Chapter - 9

SECOND AND THIRD DERIVATIVE SPECTROPHOTOMETRIC METHODS
Section (i) : Second and third derivative spectrophotometric determination of cobalt(II) using 2-acetylpyridine thiosemicarbazone (APT)

Cobalt(II) reacts with 2-acetylpyridine thiosemicarbazone (APT) to form orange yellow coloured complex in the pH range 5.0 – 8.0. The complex shows maximum absorbance at 355 nm. However, the author has made an attempt to develop a new second and third derivative method for the determination of cobalt(II) in microgram levels. The results are presented in this section.

Second and third derivative spectrum of cobalt – APT complex

The second and third derivative spectrum of Co(II) – APT complex was recorded at pH 6.0 from which the analytical wavelengths was ascertained, the derivative amplitude was measured by the peak-valley method.

The second derivative spectrum (Fig. 9.i.a) exhibits a maximum amplitude at 445 nm (peak) and a valley at 417 nm and three zero crosses at 404, 430 and 460 nm. The third derivative spectrum (Fig. 9.i.b) shows a maximum amplitude at 470 nm(peak) and valley at 428 nm and four zero crosses at 405, 450, 494 and 525 nm. At 445 (second) and 470 nm (third) the amplitude is maximum and is proportional to the amount of cobalt(II). Therefore, 445 nm (peak) and 470 nm(peak) is chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of cobalt(II) – APT complex at 445 nm and 470 nm was studied by following procedure given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was
Fig. 9.i.a.  Second derivative spectra of Co(II) – APT Vs reagent blank
[Co(II)] = 2.4 x 10^{-5} M
[APT] = 6 x 10^{-4} M
pH = 6.0

Fig. 9.i.b.  Third derivative spectra of Co(II) – APT Vs reagent blank
[Co(II)] = 2.4 x 10^{-5} M
[APT] = 6 x 10^{-4} M
pH = 6.0
Fig. 9.i.c. Second derivative spectra of Co(II) – APT Vs reagent blank
Co(II), µg/ml; a. 0.4714; b. 0.9429; c. 1.4143; d. 1.8858
[APT] = 6 X 10^4 M
pH = 6.0

Fig. 9.i.d. Third derivative spectra of Co(II) – APT Vs reagent blank
Co(II), µg/ml; a. 0.4714; b. 0.9429; c. 1.4143; d. 1.8858
[APT] = 6 X 10^4 M
pH = 6.0
made between the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 5 – fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of cobalt(II) by second and third derivatives spectrophotometry**

**Applicability of Beer’s law (second derivative)**

To explore the applicability of Beer's law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Co(II) were recorded and presented in Fig. 9.i.e. The figure shows that the peak amplitude at 445 nm, the valley amplitude at 417 nm and the peak-valley (445 – 417 nm) are all proportional to the concentration of Co(II). Further the three zero crosses at 404, 430 and 460 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of cobalt(II) were prepared. The calibration plots (fig 9.i.e) are linear and bears the relation $A_{417} = 0.0371C - 0.0003$ and $A_{445} = 0.0519C - 0.0021$.  

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Fig. 9.1.e. Second derivative Amplitude Vs amount of Co(II) µg/ml

\[ [\text{APT}] = 6 \times 10^{-4} \text{M} \]
\[ \text{pH} = 6.0 \]

Fig. 9.1.f. Third derivative Amplitude Vs amount of Co(II) µg/ml

\[ [\text{APT}] = 6 \times 10^{-4} \text{M} \]
\[ \text{pH} = 6.0 \]
The above equation suggest the peak-valley method is more sensitive and hence is adopted for the determination of Co(II).

**Third derivative spectra**

To determine the applicability of Beer’s law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Co(II) were recorded and presented in Fig. 9.i.f. The figure shows peak amplitude at 470 nm. The valley amplitude at 428 nm and the peak-valley amplitude (428 – 470 nm) are all proportional to the concentration of Co(II). Further, the four zero crosses at 405, 450, 494 and 525 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of cobalt(II) were prepared. The calibration plots (Fig. 9.i.f) are linear and bears the relation $A_{428} = 0.0749C + 0.0039$ and $A_{470} = 0.0485C - 0.0021$.

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Co(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions do not interfere in the zero order determination of Co(II) with APT also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limit is generally higher than those in the zero order determination of Co(II) with APT (Table 7.i.2). Ba(II) 27, Al(III) 25, Cu(II) 1.3, Zn(II) 0.78, Ni(II) 0.7, Fe(II) 0.6, Cr(VI) 12.4, Mn(II) 13 and Cd(II) 12 (all in µg/ml) do not interfere in second and third derivative spectrophotometric determination of Co(II).
Section (ii) : Second and third derivative spectrophotometric determination of cobalt(II) using 2-acetylpyridine semicarbazone (APS)

Cobalt(II) gives a orange yellow coloured complex with 2-acetylpyridine semicarbazone (APS) in acetic acid buffer solution (pH 5.0 – 7.0). The colour is stable for 5 hours. The zero order spectrum shows $\lambda_{\text{max}}$ at 360 nm and the second and third derivative spectrum shows $\lambda_{\text{max}}$ at 415 nm and 428 nm respectively. However, the author has made an attempt to develop a new second and third derivative method for the determination of cobalt(II) with APS in microgram levels. The results are presented in this section.

Second and third derivative spectrum of Co(II) with APS complex

The second and third derivative spectrum of Co(II) – APS complex was recorded at pH 6.0 from which the analytical wavelength was ascertained. The derivative amplitude was measured by the peak zero method.

