 \) mm min\(^{-1}\); (c) \( V_c = 10 \) and \( V_d = 10 \) mm min\(^{-1}\); and (d) \( V_c = 5 \) and \( V_d = 10 \) mm min\(^{-1}\).](image)
During the print-out of data from the derivative data store, the chart speed in the printing of the second derivative ($V_2$) only affects the differentiated curve because the kinetic curve was printed earlier. Fig. 3 shows how an increase in the chart speed of the derivative printer causes an apparent widening of the signal together with a decrease in the peak amplitude; consequently, as with the kinetic print-out, low chart speeds are superior.

Equations (2) and (3) show that intermediate differentiation intervals are best because increasing the differentiation interval, $\Delta t$, decreased the signal and an increase in the very small differentiation interval also increased the noise.

**Determination of Species**

As shown in Fig. 2(a) and (b), the peak height of the second derivative, $h$, depends on analyte concentration. This relationship was linear and gave calibration graphs for the determination of catalytic species.

The analytical data generated by electronic derivation of the response curves are easier to obtain, and are more precise than those obtained in rate methods. Electronic derivation has the additional advantages that it is more accurate and provides lower detection limits.

The increased precision and accuracy of the derivative method is largely due to the greatly improved signal reproducibility and better statistical parameters of the calibration graphs. For example, iron(II) was determined by three different procedures. Two were based on reactions with the Landolt effect; one used the tangent method (tangent I) and the other electronic derivatisation and in both, PAPH was oxidised by bromate in the presence of iodide ions. The third procedure used the tangent method for the kinetic curves obtained in the absence of iodide (tangent II). The relative standard deviations ($n = 10$) for the signals were 1.34% for the derivative method, 3.74% for the tangent method in the presence of iodide and 6.9% for the tangent method in the absence of iodide. Table 2 shows the statistical parameters for the calibration graphs. The performance of the derivative method is appreciably better than that of the other two methods. Consequently, the electronic derivatisation is about 3–4 times more precise and accurate than the tangent methods for the determination of iron(II). Table 3 also gives the values of relative standard deviation and the percentage error for each method.

The greater precision and accuracy of the electronic derivatisation technique gives lower detection limits than the conventional kinetic methods. For example, the determination of molybdenum(VI) and tungsten(VI) by the derivative technique gave detection limits of 0.020 and 0.093 pg ml⁻¹, respectively, whereas the limits for the tangent method were 0.15 and 0.67 pg ml⁻¹, respectively.

No significant differences were observed in selectivity between the derivative technique and the two tangent methods. Treatment of the samples before and during the analytical process was the same for both and the physico-chemical properties and the reactivities of all the species involved were the same. The use of different types of signals did not produce selectivity factors notably different from unity. The interference effects of ten species, nickel(II), cobalt(II), copper(II), lead(II), iron(III), nitrate, chlorate, sulphate, thiourea and EDTA, on the determination of bromide were studied using the oxidation of PAPH by bromate in the presence of iodide ions as the indicator reaction. Chemical interferences produced by the reactions between the different reactants and the interferent species present in solution were observed; both signals were equally affected.

**Resolution of Multi-component Mixtures**

Electronic derivation generates more signals in addition to those of the tangent and the kinetic curve induction period, and all of them can be obtained from one sample. The kinetic curve is recorded first, the derivative is calculated later and the print-out is obtained from the absorbance data stored in the

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### Table 2. Statistical parameters of calibration graphs for the determination of iron(II)

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>RSD, %</th>
<th>Intercept</th>
<th>RSD, %</th>
<th>$r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivative</td>
<td>1.280</td>
<td>1.430</td>
<td>0.900</td>
<td>1.600</td>
<td>1.000</td>
</tr>
<tr>
<td>Tangent I</td>
<td>0.540</td>
<td>5.020</td>
<td>0.880</td>
<td>5.960</td>
<td>0.994</td>
</tr>
<tr>
<td>Tangent II</td>
<td>1.170</td>
<td>9.420</td>
<td>0.230</td>
<td>16.600</td>
<td>0.989</td>
</tr>
</tbody>
</table>

* Relative standard deviation of slope ($n = 5$).
† Relative standard deviation of intercept ($n = 5$).
‡ Correlation coefficient.

