Fourth Derivative Spectrophotometry in Simultaneous Determination of Calcium and Magnesium in Mixture

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Abstract
The study included determination of calcium and magnesium in mixture by using 4th derivative spectrophotometry. The method is based on the red complexes formed by both metal ions with PURPURATE (MUREXIDE) indicator (violet in colour in alkaline ethanol – water mixture). The range of application were between 0 to 2.4 µg / ml and between 0 to 1.4 µg / ml at 502 & 465 nm for calcium and magnesium respectively. A statistical evaluation of the experimental results was reported.

Introduction
The absorbance peaks encountered in UV/Visible spectrophotometry are normally broad due to the overlap of the many molecular transitions taking place in the sample. Thus, in some situations; for instance the precise determination of \( \lambda_{\text{max}} \) for a broad peak or, precise quantitative and qualitative information can be satisfied. The systematic errors encountered with background or sample turbidity can cause similar problems, unless care is taken prior to sample measurement. Many methods have been proposed to overcome or reduce these problems. Unfortunately, they all suffer from numerous analytical compromises. The more relevant option can be classified into four general groups:

1. Standard Addition
2. Measurement by:
   a. Peak Wavelength shift
   b. Difference spectrophotometry
   c. Multiple Wavelength measurements
3. Mathematical correction
   a. Allen Correction
   b. Morton and Stubbs
   c. Glenn
4. Instrumental

5. Derivative spectroscopy

The spectrum of any compound may be considered as a mathematical function relating the absorbance to the wavelength of a particular compound, thus \( A = f(\lambda) \). The function includes all parameters which affect the absorbance of the compound such as the molar absorptivity of the compound at all wavelengths in the spectrum, mathematically, it is a function like any other and can be differentiated, to other curves such as \( dA/d\lambda \), \( d^2A/d\lambda^2 \), and so on. These curves are known as derivative spectra, those indicated are the first and second derivatives. The first derivative of any function shows the rate of change of that function. In spectroscopic terms, the first derivative shows the rate of change of absorbance with respect to wavelength. The second derivative shows the rate change of the first derivative, again with respect to wavelength. The process may be repeated as often as we like to obtain higher and higher order derivative spectra. Most UV/Visible spectra may be considered to be made up of a series of overlapping Gaussian peaks. It is, therefore, of interest to consider what happens to Gaussian function when it is differentiated.
The points to note from this figure are:

a. The first derivative crosses the zero axis at the same wavelength value as the maximum of the original peak (Y) (zero-order). This is true for any peak shape and is often as a way to determine the position of the peak maximum.
b. The second derivative spectrum has a negative peak (Y) corresponding to the position of the original peak maximum.
c. The second derivative spectrum has two ‘satellite’ peaks (X1 and X2) one on each side of the main negative peak.
d. The central, negative peak of the second-order spectrum is narrower than the original peak.

These facts may be generalized for higher order spectra. The central zero-crossing position remains in line with the main peak position for all odd order spectra, this peak will alternate in sign from negative to positive as each higher order spectrum is calculated. the number of satellites will increase with each even-order spectrum.

There being four in the fourth derivative at the same time the peak will become narrower and narrower.

Because odd-order spectra do not have any features corresponding to the original peak, these tend not to be used as much as even-order spectra. First derivative spectra are sometimes used because they are simple to obtain and tend not to suffer from excess noise.

Second and fourth-order spectra are used most often. Higher orders tend to become very complex and as the derivative order increases noise becomes more of a problem.

The representation of the third derivative shows an inverted sense of the first derivative the peak of the zero-order curves still being at the zero-crossing. However, the use of the fourth derivative produced peak at maximum absorbance. It also exhibits a further sharpening of the peak band. For this application, the use of second and fourth derivative gives considerable advantage. When small peaks or shoulders appear on the side of a sloping major component background, increasing derivative orders will progressively flatten the major peaks to almost straight line and resolve the minor components more sharply as peak minima with second-order derivative and maxima with fourth-order derivative. As stated previously, increasing derivative orders will increase the apparent band sharpening and will, therefore, provide an increase in analytical sensitivity up to a fourth-order derivative and to lesser extent, beyond.