The second derivative spectrum (Fig. 9.ii.a) exhibits a maximum amplitude at 415 nm (peak) and a valley at 457 nm and three zero crosses at 395, 446 and 470 nm. The third derivative spectrum (Fig. 9.ii.b) shows a maximum amplitude at 428 nm (peak) and valley at 408 nm and four zero crosses at 400, 416, 447 and 467 nm. But at 415 nm (second) and 428 nm (third), the amplitude is maximum and is proportional to the amount of cobalt(II). Therefore, 415 nm (peak) and 428 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of cobalt(II) – APS complex at 415 nm and 428 nm was studied by following procedure
Fig. 9.ii.a. Second derivative spectra of Co(II) – APS Vs reagent blank

[Co(II)] = 2.4 \times 10^{-5} \text{M}

[APS] = 4 \times 10^{-4} \text{M}

pH = 6.0

Fig. 9.ii.b. Third derivative spectra of Co(II) – APS Vs reagent blank

[Co(II)] = 2.4 \times 10^{-5} \text{M}

[APS] = 4 \times 10^{-4} \text{M}

pH = 6.0
Fig. 9.ii.c. Second derivative spectra of Co(II) - APS vs reagent blank
Co(II), μg/ml; a. 0.4714; b. 0.9429; c. 1.4143; d. 1.8858
[APS] = 4 X 10^{-4}M
pH = 6.0

Fig. 9.ii.d. Third derivative spectra of Co(II) - APS vs reagent blank
Co(II), μg/ml; a. 0.4714; b. 0.9429; c. 1.4143; d. 1.8858
[APS] = 4 X 10^{-4}M
pH = 6.0
given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 5 - fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of cobalt(II) by second and third derivatives spectrophotometry**

**Applicability of Beer's law (second derivative)**

To explore the applicability of Beer's law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Co(II) were recorded and presented in Fig. 9.ii.e. The figure shows that the peak amplitude at 415 nm, the valley amplitude at 457 nm and the peak-valley (415 – 457 nm) are all proportional to the concentration of Co(II). Further the three zero crosses at 395, 446 and 470 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of cobalt(II) were prepared. The calibration plots (fig 9.i.e) are linear and bears the relation $A_{415} = 0.2470C - 0.0486$ and $A_{457} = 0.0637C - 0.0116$. 
Second derivative Amplitude vs amount of Co(II) µg/ml

\[ [\text{APS}] = 4 \times 10^{-4} \text{M} \]

\[ \text{pH} = 6.0 \]

Third derivative Amplitude vs amount of Co(II) µg/ml

\[ [\text{APS}] = 4 \times 10^{-4} \text{M} \]

\[ \text{pH} = 6.0 \]
The above equation suggest the peak-valley method is more sensitive and hence is adopted for the determination of Co(II).

**Third derivative spectra**

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Co(II) were recorded and presented in Fig. 9.ii.f. The figure shows that the peak amplitude at 428 nm. The valley amplitude at 408 nm and the peak-valley amplitude (428 – 408 nm) are all proportional to the concentration of Co(II). Further, the four zero crosses at 400, 416, 447 and 467 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of cobalt(II) were prepared. The calibration plots (Fig. 9.ii.f) are linear and bears the relation $A_{408} = 0.1538C - 0.0002$ and $A_{428} = 0.3854C + 0.0157$.

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Co(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions that do not interfere in the zero order determination of Co(II) with APS also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limits are generally higher than those in the zero order determination of Co(II) with APS (Table 9.ii.2). W(VI) 44, Ba(II) 32, Al(III) 25 and Cd(II) 26, Sn(II) 47 Mo(IV) 23, Hg(II) 48, Cr(VI) 12, Cu(II) 1.3, Ni(II) 0.7, and V(V) 2.5 (all in µg/ml) do not interfere in second and third derivative spectrophotometric determination of Co(II).
Section (iii) : Second and third derivative spectrophotometric determination of nickel(II) using 2-acetylpyridine thiosemicarbazone (APT)

Nickel(II) reacts with 2-acetylpyridine thiosemicarbazone (APT) to form greenish yellow coloured complex in the pH range 5.0 – 7.0. The complex shows maximum absorbance at 375 nm. The author has made an attempt to develop new second and third derivative methods for the determination of nickel(II) in microgram levels. The results are presented in this section.

Second and third derivative spectrum of nickel – APT complex

The second and third derivative spectrum of Ni(II) – APT complex was recorded at pH 6.0 from which the analytical wavelengths was ascertained, the derivative amplitude was measured by the peak-valley method.

The second derivative spectrum (Fig. 9.iii.a) exhibits a maximum amplitude at 434 nm (peak) and a valley at 498 nm and three zero crosses at 395, 475 and 520 nm. The third derivative spectrum (Fig. 9.iii.b) shows a maximum amplitude at 439 nm (peak) and valley at 413 nm and four zero crosses at 400, 424, 465 and 485 nm. At 434 (second) and 439 nm (third) the amplitude is maximum and is proportional to the amount of nickel(II). Therefore, 434 nm (peak) and 439 nm (peak) were chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of nickel(II) – APT complex at 434 nm and 439 nm was studied by following procedure given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was
**Fig. 9.iii.a.** Second derivative spectra of Ni(II) - APT Vs reagent blank

- \([\text{Ni(II)}] = 4.8 \times 10^{-5}\text{M}\)
- \([\text{APT}] = 4 \times 10^{-4}\text{M}\)
- \(\text{pH} = 6.0\)

**Fig. 9.iii.b.** Third derivative spectra of Ni(II) - APT Vs reagent blank

- \([\text{Ni(II)}] = 4.8 \times 10^{-5}\text{M}\)
- \([\text{APT}] = 4 \times 10^{-4}\text{M}\)
- \(\text{pH} = 6.0\)
Fig. 9.iii.c. Second derivative spectra of Ni(II) – APT Vs reagent blank
Ni(II), µg/ml; a. 0.9381; b. 1.8762; c. 2.8142; d. 3.7523
[APT] = 4 X 10^{-4} M  
\[pH = 6.0\]

