Second derivative spectrophotometry for simultaneous determination of iron (II) and copper (II) using 2-ketobutyric acid thiosemicarbazone

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A new chromophoric reagent, 2-ketobutyric acid thiosemicarbazone (KBAT) has been used for the simultaneous determination of iron, copper and vanadium. KBAT forms yellowish-green and blue complexes with iron (II) and copper (II) respectively at pH 6.5 and a yellow complex with vanadium (V) at pH 5.5. Calibration graphs for the individual determinations by second derivative spectrophotometry were obtained. The derivative amplitudes obeyed Beer’s law at 387.2 nm for copper (II), 440 nm for iron (II) and 400 nm for vanadium (V), in the concentration range 0.30-2.40, 0.20-2.280 and 0.25-2.56 \( \mu g \) mL\(^{-1} \) respectively. Zero crossing method using the second derivative mode at the selected wavelengths was employed in the simultaneous determinations. The accuracy and reproducibility of the determination methods on known amounts of iron, copper, and vanadium in their binary mixtures were tested. The effects of foreign ions on the determinations of the metals were also studied. The recommended procedure was applied to the analysis of ferro-vanadium alloy, phosphor bronze, rice and groundnut.

Keywords: Derivative spectrophotometry, Zero crossing, Iron, Copper, Vanadium, 2-Ketobutyric acid thiosemicarbazone

Copper and iron have been thoroughly investigated because of their frequent natural occurrence and their technological significance. In addition, copper and iron in their ionic forms have metabolic importance. Daily human iron requirements are well established\(^1\) with iron (II) being better absorbed than iron (III). Trace amounts of iron and copper in various substances may be vital, objectionable or perhaps indicative of contamination or malfunction. Copper has received considerable attention owing to its uses in metallurgy and chemical industries. Moreover, it is an essential constituent of about thirty enzymes and glycoproteins and is required for the synthesis of hemoglobin and for some biological processes. It also promotes iron absorption from the gastrointestinal system, is involved in the transport of iron from tissue into plasma, helps to maintain myelin in the nervous system, is important in the formation of bone and brain tissue and is necessary for other many important functions\(^2,3\). An independent determination of copper in blood serum is less frequently required, but simultaneous evaluation of both elements gives complementary diagnostic information\(^4,5\). Moreover, industrial and commercial products may require knowledge of iron and copper content. In addition, these two elements are always together in environmental samples. The determination of these metals, therefore, in micro quantities is still challenging and in great demand in the context of increasing hazards of environmental pollution.

Derivative spectrophotometric methods have been used for the quantitative analysis of binary mixtures because of its great sensitivity and selectivity as well as a useful means of resolving two overlapping spectra and eliminating matrix interferences in the assay of two-component mixtures using the zero-crossing technique\(^6-8\). A second derivative spectrophotometric method for the determination of methyl parabins and bifonazole in commercial cream based on the measurement of acidic extract at 241.5 nm had been developed\(^9\). A novel, sensitive and highly selective first derivative spectrophotometric method has been proposed for the determination of vanadium (V) and iron (III) separately and simultaneously in various natural samples, food and biological materials using 2-hydroxy-1-naphthaldehyde benzoyl hydrazone at 540 nm for Fe and 465 nm for vanadium\(^10\). Recent developments of derivative spectrophotometry and their analytical applications have been reviewed\(^11\).
Thiosemicarbazones are one of the most important classes of reagents, which have been widely used for the spectrophotometric determination of metal ions. Murthy and Reddy, have applied 2-hydroxyacetophenone thiosemicarbazone for the determination of vanadium (V). A straightforward method for the simultaneous determination of copper and cobalt in binary mixtures by the use of diode–array detector accommodated in a flow-through sensor and first derivative spectrophotometry using pyridoxal-4-phenylthiosemicarbazone has been proposed. Pyruvic acid thiosemicarbazone (PVAT) and its derivatives, phenyl pyruvic acid thiosemicarbazone (PPVAT) and 2-keto butyric acid thiosemicarbazone (KBAT) have been synthesized, characterized and proposed as highly sensitive chromophoric reagents for the determination of several metal ions. The simultaneous determination of copper and zinc with phenyl pyruvic acid thiosemicarbazone (PPVAT) has been reported.

This work reports on a simple, sensitive, rapid and selective second order derivative spectrophotometric method for the simultaneous determination of iron and copper; copper and vanadium using the zero crossing technique. The method is based on the formation of yellowish-green, blue and yellow complexes of Fe, Cu and V ions respectively with 2-ketobutyric acid thiosemicarbazone.

**Experimental Procedure**

Reagents

All chemicals used were of analytical reagent grade and the solutions were prepared with high purity water from Millipore Mill-Q water purification system device.

