Chapter - 10

SIMULTANEOUS DERIVATIVE SPECTROPHOTOMETRIC METHODS
Section (i): An introduction to Simultaneous spectrophotometric determination of metal ions

A great number of spectrophotometric methods are reported in the literature for the determination of almost all metal ions in the periodic table. The two important characteristic features of a spectrophotometric method are sensitivity and specificity or selectivity. Sensitive methods are now a common feature for every metal ion.

A method is said to be selective if it is capable of determining only one metal ion in presence of many others. However, there exist few selective methods. On the other hand it is possible to make a method selective by controlling certain conditions such as oxidation state of the metal ion, pH and the temperature and by using suitable masking agents. Such methods are called selective methods.

The degree of selectivity of a method is dependent on the complexity of the material or the sample being analysed. In the world of ever increasing technological progress, the modern analytical chemists are confronted with the analysis of materials of more and more complex in nature. In many cases the simultaneous presence of other element is in face a potential cause of interference whenever quantitative determinations of two or more metal ions are carried out. In such cases a simultaneous determination of two or more metal ions enhances the selectivity of the method to greater extent. Therefore, in order to achieve greater degree of selectivity, the emphasis is being laid to develop direct spectrophotometric and derivative spectrophotometric procedures for the simultaneous determination of two or more metal ions when present in admixture. Simultaneous spectrophotometric analysis eliminates wavelength independent matrix system effect and permits the analysis of
two or more components in a system. It also saves the sample, time and thereby enhances the speed of analysis.

Form this point of view spectrophotometric methods for simultaneous determination of metal ions is of immense value and is receiving the attention of many chemists in recent times. Moreover, spectrophotometric and derivative spectrophotometric methods/procedures for the simultaneous determination of two metal ions are infrequently reported in the literature. Hence, the author has developed spectrophotometric methods for the simultaneous determination of two metal ions when present in admixture with each other in several biological samples. The results are presented in this chapter.

However, before this, a brief account of the theoretical aspects of the simultaneous procedures are presented here under.

**Principles of multicomponent spectrophotometric analysis**

If a system containing several absorbing components follows the principle of additivity of absorbances, i.e, the absorbance of the system is equal to the sum of the absorbances of all individual components at a particular wavelength, and if no natural interaction takes place between them, then

\[ A_j = \sum_{i=1}^{n} e_{ij} C_i \lambda_j \]

where \( e \) is the molar absorptivity of the component ‘i’ at the wavelength ‘\( J \)’ and ‘\( \lambda \)’ is the path length.
The additivity of absorbances is easily tested by calculation of absorbances for mixtures of standard solutions containing known analyte concentrations. The determination of a mixture comprising of several absorbing components in solution is carried out by measuring the absorbance at selected wavelengths and solving a set of simultaneous equations of the type

\[ A_j = \varepsilon_1 C_1 + \varepsilon_2 C_2 + \ldots + \varepsilon_n C_n \lambda_j \]

The accuracy and precision of the calculations is dependent on the number of components present and the selection of suitable wavelengths. At the wavelength selected, always one component absorbs strongly, whereas the other components absorb less intensely.

\[ \varepsilon_{ij} = \varepsilon_1 > \varepsilon_2 \varepsilon_3 \varepsilon_4 \]

The molar absorptivities of all the components at the selected wavelength are previously determined from solutions of the pure components under the same conditions as for the mixture.

The validity of Beer’s law for single component need not be maintained for their mixture. Even if the Beer’s law is not obeyed for one component of the mixture, the sum of the absorbances will still be correct for mixtures with a constant concentration of such a component.

The precision and accuracy of the calculated results depends on the number of unknown components in the mixture. The error of the resulting data considerably
increases if more than four absorbing species in unknown concentrations are to be determined.

The set of equations containing ‘l’ unknowns is solved by the familiar elimination procedures or with the aid of determinants but computer procedures are usually used if more than two absorbing components are to be determined.