Fig. 9.iii.d. Third derivative spectra of Ni(II) – APT Vs reagent blank
Ni(II), µg/ml; a. 0.9381; b. 1.8762; c. 2.8142; d. 3.7523
[APT] = 4 X 10^{-4} M  
\[pH = 6.0\]
made between the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 - fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of nickel(II) by second and third derivative spectrophotometry**

**Applicability of Beer's law (second derivative)**

To explore the applicability of Beer's law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Ni(II) were recorded and presented in Fig. 9.iii.e. The figure shows that the peak amplitude at 434 nm, the valley amplitude at 498 nm and the peak-valley (434 – 498 nm) amplitudes are proportional to the concentration of Ni(II). Further the three zero crosses at 395, 475 and 520 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of nickel(II) were prepared. The calibration plots (fig 9.iii.e) are linear and bears the relation $A_{434} = 0.2135C - 0.0088$ and $A_{498} = 0.1063C + 0.0054$. 

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Fig. 9.iii.e. Second derivative Amplitude $\nu_s$ amount of Ni(II) $\mu$g/ml

\[ [\text{APT}] = 4 \times 10^{-4} \text{M} \]

\[ \text{pH} = 6.0 \]

Fig. 9.iii.f. Third derivative Amplitude $\nu_s$ amount of Ni(II) $\mu$g/ml

\[ [\text{APT}] = 4 \times 10^{-4} \text{M} \]

\[ \text{pH} = 6.0 \]
The above equation suggest the peak-valley method is more sensitive and hence is adopted for the determination of Ni(II).

**Third derivative spectra**

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Ni(II) were recorded and presented in Fig. 9.iii.f. The figure shows that the peak amplitude at 439 nm. The valley amplitude at 413nm and the peak-valley amplitude (439 – 413nm) are all proportional to the concentration of Ni(II). Further, the four zero crosses at 400, 424, 465 and 485 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of nickel(II) were prepared. The calibration plots (Fig. 9.iii.f) are linear and bears the relation $A_{413} = 0.5192C + 0.0225$ and $A_{439} = 0.2844C + 0.0049$.

The above equations suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Ni(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ±2 percent was taken as the tolerance limit. It is noticed that all the ions that do not interfere in the zero order determination of Ni(II) with APT also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limit are generally higher than those in the zero order determination of Ni(II) with APT (Table 5.i.2). Zr(IV) 110, Ba(II) 54, W(VI) 44, Pb(II) 50, Sn(II) 50, Hg(II) 48 Ag(I) 25, Mo(VI) 23, Sr(II) 21, Cr(VI) 12, Ca(II) 10, Cu(II) 0.76, Zn(II) 0.78, Co(II) 0.6, Fe(II) 0.6 and Pd(II) 10 (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Ni(II).
Section (iv) : Second and third derivative spectrophotometric determination of nickel(II) using 2-acetylpyridine semicarbazone (APS)

Nickel(II) gives a pale yellow coloured complex with 2-acetylpyridine semicarbazone (APS) in acetic acid buffer solution (pH 8.0 – 10.0). The colour is stable for 12 hours. The zero order spectrum shows $\lambda_{\text{max}}$ at 350 nm and the second and third derivative spectrum shows $\lambda_{\text{max}}$ at 403 nm and 415 nm. However, the author has made an attempt to develop a new second and third derivative method for the determination of nickel(II) with APS in microgram levels. The results are presented in this section.

Second and third derivative spectrum of Ni(II) with APS complex

The second and third derivative spectrum of Ni(II) – APS complex was recorded at pH 9.0 from which the analytical wavelength was ascertained. The derivative amplitude was measured by the peak zero method.

The second derivative spectrum (Fig. 9.iv.a) exhibits a maximum amplitude at 403 nm (peak) and a valley at 440 nm and three zero crosses at 380, 424 and 460 nm. The third derivative spectrum (Fig. 9.iv.b) shows a maximum amplitude at 415 nm (peak) and valley at 460 nm and four zero crosses at 400, 435, 490 and 510 nm. But at 403 nm (second) and 415 nm (third), the amplitude is maximum and is proportional to the amount of nickel(II). Therefore, 403 nm (peak) and 415 nm (peak) is chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of nickel(II) – APS complex at 403 nm and 415 nm was studied by following procedure
Fig. 9.iv.a. Second derivative spectra of Ni(II) - APS vs reagent blank

[Ni(II)] = 9.6 x 10^{-6} M
[APS] = 6 x 10^{-7} M
pH = 9.0

Fig. 9.iv.b. Third derivative spectra of Ni(II) - APS vs reagent blank

[Ni(II)] = 9.6 x 10^{-6} M
[APS] = 6 x 10^{-7} M
pH = 9.0
Fig. 9.iv.c. Second derivative spectra of Ni(II) - APS vs reagent blank
Ni(II), µg/ml; a. 0.1876; b. 0.3752; c. 0.5638; d. 0.7505
[APS] = 6 \times 10^{-4} \text{M}
pH = 9.0

Fig. 9.iv.d. Third derivative spectra of Ni(II) - APS vs reagent blank
Ni(II), µg/ml; a. 0.1876; b. 0.3752; c. 0.5638; d. 0.7505
[APS] = 6 \times 10^{-4} \text{M}
pH = 9.0
given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between the second and third derivative amplitude and the pH, from which working pH 9.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 – fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of nickel(II) by second and third derivative spectrophotometry**