Apparatus

A Shimadzu UV-VIS scanning spectrophotometer (model UV-160A) equipped with 1cm quartz cells was used to record the absorbance measurements, while a Phillips digital pH meter (Model LI 613) was used for pH measurements. The second order derivative spectra were recorded with a scan speed of 2400 nm min⁻¹ using a slit with of 1 nm with nine degrees of freedom, in the required wavelength range (nm). The derivative amplitudes measured were plotted against amounts of iron (II), copper (II), and vanadium (V) to obtain the calibration graphs.

**Preparation of reagent**

A 5×10⁻³ M solution of KBAT was prepared in ethanol and stored in a dark bottle.

**Preparation of metal solutions**

Stock solution of 0.1 M Fe (II) was prepared by dissolving appropriate amount of ammonium ferrous sulphate in distilled water containing a few drops of concentrated H₂SO₄ in a 100 mL volumetric flask and standardized colorimetrically. Stock solutions of 0.1 M Cu (II) was prepared by dissolving appropriate amounts of copper chloride dihydrate in distilled water in a 100 mL volumetric flask and standardized titrimetrically. Stock solutions of 0.1 M V (V) was prepared by dissolving appropriate amounts of ammonium metavanadate in distilled water containing a few drops of ammonia in a 100 mL volumetric flask and standardized colorimetrically.

**Buffer solutions**

The buffer solutions were prepared by mixing 0.8 M sodium acetate with 1.0 M acetic acid (pH 5.5) and 0.2 M sodium hydrogen phosphate with 1.6 M sodium dihydrogen phosphate (pH 6.5) and checked using a pH meter.

**Methods**

Second derivative spectrophotometric determination of iron, copper and vanadium

Buffer solutions (5 mL each) of desired pH were taken into 10 mL volumetric flasks and various aliquots of 2.0×10⁻⁴ M solutions of iron, copper and vanadium were added separately, 2.5 mL of 5×10⁻³ M KBAT were added to each flask. The contents were made up to the mark with distilled water. The absorbances of the solutions were measured at the wavelength range 325-475 nm against reagent blank in the second derivative mode.

Simultaneous second order derivative spectrophotometric determination of binary mixtures: iron and copper; copper and vanadium

Mixed solutions of 2×10⁻⁴ M Fe (II) and Cu (II); Cu (II) and V (V) were each treated with 5 mL of buffer solutions of pH 6.5 and 5.5 respectively, 2.5 mL of 5×10⁻³ M 2-keto butyric acid thiosemicarbazone was added to each solution and the mixtures were adjusted in a 10 mL volumetric flask with distilled water. The absorbances of the mixtures were measured at the
wavelength range 325-450 nm against reagent blank solutions in the second derivative mode.

**Analysis of ferrovanadium alloy and phosphor bronze samples**

Ferrovanadium alloy and phosphor bronze samples (0.2 g each) were added separately to 3.5 mL mixture of 2:1 nitric acid: sulphuric acid and heated until dense fumes of sulphuric acid evolved. After cooling to 60°C, 3 mL of 1:1 HCl and 15 mL of water were added. The solutions were then heated to boiling for 10 min, cooled and filtered using Whatman no. 40 filter paper. The filtrates were then transferred into a 100 mL volumetric flask and made up to the mark with distilled water.

The prepared ferrovanadium and bronze sample solutions (1.0 mL each) were diluted separately to 100 mL with water in a volumetric flask. 1.0 mL of the bronze solution was taken into 10 mL volumetric flask containing 5 mL of buffer solution of pH 6.5 while 1.0 mL of ferrovanadium solution was taken into 10 mL volumetric flask containing 5 mL of buffer solution of pH 5.5. A 2.5 mL aliquot of 5×10⁻³ M KBAT was added to each flask and the contents made up to the mark with distilled water. The absorbances were measured at 387.2 nm for copper, 440 nm for iron and 400 nm for vanadium against reagent blanks in the second derivative mode. The concentrations of the metals were obtained from predetermined calibration plots.

**Analysis of groundnut and rice samples**

The groundnut and rice samples were obtained from Andhra Pradesh Agricultural Research Institute, (APARI), Hyderabad, India. 0.5 g of finely grounded rice and groundnut samples were dissolved separately in 20 mL of 70% nitric acid and the sample digested until all yellow fumes ceased. 5 mL of perchloric acid was added and the digestion continued until the white fumes ceased, followed by addition of 3 mL of 1:1 hydrochloric acid with the digestion continuing until about 0.5 mL of acid remained in the conical flask. This was then filtered into a 250 mL volumetric flask by repeated washings and made up to the mark with distilled water.

A 1 mL aliquot of the prepared groundnut and rice sample solution each was added to 5 mL of buffer solution (pH 6.5), contained in a 10 mL volumetric flask separately, 2.5 mL of the reagent (5×10⁻³ M) was then added and the contents made up to the mark with distilled water. The absorbances of iron and copper were measured at the selected wavelengths against reagent blanks in the second derivative modes. The concentrations of the metals were obtained from predetermined calibration plots.