**Two component analysis**

For a system containing two absorbing species 1 and 2, the absorbance of the systems at the selected wavelengths (\(\lambda_1\) and \(\lambda_2\)) i.e. \(A_{\lambda 1}\) and \(A_{\lambda 2}\) are given by the following equation.

\[
A_{\lambda 1} = \varepsilon_{1\lambda 1} C_1 + \varepsilon_{2\lambda 1} C_2 
\]

\[
A_{\lambda 2} = \varepsilon_{1\lambda 2} C_1 + \varepsilon_{2\lambda 2} C_2 
\]

Solving the equations (1) and (2) we get,

\[
C_1 = \frac{A_{\lambda 2} \varepsilon_{2\lambda 1} - A_{\lambda 1} \varepsilon_{2\lambda 1}}{\varepsilon_{1\lambda 1} \varepsilon_{2\lambda 2} - \varepsilon_{1\lambda 1} \varepsilon_{1\lambda 2}} \]

\[
C_2 = \frac{A_{\lambda 1} \varepsilon_{1\lambda 2} - A_{\lambda 2} \varepsilon_{1\lambda 2}}{\varepsilon_{1\lambda 1} \varepsilon_{2\lambda 2} - \varepsilon_{1\lambda 1} \varepsilon_{1\lambda 2}} 
\]

Where \(C_1\) and \(C_2\) are the concentrations of analytes 1 and 2, \(\varepsilon_{1\lambda 1}, \varepsilon_{\lambda 1}\) and \(\varepsilon_{1\lambda 2}, \varepsilon_{2\lambda 2}\) are the molar absorptivities of the components denoted by superscripts 1 and 2 at the wavelengths \(\lambda_1\) and \(\lambda_2\) respectively.
Section (ii): Review of derivative methods for the simultaneous spectrophotometric determination of metal ions

The direct spectrophotometric determination of metal ions in multicomponent systems are often complicated by interferences from the formulation matrix and spectral overlapping. Such interferences have been avoided in many ways, such as solving two simultaneous equations\(^1\) or using absorbance ratios at certain wavelengths\(^2,4\).

However, during the application of these methods\(^1,4\), the presence of spectral interferences or spectral overlap would certainly lead to erroneous results\(^5\). Other approaches aimed at solving this problem have been employed, including pH induced differential spectrophotometric\(^6\), least squares\(^7\) and orthogonal function\(^5,7,8\) methods.

Derivative spectrophotometry is a useful means of resolving two overlapping spectra and eliminating matrix interferences in the assay of two component mixtures using the zero-crossing technique\(^9,11\). In the absence of a zero-crossing point, two simultaneous equations can be solved to determine the components in such a mixture\(^12\). The latter method is based on criteria for selecting the optimum working wavelengths\(^2\). In addition, the component being determined should make a reasonable contribution to the total derivative reading of the mixture at the selective wavelengths.

Derivative spectrophotometric analysis of two-component mixtures is also carried out without the need to solve simultaneous equations. The compensation method\(^13\) is also used for the purpose. It is a non-mathematical method for detection and elimination of unwanted absorption during photometric analysis.
However, only few references are available in the literature on the determination of mixtures of metal ions by derivative spectrophotometry.

Jiang Chogqui\textsuperscript{14} et al., established first derivative spectrophotometric method for the simultaneous determination of cobalt and nickel. A second derivative spectrophotometric method was proposed by Jimenez\textsuperscript{16} et al., for the simultaneous determination of cobalt and vanadium in alloy steels.

Wang\textsuperscript{17} et al., reported a first derivative spectrophotometric method for the simultaneous determination of Fe(III) and Cu(II). A second derivative spectrophotometric method was developed for the nickel and vanadium determination in petroleum and petroleum residues by Wang\textsuperscript{18} et al using the second derivative spectrophotometry.

Bermejo-Barreta\textsuperscript{20} et al. described the application of derivative spectrophotometry to the simultaneous determination of Cu(II) and Co(II) without solving the simultaneous equations. Zhenzhuzi\textsuperscript{21} et al. determined chromium and manganese simultaneously by second derivative spectrophotometry. Cu(II) and Zn(II) were determined continuously by Li Huamin and Yan Junfeng\textsuperscript{22} using fourth derivative spectrophotometry.

Kuroda\textsuperscript{23} et al. reported the simultaneous determination of U(VI) and Th(IV) by first derivative spectrophotometric method. First derivative spectrophotometric method, has been developed for the simultaneous determination of U(VI) and Th(IV) by Ramesh\textsuperscript{24} et al. A simultaneous first derivative spectrophotometric determination
of Cu(II) and Co(II) was carried out by Vereda\textsuperscript{25} et al. Pd(II) and Co(II) were determined simultaneously by derivative spectrophotometry by Peruez\textsuperscript{26} et al.