**Applicability of Beer’s law (second derivative)**

To explore the applicability of Beer’s law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Ni(II) were recorded and presented in Fig. 9.iv.e. The figure shows that the peak amplitude at 403 nm, the valley amplitude at 440 nm and the peak-valley (403 – 440nm) are all proportional to the concentration of Ni(II). Further the three zero crosses at 380, 425 and 460 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of nickel(II) were prepared. The calibration plots (fig 9.iii.e) are linear and bears the relation \( A_{403} = 0.2437C - 0.0026 \) and \( A_{440} = 0.1579C + 0.0016 \).
Fig. 9.iv.e. Second derivative Amplitude vs amount of Ni(II) μg/ml
[APS] = $6 \times 10^{-4}$ M
pH = 9.0

Fig. 9.iv.f. Third derivative Amplitude vs amount of Ni(II) μg/ml
[APS] = $6 \times 10^{-4}$ M
pH = 9.0
The above equation suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Ni(II).

**Third derivative spectra**

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Ni(II) were recorded and presented in Fig. 9.iv.f. The figure shows that the peak amplitude at 415 nm. The valley amplitude at 460nm and the peak-valley amplitude (415 – 460nm) are all proportional to the concentration of Ni(II). Further, the four zero crosses at 400, 435, 490 and 510 nm re independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of nickel(II) were prepared. The calibration plots (Fig. 9.ii.f) are linear and bears the relation \( A_{415} = 1.0753C + 0.0093 \) and \( A_{460} = 0.7190C - 0.0049 \).

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Ni(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions do not interfere in the zero order determination of Ni(II) with APS also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limits are generally higher than those in the zero order determination of Ni(II) with APS (Table 5.ii.1). Co(II) 1.4, Cu(II) 2.5, Mg(II) 15, Cd(II) 27, and Zn(II) 1.5. (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Ni(II).
Section (v) : Second and third derivative spectrophotometric determination of copper(II) using 2-acetylpyridine thiosemicarbazone (APT)

Copper(II) reacts with 2-acetylpyridine thiosemicarbazone (APT) to form pale greenish yellow coloured complex in the pH range 5.0 – 7.0. The complex shows maximum absorbance at 376 nm. Hence, the author has made an attempt to develop a new second and third derivative method for the determination of copper(II) in microgram levels. The results are presented in this section.

Second and third derivative spectrum of copper – APT complex

The second and third derivative spectrum of Cu(II) – APT complex was recorded at pH 5.5 from which the analytical wavelengths were ascertained, the derivative amplitude was measured by the peak-valley method.

The second derivative spectrum (fig. 9.v.a) exhibits a maximum amplitude at 426 nm (peak) and a valley at 438 nm and three zero crosses at 420, 435 and 445 nm. The third derivative spectrum (fig. 9.v.b) shows a maximum amplitude at 430 nm (peak) and valley at 410 nm and four zero crosses at 400, 418, 452 and 478 nm. At 426 nm (second) and 430 nm (third) the amplitude is maximum and is proportional to the amount of copper(II). Therefore, 426 nm (peak) and 430 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of copper(II) – APT complex at 426 nm and 430 nm by following procedure given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between
Fig. 9.v.a. Second derivative spectra of Cu(II) – APT Vs reagent blank

\[
\begin{align*}
[Cu(II)] & = 2.4 \times 10^{-5} M \\
[APT] & = 6 \times 10^{-4} M \\
pH & = 5.5
\end{align*}
\]

Fig. 9.v.b. Third derivative spectra of Cu(II) – APT Vs reagent blank

\[
\begin{align*}
[Cu(II)] & = 2.4 \times 10^{-5} M \\
[APT] & = 6 \times 10^{-4} M \\
pH & = 5.5
\end{align*}
\]
Fig. 9.v.c. Second derivative spectra of Cu(II) – APT Vs reagent blank
Cu(II), µg/ml: a. 0.5083; b. 1.0166; c. 1.5249; d. 2.0332
[APT] = 6 × 10^{-4}M
pH = 5.5

Fig. 9.v.d. Third derivative spectra of Cu(II) – APT Vs reagent blank
Cu(II), µg/ml: a. 0.5083; b. 1.0166; c. 1.5249; d. 2.0332
[APT] = 6 × 10^{-4}M
pH = 5.5
the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 - fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of copper(II) by second and third derivative spectrophotometry**

**Applicability of Beer’s law (second derivative)**

To explore the applicability of Beer’s law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Cu(II) were recorded and presented in fig. 9.v.e. The figure shows that the peak amplitude at 426 nm, the valley amplitude at 438 nm and the peak-valley (426 – 438nm) are all proportional to the concentration of Cu(II). Further the three zero crosses at 420, 435 and 445 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of copper(II) were prepared. The calibration plots (fig 9.v.e) are linear and bears the relation $A_{426} = 0.1762C - 0.0035$ and $A_{438} = 0.04C + 0.0001$.

The above equation suggest the peak-valley method is more sensitive and hence is adopted for the determination of Cu(II).
**Fig. 9.v.e.** Second derivative Amplitude Vs amount of Cu(II) µg/ml
\[
\text{[APT]} = 6 \times 10^{-4} M \\
P\text{H} = 5.5
\]

\begin{align*}
\text{a: } A_{40} &= 0.1762C - 0.0035 \\
\text{b: } A_{40} &= 0.04C + 0.0001
\end{align*}

**Fig. 9.v.f.** Third derivative Amplitude Vs amount of Cu(II) µg/ml
\[
\text{[APT]} = 6 \times 10^{-4} M \\
P\text{H} = 5.5
\]

\begin{align*}
\text{a: } A_{430} &= 0.2046C + 0.004 \\
\text{b: } A_{410} &= 0.4076C - 0.004
\end{align*}
Third derivative spectra

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Cu(II) were recorded and presented in fig. 9.v.f. The figure shows that the peak amplitude at 430 nm. The valley amplitude at 410 nm and the peak-valley amplitude (430–410nm) are all proportional to the concentration of Cu(II). Further, the four zero crosses at 400, 419, 452 and 475 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of copper(II) were prepared. The calibration plots (fig. 9.v.f) are linear and bears the relation $A_{410} = 0.4106C - 0.004$ and $A_{430} = 0.2046C + 0.004$.