The data on the amounts of Fe, Cu and V were obtained in multiple measurements (n = 5) and were reported as the mean and percent relative error.

**Results and Discussion**

**Derivative spectra**

2-Ketobutyric acid thiosemicarbazone (KBAT) reacts with iron (II), forming a yellowish green coloured complex and with copper (II) forming a blue complex at pH 6.5 while forming a yellow coloured complex with vanadium at pH 5.5. The proposed reaction mechanism of KBAT with various metals is presented in Scheme 1.

The second derivative spectra of ([Fe (II) - KBAT], [Cu (II)-KBAT] and [V(V)-KBAT]) at their optimum pH were recorded in the wavelength region 325-475 nm as shown in Figs 1-3. From the derivative spectra (Fig. 1), it can be seen that the second derivative spectrum of Fe (II) complex shows three zero crossing points occur at 349.8, 387.2 and 425 nm with maximum and sufficient amplitude at 370 and 440 nm respectively, although a slight shift in the zero-crossing point for the higher concentration (2.23 µg mL⁻¹) is observed. At pH 5.5 and 6.5, the spectrum of Cu (II) complex shows a zero crossing point at 349.8, 387.2 and 425 nm with maximum and sufficient amplitude at 370 and 440 nm respectively, although a slight shift in the zero-crossing point for the higher concentration (2.23 µg mL⁻¹) is observed. At pH 5.5 and 6.5, the spectrum of Cu (II) complex shows a zero crossing point at 348.6 nm with maximum amplitude at 365 nm (Fig. 2). The spectrum of V (V) complex at pH 5.5 exhibit two zero crossing points at 350 and 380 nm with maximum amplitude at 400 nm (Fig. 3).

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*Fig. 1—Second order derivative spectra for iron (II) - KBAT against blank. Conditions: Fe (II) µg mL⁻¹: 1 = 1.270, 2 = 1.670, 3 = 2.232, pH = 5.5 and 6.5*
This will allow for simultaneous determination of Fe (II) and Cu (II) by measuring the derivative amplitudes of Cu (II) at 387.2 nm (iron zero cross) and Fe (II) at 440 nm, where Cu (II) has no absorption. Similarly, the simultaneous determination of V (V) and Cu (II) can be achieved by measuring the derivative amplitudes of V (V) at 400 nm and Cu (II) at 380 nm (vanadium zero cross) respectively. For the simultaneous determination of Fe (II) and V (V), the derivative amplitude of iron can be measured at 365 nm where vanadium exhibits negative amplitude while vanadium can be determined at 400 nm where iron also exhibits negative amplitude. It is reported\(^8\) that the use of zero-crossing points in derivative spectrophotometry accounts for improved selectivity; it is necessary that the zero crossing wavelengths do not change with varying concentrations of the related species. To evaluate this condition, changes in the pre-mentioned zero crossing wavelengths were tested in the presence of different concentrations of Fe (II) and V (V).
of different concentrations of the individual ions. As shown in the derivative spectra, no shifts in the zero crossing points were observed in the lower concentration but at the higher concentration (2.232 µg mL\(^{-1}\), Fig. 1) and (2.048 µg mL\(^{-1}\), Fig. 3). Although slight shifts in the zero crossing points were observed at the higher concentrations, the calibration graphs were linear within the concentration range of determination.

**Calibration, accuracy and precision**

Individual calibration graphs were constructed by plotting the second derivative amplitudes measured at 440, 387.2 and 400 nm against the amounts of Fe (II), Cu (II) and V (V) respectively. The plots reveal that Beer’s law is obeyed in the ranges 0.20-2.80 µg mL\(^{-1}\) of Fe (II), 0.30-2.40 µg mL\(^{-1}\) of Cu (II) and 0.25-2.56 µg mL\(^{-1}\) of V (V).

The results of mixtures of 0.558-3.348 µg mL\(^{-1}\) iron (II) and 0.635-3.810 µg mL\(^{-1}\) copper (II) obtained from the second derivative spectra are presented in Table 1. From the results, both metals can be analyzed with a relative error of 0.15% for concentrations of up to 3.804 µg mL\(^{-1}\) Cu (II). The results of the mixtures containing 0.509-3.06 µg mL\(^{-1}\) V (V) and 0.635-3.810 µg mL\(^{-1}\) Cu (II) indicate that both metals can be analyzed with a relative error of 0.39% for vanadium and 0.15% for copper.