Simultaneous determination of Ni(II), Zn(II) and Cu(II) by second derivative spectrophotometry was reported by Gallardo Maelgarejo\textsuperscript{27} et al. Perikov\textsuperscript{28} et al. reported simultaneous extraction spectrophotometric determination of iron, nickel, copper, zinc and cadmium. Recently, Fernandezalba\textsuperscript{29} et al presented simultaneous second derivative spectrophotometric determination of Mn and Cu.

Simultaneous spectrophotometric determination of zinc(II) and nickel(II) was reported by Blanco\textsuperscript{30} et al. Wang\textsuperscript{31} et al. reported simultaneous third derivative spectrophotometric determination of Cu(II) and Ni(II) in iron-alloys and aluminium alloys. Costa\textsuperscript{32} et al reported determination of nickel and iron in copper base alloy by simultaneous method. Sayed\textsuperscript{33} et al. reported simultaneous first derivative spectrophotometric determination of Fe(III) and Mo(VI) in Co-Cr and Ni-Cr alloys. Ines\textsuperscript{34} et al. determined Cu(II) and Fe(II) by second derivative simultaneous spectrophotometry using mixtures of ligands. Simultaneous determination of Cu and Fe was reported by Baraj\textsuperscript{35}. Recently, simultaneous first and second derivative spectrophotometric determination of Ni(II) and Cu(II) using diacetylmonoxime benzoylhydrazone (DMBH) was carried out in our laboratory.\textsuperscript{36,37} Ni(II) and Cu(II) were also determined by simultaneous second derivative spectrophotometry employing diacetylmonoxime isonicotinoylhydrazone (DMIH).

Simultaneous determination of Cd(II) and Hg(II) was reported by Elham\textsuperscript{38} et al. Simultaneous spectrophotometric determination of Cu(II) and Ni(II) was reported by Prasad\textsuperscript{39} et al. Non extractive trace level simultaneous determination of Hg(II) and
Zn(II) in environmental samples was reported by Agnihotri\textsuperscript{40} et al. Simultaneous first derivative determination of Cu(II) and Ni(II) was reported by Prasad\textsuperscript{41} et al. Simultaneous determination of Co(II) and Pd(II) in alloy samples was reported by Bagherian\textsuperscript{42}.

Simultaneous first derivative spectrophotometric determination of Ni(II) and Cu(II) in alloys was reported by Chandrasekhar\textsuperscript{43} et.al. Derivative spectrophotometric determination of Ni(II) and Cu(II) in alloys was proposed by Chandrasekhar\textsuperscript{44} et.al. Simultaneous second derivative spectrophotometric determination of Ni(II) and Cu(II) was reported by Chandrasekhar\textsuperscript{40} et. al. Simultaneous Second derivative spectrophotometric determination of Ni(II) and Cu(II) in alloys was also reported by chandrasekhar\textsuperscript{46} et. al. Using diacetylmonoxime benzoylhydrazone.
Section (iii) : Simultaneous second derivative spectrophotometric determination of cobalt (II) and copper (II) using 2-acetylpyridine semicarbazone (APS)

The second derivative spectra of cobalt(II) and copper(II) complexes with 2-acetylpyridine semicarbazone (APS) (Section (i) and Section (iii) of chapter 9) show maximum amplitude at 417 nm (peak) and at 460 nm (valley) for cobalt(II), and 440 nm (peak) for copper(II) respectively. The derivative amplitude increases proportionately with the concentration of the metal ions concerned at the wavelengths specified. Investigations were therefore carried out to explore the possibility of simultaneous second derivative spectrophotometric determination of cobalt(II) and copper(II).

Second derivative spectra of Co(II) and Cu(II) complex

The second derivative spectra of Co(II) and Cu(II) were recorded at pH 6.0 by employing the procedure given in 2.IV.a.

From the spectra in Fig 10.iii.a it is noticed that cobalt(II) shows maximum amplitudes at 417 nm and 460 nm and copper(II) at 447 nm. At 417 nm copper has zero amplitude (zero cross). Further at this wavelength cobalt(II) has maximum amplitude. cobalt has a zero cross at 447 nm while at this wavelength Cu(II) has appreciable amplitude and proportional to the concentration of the metal ion.