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Cu(II).

Effect of foreign ions on the derivative amplitude of the complex

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions do not interfere in the zero order determination of Cu(II) with APT also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limits are generally higher than those in the zero order determination of Cu(II) with APT (Table 6.i.1). Ni(II) 0.7, Co(II) 0.12, Zn(II) 0.8, Fe(II) 0.67, Zr(IV) 110, Mo(VI) 116, Sr(II) 21, Ca(II) 10, Ba(II) 5.4, and Pb(II) 2.7 (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Cu(II).
Section (vi) : Second and third derivative spectrophotometric determination of copper(II) using 2-acetylpypyridine semicarbazone (APS)

Copper(II) gives a yellow coloured complex with 2-acetylpypyridine semicarbazone (APS) in acetic acid buffer solution (pH 5.0 – 7.0). The colour is stable for 75 minutes. The zero order spectrum shows $\lambda_{\text{max}}$ at 355 nm and the second and third derivative spectrum shows $\lambda_{\text{max}}$ at 440 nm and 439 nm respectively. However, the author has made an attempt to develop a new second and third derivative method for the determination of copper(II) with APS in microgram levels. The results are presented in this section.

Second and third derivative spectrum of Cu(II) with APS complex

The second and third derivative spectrum of Cu(II) – APS complex was recorded at pH 6.0 from which the analytical wavelength was ascertained. The derivative amplitude was measured by the peak zero method.

The second derivative spectrum (fig. 9.vi.a) exhibits a maximum amplitude at 440 nm (peak) and a valley at 415 nm and four zero crosses at 405, 424, 460 and 480 nm. The third derivative spectrum (fig. 9.vi.b) shows a maximum amplitude at 438 nm (peak) and valley at 410 nm and four zero crosses at 395, 425, 465 and 480 nm. But at 440 nm (second) and 439 nm (third), the amplitude is maximum and is proportional to the amount of copper(II). Therefore, 440 nm (peak) and 439 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of copper(II) – APS complex at 440 nm and 439 nm was studied by following procedure
Fig. 9.vi.a. Second derivative spectra of Cu(II) – APS Vs reagent blank
[Cu(II)] = 4.8 x 10^{-5} M
[APS] = 6 x 10^{-4} M
pH = 6.0

Fig. 9.vi.b. Third derivative spectra of Cu(II) – APS Vs reagent blank
[Cu(II)] = 4.8 x 10^{-5} M
[APS] = 6 x 10^{-4} M
pH = 6.0
Fig. 9.vi.c. Second derivative spectra of Cu(II) – APS Vs reagent blank
Cu(II), μg/ml; a. 1.0166; b. 2.0332; c. 3.0499; d. 4.0665
[APS] = 6 \times 10^{-4} M
pH = 6.0

Fig. 9.vi.d. Third derivative spectra of Cu(II) – APS Vs reagent blank
Cu(II), μg/ml; a. 1.0166; b. 2.0332; c. 3.0499; d. 4.0665
[APS] = 6 \times 10^{-4} M
pH = 6.0
given in 2.iv.b. The second and third derivative spectra were recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between the second and third derivative amplitude and the pH, from which working pH 9.0 was selected.

Effect of the reagent concentration on the derivative amplitude

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 – fold molar excess of the reagent is enough to achieve maximum amplitude.

Determination of copper(II) by second and third derivatives spectrophotometry

Applicability of Beer’s law (second derivative)

To explore the applicability of Beer’s law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Cu(II) were recorded and presented in fig. 9.vi.e. The figure shows that the peak amplitude at 440 nm, the valley amplitude at 415 nm and the peak-valley (440 – 415 nm) are all proportional to the concentration of Cu(II). Further the four zero crosses at 405, 425, 460 and 480 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of copper(II) were prepared. The calibration plots (fig 9.v.e) are linear and bears the relation $A_{415} = 0.01C + 0.0001$ and $A_{440} = 0.1009C + 0.0015$. 

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Fig. 9.vi.e. Second derivative Amplitude Vs amount of Cu(II) µg/ml

\[ \text{[APS]} = 6 \times 10^{-4} \text{M} \]
\[ \text{pH} = 6.0 \]

\[ a : A_{440} = 0.1009C + 0.0015 \]
\[ b : A_{415} = 0.01C + 0.0001 \]

Fig. 9.vi.f. Third derivative Amplitude Vs amount of Cu(II) µg/ml

\[ \text{[APS]} = 6 \times 10^{-4} \text{M} \]
\[ \text{pH} = 6.0 \]

\[ a : A_{439} = 0.12C - 0.005 \]
\[ b : A_{410} = 0.2309C + 0.0015 \]
The above equation suggest the peak-valley method is more sensitive and hence is adopted for the determination of Cu(II).

Third derivative spectra

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Cu(II) were recorded and presented in fig. 9.vi.f. The figure shows that the peak amplitude at 439 nm. The valley amplitude at 410 nm and the peak-valley amplitude (439 – 410 nm) are all proportional to the concentration of Cu(II). Further, the four zero crosses at 395, 425, 465 and 480 nm re independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of copper(II) were prepared. The calibration plots (fig. 9.ii.f) are linear and bears the relation $A_{410} = 0.2309C + 0.0015$ and $A_{439} = 0.12C - 0.005$.