In recent years, the use of derivative spectrophotometry (DS) has become more practical owing to the increase in the resolving power of the analytical instrumentation and easier access to microcomputers with appropriate software, which allows the almost instantaneous generation of the derivative spectra.
. This facility in the collection and treatment of the spectra has allowed analysis of multi-component mixture of analyte with overlapping spectra.

Young, Sweet, and Baker have studied the simultaneous spectrophotometric determination of calcium and magnesium by Erichrome Black – T. depends on obtaining absorbance measurement at a wave length of 630 nm. and at pH of both 9.5 and 11.7. On the basis of 43 known aqueous mixture of calcium and magnesium. Tammelene and Mogensen have investigated the 1 to 1 complex between calcium and murexide in basic medium ranging in colour from yellow-orange to red.

Al-Sindy have studied the simultaneous spectrophotometric determination of calcium and magnesium by first and second derivative spectrophotometry using Bromopyrogallol Red (BPR).

Experimental

Apparatus:
A shimadzu UV/Visible recording spectrophotometer (Model UV-160) and 1-cm quartz cells were used for the normal and derivative spectrophotometric measurements.

Reagents:
All experiments were performed with analytica reagent grade chemicals and doubly distilled water was used through out.

Solution: 0.1 N NaOH (only) to get pH 12

A 4 ml of the reagent was selected because of the highest absorbance.

Effect of Buffer concentration:

Table 1: Effect of reagent concentration on absorbance

<table>
<thead>
<tr>
<th>Ml of 0.001 M murexide</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>λ max (nm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of magnesium</td>
<td>0.231</td>
<td>0.381</td>
<td>0.498</td>
<td>0.584</td>
<td>0.564</td>
<td>465</td>
</tr>
<tr>
<td>Absorbance of calcium</td>
<td>0.269</td>
<td>0.604</td>
<td>0.766</td>
<td>0.746</td>
<td>0.731</td>
<td>502</td>
</tr>
<tr>
<td>Absorbance of Ca²⁺ &amp; Mg²⁺</td>
<td>0.251</td>
<td>0.347</td>
<td>0.617</td>
<td>0.705</td>
<td>0.660</td>
<td>482</td>
</tr>
</tbody>
</table>

A 0.5 ml of sodium hydroxide was selected for the subsequent experiments due to magnesium is precipitated in strong alkaline solution.

Calcium Solution, 0.001 M (40 ppm). A 0.0100 gram sample of calcium carbonate solution (Fluka Co. 99.9 %) was dissolved in 5 ml of concentrated hydrochloric acid and the solution was diluted to 100 ml.

Magnesium Solution, 0.001 M (24 ppm). A 0.0040 gram sample of magnesium oxide solution (Merk Co. 99.7 %) was dissolved in 5 of concentrated hydrochloric acid and the solution was diluted to 100 ml.

Dye Solution, 0.001 M. A 0.0284 gram sample of pure & dry purpurate ion(murexide) is dissolved in 75 ml of pure ethanol, and diluted with 25 ml of water, store in refrigerator. This solution should be use able for ten days at 5°C.

Interfering ion solution, 1000 ppm. A 1000 µg/ml solution of each ion prepared in double distilled water.

Results and Discussion

Spectrophotometric Measurements

Fig. 2 shows the absorbance spectrum of magnesium only which has an absorption maximum at 465 nm. The corresponding spectrum of the calcium at 502 nm.

Study of optimum condition

Effect of Reagent Amount

The influence of the concentration of murexide reagent on maximum formation of the colored complex on each of 0.001 M magnesium and 0.001 M calcium at pH 11.3 was investigated and the results illustrated.

Table 2: Effect of buffer amount on absorbance

<table>
<thead>
<tr>
<th>Ml of 0.1 M NaOH</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
<th>0.75</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance of magnesium</td>
<td>0.000</td>
<td>0.201</td>
<td>0.299</td>
<td>0.252</td>
<td>0.183</td>
<td>0.133</td>
<td>0.035</td>
<td>0.008</td>
</tr>
<tr>
<td>Absorbance of calcium</td>
<td>0.040</td>
<td>0.321</td>
<td>0.680</td>
<td>0.692</td>
<td>0.704</td>
<td>0.672</td>
<td>0.718</td>
<td>0.678</td>
</tr>
<tr>
<td>Absorbance of Ca²⁺ &amp; Mg²⁺</td>
<td>0.341</td>
<td>0.415</td>
<td>0.817</td>
<td>0.792</td>
<td>0.768</td>
<td>0.759</td>
<td>0.731</td>
<td>0.700</td>
</tr>
<tr>
<td>λ max (nm.) for mixture</td>
<td>472</td>
<td>475</td>
<td>484</td>
<td>485</td>
<td>488</td>
<td>493</td>
<td>489</td>
<td>482</td>
</tr>
</tbody>
</table>

A 0.5 ml of sodium hydroxide was selected for the subsequent experiments due to magnesium is precipitated in strong alkaline solution.

