Spectrophotometric Method for the Determination of Copper (II) in Leafy Vegetable, soil, Alloys and Pharmaceutical Samples Using 3-methylthiophene-2-carbaxaldehyde-3-thiosemicarbazone (3-MTAT)

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ABSTRACT. A highly sensitive spectrophotometric method has been developed for the determination of copper (II) using 3-methylthiophene-2-carbaxaldehyde-3-thiosemicarbazone (3-MTAT) as an analytical reagent. The 3-MTAT forms reddish brown species of copper (II) at a pH range of 5.0-7.0. The Cu (II)-3-MTAT complex shows maximum absorbance at 430 nm, with molar absorptivity and Sandell’s sensitivity being 1.6 x 10^4 L Mol^-1 Cm^-1 and 3.6 x 10^-3 µg cm^-2 respectively. The system obeys Beer’s law in the range of 0.35-3.53 mg/L. The regression coefficient of the Beer’s law straight line is 0.338, and the correlation coefficient is 0.999. The detection limit of the method is 0.021 µg mL^-1. Most of the common metal ions generally found associated with copper do not interfere. The repeatability of the method was checked by finding the relative standard deviation. The developed method has been successfully employed for the determination of copper (II) in leafy vegetable and pharmaceutical samples and this method was inter comparison of experimental values using AAS.

1. INTRODUCTION

Copper is one of the metal ions that play an important role in the biological system. It plays a key role during cell respiration, in the blood of invertebrate animals, and in the formation of hemocyanin, an important respiratory protein, found in the lymph of most animals belonging to Phyla Mollusca and Arthropoda. From the standpoint of human health, its role in three physiological functions is of prime importance. Copper is involved in hemopoiesis and in maintenance of vascular and skeletal integrity in addition to the structure and function of the central nervous system. Copper occurs naturally in most vegetables, meats, and grains. The study of copper in food items is of great concern, because it plays a definitive role in the intrinsic mechanisms regulating vital biological processes [1]. Over exposure to copper causes metallic taste, ptyalism, nausea, vomiting, epigastric burning, and diarrhea. Heavy doses of copper cause a series of systematic toxic effects such as hemolysis, hepatic neurosis, gastrointestinal, bleeding, oliguria azotemia, hemoglobinuria, hematuria, proteinuria, hypertension, tachycardia, convulsions, and coma. When a congenital deficiency in the homeostatic mechanism for copper exists, the metal accumulates in the liver, discrete areas of the brain, the cornea of the eye, and other tissues, causing Wilson’s disease. A wide variety of clinical disorders have been associated with a dietary deficiency of copper, which respond to copper therapy. They include anemia, depressed growth, bone disorders, depigmentation of hair or wool, abnormal wool growth, neonatal ataxia, impaired reproductive performance, heart failure, and gastrointestinal disturbances [2]. In view of this, the separation and determination of copper from associated elements is indispensable. For the determination of copper at micro levels there are several frequently adopted methods using analytical techniques such as AAS, ICP-OES, X-ray fluorescence spectroscopy, spectrophotometry, spectrofluorometry, and other such techniques. Among these, the spectrophotometric methods are preferred as they are cheaper and easier to handle and have comparable sensitivity.
A number of spectrophotometric reagents have been used for the determination of copper (II), but a very few number are used for the separation and determination of it. Thiosemicarbazones are important sulfur- and nitrogen-containing organic reagents, where copper coordinates with these reagents to form stable complexes, it is more stable in divalent state. The metal chelates of these sulfur- and nitrogen-containing organic reagents find a wide range of applications in medicine [3] and agriculture. The reviewed [4, 5] literature revealed that only a few thiosemicarbazones were employed for determination of copper (II) [6-45]. Hence, the authors introduced a new reagent, 3-methylthiophene-2-carbaxaldehyde-3-thiosemicarbazone (3-MTAT), for the selective and spectrophotometric determination of Cu (II) in leafy vegetable, soils pharmaceutical, and standard alloy samples.

2. EXPERIMENTAL

2.1. Apparatus

A Shimadzu 2450 UV-VIS spectrophotometer with 1.0 cm quartz cell is used for absorbance studies. An Elico LI-120 digital pH meter is used for pH adjustment. A Perkin-Elmer 170-30 atomic absorption spectrometer is used for the comparison of results. A Nicolet FT-IR 560 Magna spectrometer using KBr was used to obtain the infrared spectra of the compound (3-MTAT). The Bruker 300MHz NMR spectrometer was used to obtain the $^1$H-NMR spectra of the ligand. The Micro Mass VG 7070 H Mass spectrometer was used to obtain the mass of the 3-MTAT.