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Cu(II).

Effect of foreign ions on the derivative amplitude of the complex

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions that do not interfere in the zero order determination of Cu(II) with APS also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limits are generally higher than those in the zero order determination of Cu(II) with APS (Table 6.ii.3). Zn(II) 1.5, Ni(II) 1.4, Co(II) 1.4, W(VI) 4.4, Sr(II) 21, Mo(VI) 2.3, Cd(II) 2.6, Fe(II) 1.4, Sn(II) 2.8 and Ag(I) 4.7 (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Cu(II).
Section (vii) : Second and third derivative spectrophotometric determination of zinc(II) using 2-acetylpyridine thiosemicarbazone (APT)

Zinc(II) reacts with 2-acetylpyridine thiosemicarbazone (APT) to form greenish yellow coloured complex in the pH range 5.0 – 10.0. The complex shows maximum absorbance at 360 nm. Hence, the author has made an attempt to develop a new second and third derivative method for the determination microgram quantities of zinc(II). The results are presented in this section.

Second and third derivative spectrum of zinc – APT complex

The second and third derivative spectrum of Zn(II) – APT complex was recorded at pH 6.0 from which the analytical wavelengths were ascertained, the derivative amplitude was measured by the peak-valley method.

The second derivative spectrum (Fig. 9.vii.a) exhibits a maximum amplitude at 465 nm (peak) and a valley at 522 nm and three zero crosses at 430, 500 and 540 nm. The third derivative spectrum (Fig. 9.vii.b) shows a maximum amplitude at 428 nm (peak) and valley at 477 nm and four zero crosses at 404, 460, 492 and 524 nm. At 465 nm (second) and 428 nm (third) the amplitude is maximum and is proportional to the amount of zinc(II). Therefore, 465 nm (peak) and 428 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of zinc(II) – APT complex at 465 nm was studied and 428 nm by following procedure given in 2.iv.b. The second and third derivative spectra were recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between
Fig. 9.vii.a. Second derivative spectra of Zn(II) – APT Vs reagent blank

\[
\begin{align*}
[Zn(II)] & = 9.6 \times 10^{-6} \text{M} \\
[APT] & = 6 \times 10^{-4} \text{M} \\
pH & = 6.0
\end{align*}
\]

Fig. 9.vii.b. Third derivative spectra of Zn(II) – APT Vs reagent blank

\[
\begin{align*}
[Zn(II)] & = 9.6 \times 10^{-6} \text{M} \\
[APT] & = 6 \times 10^{-4} \text{M} \\
pH & = 6.0
\end{align*}
\]
Fig. 9.vii.c. Second derivative spectra of Zn(II) – APT Vs reagent blank
Zn(II), µg/ml; a. 0.2092; b. 0.4185; c. 0.6277; d. 0.8370
[APT] = 6 X 10^4 M
pH = 6.0

Fig. 9.vii.d. Third derivative spectra of Zn(II) – APT Vs reagent blank
Zn(II), µg/ml; a. 0.2092; b. 0.4185; c. 0.6277; d. 0.8370
[APT] = 6 X 10^4 M
pH = 6.0
the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

**Effect of the reagent concentration on the derivative amplitude**

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 - fold molar excess of the reagent is enough to achieve maximum amplitude.

**Determination of zinc(II) by second and third derivative spectrophotometry**

**Applicability of Beer's law (second derivative)**

To explore the applicability of Beer's law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Zn(II) were recorded and presented in Fig. 9.vii.e. The figure shows that the peak amplitude at 465 nm, the valley amplitude at 522 nm and the peak-valley (465 – 522 nm) are all proportional to the concentration of Zn(II). Further the three zero crosses at 430, 500 and 540 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of zinc(II) were prepared. The calibration plots (fig 9.vii.e) are linear and bears the relation $A_{465} = 0.76C - 0.002$ and $A_{522} = 0.523C + 0.006$. 

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Fig. 9.vii.e. Second derivative Amplitude Vs amount of Zn(II) μg/ml

\[ [APT] = 6 \times 10^{-4} M \]
\[ \text{pH} = 6.0 \]

Fig. 9.vii.f. Third derivative Amplitude Vs amount of Zn(II) μg/ml

\[ [APT] = 6 \times 10^{-4} M \]
\[ \text{pH} = 6.0 \]
The above equation suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Zn(II).

**Third derivative spectra**

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Zn(II) were recorded and presented in Fig. 9.vii.f. The figure shows that the peak amplitude at 428 nm. The valley amplitude at 477 nm and the peak-valley amplitude (428 - 477 nm) are all proportional to the concentration of Zn(II). Further, the four zero crosses at 404, 460, 492 and 520 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of zinc(II) were prepared. The calibration plots (Fig. 9.vii.f) are linear and bears the relation $A_{428} = 0.404C - 0.0005$ and $A_{477} = 0.1365C + 0.0015$.

The above equations suggest the peak-valley method is more sensitive and hence is adopted for the determination of Zn(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions do not interfere in the zero order determination of Zn(II) with APT also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limit is generally higher than those in the zero order determination of Zn(II) with APT (Table 8.i.3). Co(II) 0.2, Fe(II) 0.6, Pb(II) 41, Sn(II) 47, Ni(II) 1.4, Cd(II) 26, Mo(VI) 19, Mn(II) 11, Zr(IV) 3.8 and Hg(II) 2.8 (all in µg/ml) do not interfere in second and third derivative spectrophotometric determination of Zn(II).
Section (viii) : Second and third derivative spectrophotometric determination of zinc(II) using 2-acetylpyridine semicarbazone (APS)

Zinc(II) gives a greenish yellow coloured complex with 2-acetylpyridine semicarbazone (APS) in acetic acid buffer solution (pH 5.0 – 10.0). The colour is stable for 12 hours. The zero order spectrum shows $\lambda_{\text{max}}$ at 355 nm and the second and third derivative spectrum shows $\lambda_{\text{max}}$ at 434 nm and 438 nm. Hence, the author has made an attempt to develop a new second and third derivative method for the determination of microgram quantities of zinc(II) with APS. The results are presented in this section.