Order of addition: Both metal ions (M), mixed with reagent (R) and buffer solution (B) which are shown in Table 3 by using zero order.

Table 3: Order of addition of the reagents

<table>
<thead>
<tr>
<th>Reaction component</th>
<th>Order number</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>M+R+B</td>
<td>I</td>
<td>0.816</td>
</tr>
<tr>
<td>M+B+R</td>
<td>II</td>
<td>0.302</td>
</tr>
</tbody>
</table>

Order I was selected for the subsequent experiments

Quantitative and Qualitative Resolution of Multi-Component Systems:

One of the classic analytical problems for any researcher in the field of UV/Visible spectrophotometry is the resolution of a number of components in a mixture, particularly when the absorbance of those component is extremely variable and/or components with naturally narrow absorption bands. The problems is to resolve small shoulders or peaks from the total spectrum. Fig. 2 (A and B) shows the idealized representation of 4th derivative in the mixture.
Mixture

![Graph showing absorbance vs wavelength for Mg II and Ca II](image)

Fig. 2 a,b: shows fourth- derivative absorption spectra of the complexes of Mg II and Ca II with murexide and a mixture of both complexes. It can be seen two closeness overlapping spectra (+ ve maxima) of two ions at 465 and 502 nm, respectively.

**Recommended procedure and calibration curve:**

After establishment of the optimum reaction conditions, a calibration curve was constructed by transferring increasing volumes of 24 µg/ml and 40 µg/ml of magnesium, calcium respectively into 25-ml volumetric flasks to cover the range 0 to 2.4 µg/ml for magnesium and 0.16 to 3.2 µg/ml for calcium, then 4 ml of 0.001 M ammonium purpurate (Murexide) reagent and 0.5 ml of 0.1 M sodium hydroxide. Dilute to volume by double distilled water and read the absorbance immediately against a blank. A plot of absorbance versus determinant concentration shows that Beer's law was obeyed over the concentration 0 to 1.4 and 0.16 to 2.4 for Mg and Ca respectively.

![Graph showing absorbance vs concentration for Mg II and Ca II](image)

**Fig. 3:** Calibration curve of magnesium and calcium from 0 to 1.4 and 2.4 ppm respectively

**Effect of each ion to other:**

To an aliquot of solution containing 1 ml of 0.001M calcium(1.6 ppm), various amount of 0.001M magnesium were added, then 4 ml of 0.001 M reagent, the mixture was diluted in to 25 ml with double distilled water. The absorbance was measured at wavelength range 487 nm, (for total spectrum of mixture magnesium and calcium at zero order derivative), and at 470 nm, 502 nm (for each magnesium, calcium respectively by using fourth order derivative). The results are summarised in Table 4 a.
Table 4a: Effect of calcium on calibration curve of magnesium at fourth order derivative

<table>
<thead>
<tr>
<th>Concentration Mg$^{2+}$ (ppm)</th>
<th>Absorbance of Mg$^{2+}$ at zero order</th>
<th>Absorbance of mixture at zero order</th>
<th>Absorbance of each ion in mixture using fourth order derivative</th>
<th>Absorbance of Mg$^{2+}$ only using 4th order derivative</th>
<th>Recovery of Mg$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mg$^{2+}$ ( \lambda_{max} = 465 )</td>
<td>Ca$^{2+}$ ( \lambda_{max} = 503 )</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>0.157</td>
<td>0.638</td>
<td>0.000</td>
<td>0.122</td>
<td>0.070</td>
</tr>
<tr>
<td>0.24</td>
<td>0.222</td>
<td>0.672</td>
<td>0.000</td>
<td>0.122</td>
<td>0.095</td>
</tr>
<tr>
<td>0.48</td>
<td>0.377</td>
<td>0.736</td>
<td>0.038</td>
<td>0.105</td>
<td>0.137</td>
</tr>
<tr>
<td>0.72</td>
<td>0.477</td>
<td>0.838</td>
<td>0.073</td>
<td>0.093</td>
<td>0.173</td>
</tr>
<tr>
<td>0.96</td>
<td>0.592</td>
<td>0.915</td>
<td>0.108</td>
<td>0.081</td>
<td>0.215</td>
</tr>
<tr>
<td>1.44</td>
<td>0.823</td>
<td>1.027</td>
<td>0.170</td>
<td>0.061</td>
<td>0.270</td>
</tr>
<tr>
<td>1.92</td>
<td>0.970</td>
<td>1.171</td>
<td>0.245</td>
<td>0.031</td>
<td>0.345</td>
</tr>
<tr>
<td>2.40</td>
<td>1.057</td>
<td>1.232</td>
<td>0.315</td>
<td>0.021</td>
<td>0.383</td>
</tr>
</tbody>
</table>