Hence, a simultaneous second derivative spectrophotometric determination of Co(II) and Cu(II) was carried out by measuring the peak amplitudes at 417 nm and 447 nm respectively. Typical spectra are shown in Fig 10.iii.a.
Fig.10.iii.a Second derivative spectra of a. Co(II) – APS system; b. Cu(II) – APS system

[Co(II)] = 2.4 x 10^{-3} M
[Cu(II)] = 4.8 x 10^{-3} M
[APS] = 5 x 10^{-4} M
pH = 5.0
Calibration plots

To determine the linear relationship between the derivative amplitude and concentration of Co(II) and Cu(II) ions at 417 nm and 447 nm respectively, individual calibration plots were constructed. The plots are shown in Figs 10.iii.b and 10.iii.c.

The plots are linear and obey the following relationships

\[ A_{447} = 0.0576C - 0.0001 \]

and

\[ A_{417} = 0.1221C + 0.0015 \]

for Co(II) and Cu(II) respectively. The amplitude is proportional to the metal ion concentration in the range of 0.23 – 1.88 µg/ml of Co(II) and 0.51 – 5.18 µg/ml of Cu(II) at 417 nm and 447 nm respectively.

The simultaneous second derivative spectrophotometric determination of Co(II) and Cu(II) in the synthetic mixtures was carried out by adopting the procedure given below

Procedure

To a known aliquot of the synthetic mixture of Co(II) and Cu(II) taken in a 25-ml volumetric flask, 10 ml buffer solutions (pH 6.0), and 1ml of APS (1x10^{-2} M) solution were added. The contents of the flask were made upto the mark with distilled water and the second derivative spectrum were recorded and presented in Figs 10.iii.b and 10.iii.c. The derivative amplitudes at 417 nm and 447 nm were measured and the
Fig. 10.iii.b. Second derivative Amplitude Vs amount of Cu(II) µg/ml
\begin{align*}
\text{pH} & = 6.0 \\
[\text{APS}] & = 6 \times 10^{-4} \text{M}
\end{align*}

Amplitude
\begin{align*}
A_{441} &= 0.0576C - 0.0001
\end{align*}

\[
\begin{array}{c}
\text{Amount of Cu(II) (µg/ml)} \\
0 & 1 & 2 & 3 & 4 & 5
\end{array}
\]

Fig. 10.iii.c. Second derivative Amplitude Vs amount of Co(II) µg/ml
\begin{align*}
\text{pH} & = 6.0 \\
[\text{APS}] & = 6 \times 10^{-4} \text{M}
\end{align*}

Amplitude
\begin{align*}
A_{441} &= 0.1221C + 0.0015
\end{align*}

\[
\begin{array}{c}
\text{Amount of Co(II) (µg/ml)} \\
0 & 0.2 & 0.4 & 0.6 & 0.8 & 1.0 & 1.2 & 1.4 & 1.6 & 1.8 & 2.0
\end{array}
\]
amount of Co(II) and Cu(II) were computed from the predetermined calibration plots. The results are presented in Table 10.iii.1.

The present method does not involve either solving of simultaneous equations or computation of the amplitude of a particular species. Further, it has been successfully employed for the accurate determination of cobalt(II) and copper(II).

Table 10.iii.1
Simultaneous second derivative determination of Co(II) and Cu(II) in synthesis mixtures*

<table>
<thead>
<tr>
<th>Amount in (µg/ml)</th>
<th>Error</th>
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<tbody>
<tr>
<td>1.414</td>
<td>0.508</td>
</tr>
<tr>
<td>1.414</td>
<td>1.16</td>
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<td>1.414</td>
<td>2.033</td>
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</tr>
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</tr>
<tr>
<td>1.414</td>
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<tr>
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<td>4.574</td>
</tr>
<tr>
<td>0.235</td>
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<tr>
<td>0.471</td>
<td>3.049</td>
</tr>
<tr>
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</tr>
<tr>
<td>0.942</td>
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</tr>
<tr>
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</tr>
<tr>
<td>1.414</td>
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<tr>
<td>1.650</td>
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</tr>
<tr>
<td>1.88</td>
<td>3.049</td>
</tr>
<tr>
<td>2.12</td>
<td>3.049</td>
</tr>
</tbody>
</table>

* Average of five determinations.
Section (iv) : Simultaneous second derivative spectrophotometric determination of mercury(II) and zinc(II) using 2-acetylpyridine thiosemicarbazone (APT)

The second derivative spectra of mercury(II) and zinc(II) complexes with 2-acetylpyridine thiosemicarbazone (APT) (Section (ix) and Section (viii) of chapter 9) show maximum amplitude at 410 nm (peak) and at 484 nm (valley) for mercury(II) and 466 nm (peak) for zinc(II) respectively. At zero crossing points derivative amplitude increases proportionately with the concentration of concerned metal ions. Investigations were therefore carried out to explore the possibility of simultaneous second derivative spectrophotometric determination of mercury(II) and zinc(II).