Second and third derivative spectrum of Zn(II) with APS complex

The second and third derivative spectrum of Zn(II) – APS complex was recorded at pH 6.0 from which the analytical wavelength was ascertained. The derivative amplitude was measured by the peak zero method.

The second derivative spectrum (Fig. 9.viii.a) exhibits a maximum amplitude at 434 nm (peak) and two valleys at 414 and 474 nm, along with four zero crosses at 404, 425, 460 and 490 nm respectively. The third derivative spectrum (Fig. 9.viii.b) shows a maximum amplitude at 438 nm (peak) and two valleys at 412 and 472 nm and four zero crosses at 400, 425, 458 and 490 nm. But at 434 nm (second) and 438 nm (third), the amplitude is maximum and is proportional to the amount of zinc(II). Therefore, 428 nm (peak) and 440 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of zinc(II) – APS complex at 434 nm and 438 nm was studied by following procedure given in
Amplitude vs Amplitude

9.viii.a. Second derivative spectra of Zn(II) - APS vs reagent blank

[Zn(II)] = 4.8 x 10^{-5} M
[APS] = 6 x 10^{-4} M
pH = 6.0

![Second derivative spectra of Zn(II) - APS vs reagent blank](image)

9.viii.b. Third derivative spectra of Zn(II) - APS vs reagent blank

[Zn(II)] = 4.8 x 10^{-5} M
[APS] = 6 x 10^{-4} M
pH = 6.0

![Third derivative spectra of Zn(II) - APS vs reagent blank](image)
**Fig. 9.viii.c.** Second derivative spectra of Zn(II) - APS Vs reagent blank

Zn(II), μg/ml; a. 1.0462; b. 2.0925; c. 3.1387; d. 4.1850

[APS] = 6 x 10⁻⁴M

pH = 6.0

**Fig. 9.viii.d.** Third derivative spectra of Zn(II) - APS Vs reagent blank

Zn(II), μg/ml; a. 1.0462; b. 2.0925; c. 3.1387; d. 4.1850

[APS] = 6 x 10⁻⁴M

pH = 6.0
2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak - zero method. A plot was made between the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

Effect of the reagent concentration on the derivative amplitude

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectrum of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 – fold molar excess of the reagent is enough to achieve maximum amplitude.

Determination of zinc(II) by second and third derivative spectrophotometry

Applicability of Beer’s law (second derivative)

To explore the applicability of Beer’s law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Zn(II) were recorded and presented in Fig. 9.vii.e. The figure shows that the peak amplitude at 434 nm, two valley amplitude at 414nm and 474 nm and the peak-valley (434 – 414nm) are all proportional to the concentration of Zn(II). Further the four zero crosses at 404, 425, 460 and 490 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of zinc(II) were prepared. The calibration plots (fig 9.vii.e) are linear and bears the relation $A_{434} = 0.0116C - 0.0005$ and $A_{414} = 0.005C - 0.0007$. 
Fig. 9.viii.e. Second derivative Amplitude Vs amount of Zn(II) μg/ml
[APS] = 6 X 10^{-4}M
pH = 6.0

Fig. 9.viii.f. Third derivative Amplitude Vs amount of Zn(II) μg/ml
[APS] = 6 X 10^{-4}M
pH = 6.0
The above equation suggest the peak-valley method is more sensitive and hence used in the determination of Zn(II).

**Third derivative spectra**

To determine the applicability of Beer's law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentrations of Zn(II) were recorded and presented in Fig. 9.viii.f. The figure shows that the peak amplitude at 438 nm. Two valley amplitude at 412 and 472 nm and the peak-valley amplitude (438 - 412nm) are all proportional to the concentration of Zn(II). Further, the four zero crosses at 400, 425, 458 and 490 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of zinc(II) were prepared. The calibration plots (Fig. 9.ii.f) are linear and bears the relation $A_{412} = 0.0601C - 0.0096$ and $A_{438} = 0.0303C + 0.0015$.

The above equations suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Zn(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by following the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ± 2 percent was taken as the tolerance limit. It is noticed that all the ions that do not interfere in the zero order determination of Zn(II) with APS also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limit is generally higher than those in the zero order determination of Zn(II) with APS (Table 8.ii.2). W(VI) 32, Co(II) 0.7, Ni(II) 0.1, Fe(II) 0.67, Mn(II) 2.1, Al(III) 26, Ag(I) 12 and Cd(II) 2. (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Zn(II).
Section (ix) : Second and third derivative spectrophotometric determination of mercury(II) using 2-acetylpyridine thiosemicarbazone (APT)

Mercury(II) reacts with 2-acetylpyridine thiosemicarbazone (APT) to form yellow coloured complex in the pH range 5.0 – 7.0. The complex shows maximum absorbance at 351 nm. Hence, the author has made an attempt to develop a new second and third derivative method for the determination of mercury(II) in microgram levels. The results are presented in this section.

Second and third derivative spectrum of mercury – APT complex

The second and third derivative spectrum of Hg(II) – APT complex was recorded at pH 6.0 from which the analytical wavelengths was ascertained, the derivative amplitude was measured by the peak-valley method.