<table>
<thead>
<tr>
<th>Diverse ions</th>
<th>In 2.79 µg mL(^{-1})Fe (II)</th>
<th>In 2.40 µg mL(^{-1})Cu (II)</th>
<th>In 2.55 µg mL(^{-1})V (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+), K(^+), Li(^+), Mg(^{2+}), Ca(^{2+}), Ba(^{2+}), Sr(^{2+})</td>
<td>2000</td>
<td>2000</td>
<td>2000</td>
</tr>
<tr>
<td>Thiocyanate, F(^-), Cl(^-), Tartrate</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Oxalate, citrate, ascorbate, phosphate, thiosulphide</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Al (III), Pd (II), Pb (II), Sn (II), Hg (II), Ti (IV), Ce (II), Mn (II), Cr (III)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>EDTA</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1—Simultaneous second order derivative determination of Fe (II) and Cu (II); V (V) and Cu (II) with KBAT in binary mixtures

<table>
<thead>
<tr>
<th>Amount taken (µg mL(^{-1}))</th>
<th>Amount found (µg mL(^{-1}))</th>
<th>Relative error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.558 1.27</td>
<td>0.558 1.268</td>
<td>0 0.15</td>
</tr>
<tr>
<td>1.116 2.54</td>
<td>1.116 2.536</td>
<td>0 0.16</td>
</tr>
<tr>
<td>1.116 3.81</td>
<td>1.116 3.804</td>
<td>0 0.16</td>
</tr>
<tr>
<td>1.116 0.635</td>
<td>1.116 0.634</td>
<td>0 0.16</td>
</tr>
<tr>
<td>1.116 1.27</td>
<td>1.116 1.268</td>
<td>0 0.15</td>
</tr>
<tr>
<td>2.232 1.27</td>
<td>2.232 1.268</td>
<td>0 0.15</td>
</tr>
<tr>
<td>3.348 1.27</td>
<td>3.404 1.268</td>
<td>1.6 0.15</td>
</tr>
<tr>
<td>0.509 0.27</td>
<td>0.512 1.268</td>
<td>0.59 0.15</td>
</tr>
<tr>
<td>1.02 2.54</td>
<td>1.024 2.536</td>
<td>0.39 0.16</td>
</tr>
<tr>
<td>1.02 3.81</td>
<td>1.024 3.804</td>
<td>0.39 0.16</td>
</tr>
<tr>
<td>1.02 0.635</td>
<td>1.024 0.634</td>
<td>0.39 0.16</td>
</tr>
<tr>
<td>1.02 1.27</td>
<td>1.024 1.268</td>
<td>0.39 0.15</td>
</tr>
<tr>
<td>2.04 1.27</td>
<td>2.048 1.268</td>
<td>0.39 0.15</td>
</tr>
<tr>
<td>3.06 1.27</td>
<td>3.072 1.268</td>
<td>0.39 0.15</td>
</tr>
</tbody>
</table>

Table 2—Tolerance limit of diverse ions (µg mL\(^{-1}\))

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount taken (%)</th>
<th>Amount found (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphor bronze7A</td>
<td>Cu 88</td>
<td>Fe 1.9</td>
</tr>
<tr>
<td>Ferrovanadium alloy (BCS 205/3)</td>
<td>Fe 47.22</td>
<td>V 50.16</td>
</tr>
</tbody>
</table>

Table 3—Determination of Phosphor bronze, Ferrovanadium alloy, rice and groundnut

Effect of diverse ions

The effects of diverse ions on the determination of Fe (II), Cu (II) and V (V) were studied to determine the tolerance levels of these ions in the present method. Solutions containing 2.79 µg mL\(^{-1}\) Fe (II), 3.17 µg mL\(^{-1}\) Cu (II) and 2.55 µg mL\(^{-1}\) V (V) and the various amounts of other ions were prepared, and the proposed second derivative procedure for their determination was followed. The results are presented in Table 2. Based on these results, the selectivity for the iron, copper and vanadium determination methods were observed. The only serious interfering species was EDTA. The other ions such as Na\(^+\), K\(^+\), Li\(^+\), Mg\(^{2+}\), Ca\(^{2+}\), Ba\(^{2+}\) and Sr\(^{2+}\), at a concentration of 2000 µg mL\(^{-1}\) had no interference effect.
Applications
The results of known amounts of iron, copper and vanadium determined in order to assess the proposed method, demonstrated the applicability of the method for simultaneous determination of iron and copper, copper and vanadium in binary mixtures. Two alloys and some food samples were analyzed using the proposed method. The results of the analysis of ferrovanadium and phosphor bronze alloys, rice and groundnut samples are presented in Table 3.

Conclusion
A simple, rapid and selective procedure for the simultaneous determination of iron and copper, copper and vanadium by second order derivative spectrophotometry using the zero crossing method has been developed with KBAT. The proposed procedures were applied satisfactorily to assays of iron and copper, vanadium and copper in synthetic binary mixtures, phosphor bronze, rice and groundnut, and iron and vanadium in industrial alloy samples.

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References