### Table 3. Precision and accuracy in the determination of iron(II).

<table>
<thead>
<tr>
<th>Method</th>
<th>$x^*$</th>
<th>$s^+$</th>
<th>RSD, %</th>
<th>$E$, %</th>
<th>$%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivative</td>
<td>1.010</td>
<td>0.037</td>
<td>3.72</td>
<td>2.62</td>
<td></td>
</tr>
<tr>
<td>Tangent I</td>
<td>0.910</td>
<td>0.078</td>
<td>8.60</td>
<td>6.32</td>
<td></td>
</tr>
<tr>
<td>Tangent II</td>
<td>0.887</td>
<td>0.102</td>
<td>11.53</td>
<td>8.21</td>
<td></td>
</tr>
</tbody>
</table>

* Mean of ten determinations (µg ml⁻¹).
† Standard deviation (µg ml⁻¹).
‡ Relative standard deviation ($n = 10$).
§ Relative error.

### Table 4. Statistical parameters of calibration graphs for the determination of molybdenum

<table>
<thead>
<tr>
<th>Method</th>
<th>Slope</th>
<th>RSD, %</th>
<th>Intercept</th>
<th>RSD, %</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Derivative</td>
<td>4.930</td>
<td>0.670</td>
<td>0.785</td>
<td>3.010</td>
<td>0.999</td>
</tr>
<tr>
<td>Tangent I</td>
<td>0.750</td>
<td>3.990</td>
<td>0.110</td>
<td>30.610</td>
<td>0.998</td>
</tr>
<tr>
<td>Tangent II</td>
<td>0.530</td>
<td>1.500</td>
<td>0.022</td>
<td>31.920</td>
<td>0.997</td>
</tr>
</tbody>
</table>

* Relative standard deviation of slope ($n = 5$).
† Relative standard deviation of intercept ($n = 5$).
‡ Correlation coefficient.

### Table 5. Simultaneous determination of molybdenum and tungsten in synthetic samples

<table>
<thead>
<tr>
<th>Molybdenum/µg ml⁻¹</th>
<th>Tungsten/µg ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>$h^*$</td>
<td>$r^t$</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
</tr>
<tr>
<td>6.65</td>
<td>5.43</td>
</tr>
<tr>
<td>6.13</td>
<td>5.07</td>
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<tr>
<td>10.85</td>
<td>8.45</td>
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<tr>
<td>8.45</td>
<td>1.20</td>
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<tr>
<td>10.80</td>
<td>8.45</td>
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<tr>
<td>19.45</td>
<td>14.65</td>
</tr>
<tr>
<td>19.50</td>
<td>14.60</td>
</tr>
<tr>
<td>19.45</td>
<td>14.65</td>
</tr>
</tbody>
</table>

* Peak height of derivative signal (cm).
† Slope of kinetic curve (cm).
‡ Mean ± standard deviation ($n = 3$).
memory. The previously recorded signals are used to establish and calculate several equation systems which, in turn, are used to determine several species simultaneously. In this work, simultaneous determinations were carried out for (1) iodide and bromide and (2) tungsten and molybdenum; the respective indicator reactions were PAPH oxidation by bromate and iodide oxidation by hydrogen peroxide.

Table 5 presents the results of the simultaneous determinations of three different concentrations of molybdenum and tungsten in three synthetic mixtures. As the derivative method is very simple and the signals are measured indirectly by the graphical technique, the approach proposed here would be considerably enhanced by combining it with computer measurement.

The authors thank D. W. Schofield for translating the manuscript.

References

Received December 18th, 1989
Accepted August 3rd, 1990