The second derivative spectrum (Fig. 9.ix.a) exhibits a maximum amplitude at 410 nm (peak) and a valley at 473 nm and three zero crosses at 380, 450 and 505 nm. The third derivative spectrum (Fig. 9.ix.b) shows a maximum amplitude at 420 nm (peak) and valley at 480 nm and four zero crosses at 390, 452, 515 and 560 nm. At 410 nm (second) and 420 nm (third) the amplitude is maximum and is proportional to the amount of mercury(II). Therefore, 410 nm (peak) and 420 nm (peak) are chosen for further studies.

Effect of pH on the derivative amplitude

The effect of pH on the second and third derivative peak amplitude of mercury(II) – APT complex at 410 nm and 420 nm by following procedure given in 2.iv.b. The second and third derivative spectrum was recorded. The derivative amplitude was measured by following peak – zero method. A plot was made between
Fig. 9.ix.a. Second derivative spectra of Hg(II) - APT Vs reagent blank

[Hg(II)] = 7.2 x 10^{-6} M

[APT] = 6 x 10^{-4} M

pH = 6.0

Fig. 9.ix.b. Third derivative spectra of Hg(II) - APT Vs reagent blank

[Hg(II)] = 7.2 x 10^{-6} M

[APT] = 6 X 10^{-4} M

pH = 6.0
Fig. 9.ix.c. Second derivative spectra of Hg(II) – APT Vs reagent blank
Hg(II), μg/ml; a. 0.4814; b. 0.9628; c. 1.4442; d. 1.9257
[APT] = 6 × 10^-5 M
pH = 6.0

Fig. 9.ix.d. Third derivative spectra of Hg(II) – APT Vs reagent blank
Hg(II), μg/ml; a. 0.4814; b. 0.9628; c. 1.4442; d. 1.9257
[APT] = 6 × 10^-5 M
pH = 6.0
the second and third derivative amplitude and the pH, from which working pH 6.0 was selected.

Effect of the reagent concentration on the derivative amplitude

To determine the number of times of the reagent concentration to the metal ion concentration required to achieve maximum peak amplitude, the derivative spectra at various concentrations of reagent were recorded by adopting the procedure given in 2.iv.c. A second and third derivative spectra of each solution was recorded and derivative amplitude was measured at selected wavelengths. The results obtained from this method indicates that a 10 - fold molar excess of the reagent is enough to achieve maximum amplitude.

Determination of mercury(II) by second and third derivative spectrophotometry

Applicability of Beer's law (second derivative)

To explore the applicability of Beer's law for the present system by second derivative spectrophotometric method, the procedure given in 2.iv.f was used. The derivative spectra at different concentration of Hg(II) were recorded and presented in Fig. 9.ix.e. The Figure shows that the peak amplitude at 410 nm, the valley amplitude at 473 nm and the peak-valley (410 – 473 nm) are all proportional to the concentration of Hg(II). Further the three zero crosses at 380, 350 and 505 nm are independent of concentration. Plots between derivative amplitude (peak zero, valley zero and peak valley) and the amount of mercury(II) were prepared. The calibration plots (fig 9.ix.e) are linear and bears the relation $A_{410} = 0.664C - 0.0286$ and $A_{473} = 0.08C + 0.008$. 
**Fig. 9.i.e.**  Second derivative Amplitude $\nu$s amount of Hg(II) $\mu$g/ml

\[
[A_{PT}] = 6 \times 10^{-4}M
\]

$\text{pH} = 6.0$

**Fig. 9.ix.e.**  Second derivative Amplitude $\nu$s amount of Hg(II) $\mu$g/ml

\[
a : A_{410} = 0.664C - 0.0286
\]

\[
b : A_{473} = 0.08C + 0.008
\]

**Fig. 9.ix.f.**  Third derivative Amplitude $\nu$s amount of Hg(II) $\mu$g/ml

\[
a : A_{420} = 0.6344C - 0.0056
\]

\[
b : A_{490} = 0.3814C - 0.0159
\]

\[
[A_{PT}] = 6 \times 10^{-4}M
\]

$\text{pH} = 6.0$
The above equations suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Hg(II).

**Third derivative spectra**

To determine the applicability of Beer’s law for the present system by third derivative spectrophotometric method, the procedure given in 2.iv.f was adopted. The derivative spectra at different concentration of Hg(II) were recorded and presented in Fig. 9.ix.f. The figure shows that the peak amplitude at 420 nm. The valley amplitude at 480 nm and the peak-valley amplitude (420 – 480 nm) are all proportional to the concentration of Hg(II). Further, the four zero crosses at 390, 452, 515 and 560 nm are independent of concentration. Plots between derivative amplitudes (peak zero, valley zero and a peak valley) and the amount of mercury(II) were prepared. The calibration plots (Fig. 9.ix.f) are linear and bears the relation $A_{420} = 0.6344C - 0.0056$ and $A_{480} = 0.3814C - 0.0159$.

The above equations suggest that the peak-valley method is more sensitive and hence is adopted for the determination of Hg(II).

**Effect of foreign ions on the derivative amplitude of the complex**

The effect of the foreign ions was studied by adopting the procedure given in 2.iv.g. The amount of foreign ions which brings about the change in amplitude by ±2 percent was taken as the tolerance limit. It is noticed that all the ions that do not interfere in the zero order determination of Hg(II) with APT also do not interfere in the second and third derivative spectrophotometric analysis. Further, their tolerance limit is generally higher than those in the zero order determination of Hg(II) with APT (Table 4.i.1). W(VI) 36, Ba(II) 54, Cd(II) 26, Zn(II) 0.1, Ni(II) 0.4, Ag(I) 0.2, Mn(II) 109, Co(II) 0.4, Au(III) 0.4, Pt(IV) 10, Tl(III) 10 and Pb(II) 4.1 (all in μg/ml) do not interfere in second and third derivative spectrophotometric determination of Hg(II).