Slope 7.08  7.16  0.99  0.99  0.19  -0.48
Intercept -0.37  -0.324

From the above table shown that calcium is affected on magnesium at zero order, but it eliminated in fourth order derivative. The same study was applied for the effect of magnesium in presence of calcium, which summarized in Table 4b.

Table 4b: Effect of magnesium on calibration curve of calcium at fourth order derivative

<table>
<thead>
<tr>
<th>Concentration of Ca$^{2+}$ (ppm)</th>
<th>Absorbance of Ca$^{2+}$ at zero order</th>
<th>Absorbance of mixture at zero order</th>
<th>Absorbance of each ion in mixture using fourth order derivative</th>
<th>Absorbance of Ca$^{2+}$ only using 4th order derivative</th>
<th>Recovery of Ca$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mg$^{2+}$ ( \lambda_{max} = 465 )</td>
<td>Ca$^{2+}$ ( \lambda_{max} = 502 )</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.088</td>
<td>0.656</td>
<td>0.215</td>
<td>0.000</td>
<td>0.028</td>
</tr>
<tr>
<td>0.40</td>
<td>0.186</td>
<td>0.595</td>
<td>0.177</td>
<td>0.015</td>
<td>0.045</td>
</tr>
<tr>
<td>0.80</td>
<td>0.369</td>
<td>0.667</td>
<td>0.155</td>
<td>0.035</td>
<td>0.074</td>
</tr>
<tr>
<td>1.20</td>
<td>0.549</td>
<td>0.814</td>
<td>0.108</td>
<td>0.060</td>
<td>0.100</td>
</tr>
<tr>
<td>1.60</td>
<td>0.638</td>
<td>0.904</td>
<td>0.085</td>
<td>0.080</td>
<td>0.123</td>
</tr>
<tr>
<td>2.40</td>
<td>0.831</td>
<td>1.130</td>
<td>0.025</td>
<td>0.130</td>
<td>0.170</td>
</tr>
</tbody>
</table>

Slope 2.89  17.3  15.7  0.97  0.166  -0.324
Intercept -0.188

In order to eliminate the effect of interference of each ion to other, it will be introducing a term called **inter element correction factor** (IECF). The value of effect of calcium on magnesium equal (1.3) and for magnesium on calcium equal (0.35), then applied this equation:

\[
\text{The true value of magnesium(from table 4a)} = (1.3 \times \text{absorbance of calcium affected at any point + absorbance of magnesium with interference}) \times \text{Concentration of magnesium from calibration curve/absorbance of magnesium with out interference}
\]

**For example:**

1. **Determination of pure magnesium in mixture:**
   From table 4a by using 4th derivative spectrophotometer
   \[
   (1.3 \times A_{\text{Ca with interference}} + A_{\text{Mg with interference}})
   \]
   True value of Mg II in ppm = ----------------------
   \[
   A_{\text{Mg with out interference}}
   \]
   Absorbance of Calcium interfere = 0.081
   Absorbance of Mg in mixture = 0.108
   Concentration of Mg at this point = 0.96 ppm
   Absorbance of Magnesium free = 0.215

   **Solution:**
   \[
   (1.3 \times 0.081 + 0.108) \times 0.96
   \]
   Mg II (ppm) = ------------------------------ = 0.9524 ppm
   0.215
   Recovery of Mg II = 0.95 / 0.96 \times 100 = 99.2 %