Second derivative spectra of Hg(II) and Zn(II) complex

The second derivative spectra of Hg(II) and Zn(II) were recorded at pH 6.0 by employing the procedure given in 2.IV.a.

From the spectra in Fig 10.iii.a it is noticed that mercury(II) shows maximum amplitudes at 410 nm and 484 nm and zinc(II) at 466 nm. At 430 nm zinc has zero amplitude (zero crossing). Further at this wavelength mercury(II) has an appreciable amplitude. Mercury has a zero cross at 462 nm while at this wavelength the amplitude of Zn(II) is appreciable and proportional to the concentration of the metal ion.

Hence, a simultaneous second derivative spectrophotometric determination of Hg(II) and Zn(II) was carried out by measuring the peak amplitudes at 430 nm and 462 nm respectively. Typical spectra are shown in Figs 10.iv.b and 10.iv.c.
Fig. 10.iv.a  Second derivative spectra of  a. Hg(II) - APT system;  b. Zn(II) - APT system

[Hg(II)] = 7.2 \times 10^{-5} M
[Zn(II)] = 9.6 \times 10^{-6} M
[APT] = 6 \times 10^{-4} M
pH = 6.0
Calibration plots

To determine the linear relationship between the derivative amplitude and concentration of Hg(II) and Zn(II) ions at 430 nm and 462 nm respectively, individual calibration plots were constructed by plotting the derivative amplitudes against the corresponding concentrations for Hg(II) and Zn(II). The plots thus obtained are shown in Figs 10.iv.b and 10.iv.c.

The plots are linear and obeys the relationships

\[ A_{462} = 0.496C - 0.0005 \]

and

\[ A_{430} = 0.2486C - 0.0146 \]

for Hg(II) and Zn(II) respectively. The amplitude is proportional to the metal ion concentration in the range of 0.240 – 2.407 μg/ml of Hg(II) and 0.105 – 1.046 μg/ml of Zn(II) at 430 nm and 462 nm respectively.

The simultaneous second derivative spectrophotometric determination of Hg(II) and Zn(II) in the synthetic mixtures was carried out by adopting the procedure given below
Fig. 10.iv.b. Second derivative Amplitude Vs amount of Zn(II) μg/ml
pH = 6.0
[APT] = 6 x 10^-4 M

Fig. 10.iv.c. Second derivative Amplitude Vs amount of Hg(II) μg/ml
pH = 6.0
[APT] = 6 x 10^-4 M
**Procedure**

To a known aliquot of the synthetic mixture of Hg(II) and Zn(II) taken in a 25-ml volumetric flask, 10 ml buffer solutions (pH 6.0), and 1ml of APT (1x10^{-2} M) solution were added. The contents of the flask were made upto the mark with distilled water and the second derivative spectra were recorded and presented in Fig 10.iii.a. The derivative amplitude at 417 nm and 462 nm were measured and the amount of Hg(II) and Zn(II) were computed from the predetermined calibration plots. The results are presented in Table 10.iv.1.

The present method does not involve either solving of simultaneous equations or computation of the amplitude of a particular species.
Table 10.4v.1
Simultaneous second derivative determination of Hg(II) and Zn(II) in synthesis mixture*

<table>
<thead>
<tr>
<th>Amount in (µg/ml)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Taken</strong></td>
<td><strong>Hg(II)</strong></td>
</tr>
<tr>
<td>Hg(II)</td>
<td>Zn(II)</td>
</tr>
<tr>
<td>1.444</td>
<td>0.104</td>
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<tr>
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<td>0.209</td>
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<tr>
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</tr>
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<tr>
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<tr>
<td>2.166</td>
<td>0.627</td>
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</tbody>
</table>

- Average of five determinations
Reference