2. **Determination of pure calcium in mixture:**
   From table 4b by using 4th derivative spectrophotometer
   \[
   (0.35 \times A_{\text{Mg with interference}} + A_{\text{Ca with interference}})
   \]
   True value of Ca II in ppm = ----------------------
   \[
   A_{\text{Ca with out interference}}
   \]
   Absorbance of Magnesium interfere = 0.081
   Absorbance of Mg in mixture = 0.108
   Concentration of Mg at this point = 0.96 ppm
   Absorbance of Magnesium free = 0.215

   **Solution:**
   \[
   (0.35 \times 0.081 + 0.108) \times 0.96
   \]
   Mg II (ppm) = ------------------------------ = 0.9524 ppm
   0.215
   Recovery of Mg II = 0.95 / 0.96 \times 100 = 99.2 %
Absorbance of Ca in mixture = 0.060
Concentration of Ca at this point = 1.2 ppm
Absorbance of Calcium free = 0.100

Solution:
\[(0.35 \times 0.108 + 0.06) \times 1.2 = 0.100\]
Ca II (ppm) = 1.173 ppm

Recovery of Ca II = 1.17 / 1.2 * 100 = 97.8 %

Application
Determination of magnesium and calcium in drinking water:

The proposed method for determination of two ions without separation in drinking water, the successful results were obtained in Table 5.

Determination of magnesium and calcium in dolomite
A 0.1 g of dolomite was wet digestion in acidic medium, the sample was cooled and filtered up to 25 ml distilled water, then 0.25 ml of aliquot was taken for determination of Mg and Ca in a mixture by derivative spectrophotometer. Table 5 show the results.

Determination of magnesium and calcium in paracetamol tablet
The above procedure is applied.

Table 5: Determination of magnesium and calcium in various samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mg^{2+}</th>
<th>Ca^{2+}</th>
<th>Mg^{2+}</th>
<th>Ca^{2+}</th>
<th>Mg^{2+}</th>
<th>Ca^{2+}</th>
<th>Mg^{2+}</th>
<th>Ca^{2+}</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Atomic</td>
<td>4 th.</td>
<td>Complex</td>
<td>Recovery %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Absorption Spectrophotometry</td>
<td>Derivative Spectrophotometry</td>
<td>formation titration with EDTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drinking Water (ppm)</td>
<td>35</td>
<td>40</td>
<td>40</td>
<td>36</td>
<td>Accep</td>
<td>Accep</td>
<td>24</td>
<td>24</td>
<td>Accep</td>
</tr>
<tr>
<td>Dolomite %</td>
<td>12.4</td>
<td>21.6</td>
<td>13.1</td>
<td>20</td>
<td>105</td>
<td>92.5</td>
<td>13.8</td>
<td>22</td>
<td>111</td>
</tr>
<tr>
<td>Paracetamol %</td>
<td>≤0.5</td>
<td>≤0.5</td>
<td>0.4</td>
<td>0.5</td>
<td>Accep</td>
<td>Accep</td>
<td>0.5</td>
<td>0.5</td>
<td>Accep</td>
</tr>
</tbody>
</table>
References
12. L.J. Al-Sindy, Determination of calcium and magnesium using Molecular Absorption Flow – Injection and Derivative Spectrophotometry in Aqueous Solution, PhD., Dohuk University, College of Medicine, (2002).

otation الكالسيوم والمغنيسيوم في المزيج باستخدام مطيافية المشتقة الرابعة
معاذ عبد الله الحجار و محمد سالم العنزي و سهير محفوظ
شعبة العلوم الأساسية ، كلية الزراعة والغابات ، جامعة الموصل ، الموصل ، العراق

الملخص
شملت الدراسة تقدير الكالسيوم والمغنيسيوم كخلط باستخدام مطيافية المشتقة الرابعة واعتمدت الطريقة على تفاضل الامتصاص مع الطول الموجي إلى حد الدّرجة الرابعة والحصول على ندرين منفصلتين حادتين باستخدام كاشف الميروكسيد والحصول على معقد احمر اللون في وسط كحلي والماء والذان يعطيان اعلى شدة امتصاص عند الطول الموجي 565 و410 نانومتر على التوالي وضمن مداي 4،0-4,4-1.4 جزء بالمليون.