2.2. Reagents and chemicals

All reagents used conform to analytical reagent grade, unless otherwise stated. 3-methylthiophene-2-carbaxaldehyde-3-thiosemicarbazone (3-MTAT) is prepared employing the following the procedure. (3ml, 0.0030 mol) of 3-methylthiophene-2-carbaxaldehyde is dissolved in 10 ml of absolute ethanol and mixed in a round bottomed flask with (0.284 g, 0.0030 mol) of thiosemicarbazide dissolved in 20ml of hot water. The mixture was heated under reflux for 30 minutes and then allowed to cool at room temperature for two hours. The crystals obtained were subjected to filtration, washed with cold ethanol and then recrystallized from ethanol (Scheme I). The melting point was 188-190°C. 3-MTAT dissolve in N,N-dimethylformamide (DMF) and dimethyl sulphoxide. The characterization of 3-MTAT was carried out by IR $^1$H NMR and Mass spectroscopy. The IR spectrum of 3-MTAT shows absorption bands around 1485 cm$^{-1}$ (C = S) 1540 cm$^{-1}$ (C = N) 2980 cm$^{-1}$ (Sp3 C-H) 3030 cm$^{-1}$ (Sp2 C-H) and 3300 cm$^{-1}$ (-NH). The $^1$H NMR (DMSO, ppm): 11.4 (-N-H), 7.6 (-NH$_2$), 9.85 (sp2 C-H), 2.6 (-CH$_3$), 7.2-7.8 (thiophene) and Mass spectrum of 3-MTAT shows signal at 200 (M +1) corresponding to its molecular ion peak. The molecular formula and molecular weight of the reagent C$_7$H$_9$N$_2$O$_2$ and 199 is respectively.

\[
\begin{align*}
\text{3-methylthiophene-2-carbaxaldehyde} & \quad + \quad \text{H}_2\text{N-C-N-NH}_2 \\
& \xrightarrow{\text{H}_3\text{C}-\text{O} \text{H \ loss of H}_2\text{O}} \quad \text{3-methylthiophene-2-carbaxaldehyde-3-thiosemicarbazone}
\end{align*}
\]

**Scheme-1** Preparation of 3-methylthiophene-2-carbaxaldehyde thiosemicarbazone (3-MTAT)

2.3. Preparation of standard Copper (II) solution

The stock solution was prepared by dissolving 3.93 g of copper sulfate pentahydrate (CuSO$_4$ 5H$_2$O) in double-distilled water containing a few drops of concentrated sulfuric acid. The solution was made up to 1 L and standardized by iodometry [46]. This stock solution was diluted further, whenever necessary, with double-distilled water.
2.4. **Buffer solutions**
Solutions of 1.0 M sodium acetate and 1.0 M acetic acid were prepared in double distilled water. Suitable portions of these solutions were mixed to get the desired pH of the solution.

2.5. **Analytical procedure for leafy vegetable samples**
The leafy vegetables were brought from the local market during the month of January. The samples were cleaned and dried in open air, protecting them from mineral contamination. The dried sample was pulverized in a mortar for the purpose of analysis, to a convenient size. Ten grams of each powdered sample was taken into a silica crucible, heated to oxidize the organic matter, and ashed at 550 °C in a muffle furnace for 4-5 h. The ash was dissolved by heating with 10 mL of 2 N hydrochloric acid and filtered through an acid-washed filter paper (Whatman no. 41), and then the residue was washed with hot water. The filtrate and washings were collected in a 25 mL volumetric flask and finally made up to the mark with double-distilled water.

2.6. **Analytical procedure for pharmaceutical samples**
Pharmaceutical samples such as Supradyn, Thesagram-M, Vimgram, and Fersolate were analyzed for copper (II). All of the pharmaceutical samples were brought into solution by adapting the following procedure. The samples were treated separately with concentrated nitric acid on a hot plate, at a low temperature, to avoid violent spurring. The residue of each sample was cooled, and again nitric acid was added. The temperature of the hot plate was kept low to avoid violent spurring. The residue of each sample was cooled, and again nitric acid was added. The temperature of the hot plate was increased to 300 °C. The residue obtained was dissolved in nitric acid (1:1) and then slowly heated for 2 h to procure a dry mass. Finally, the residue was dissolved in a minimum amount of double-distilled water. The same solution was quantitatively transferred into a 500 mL volumetric flask and then made up to the mark with double-distilled water.

2.7. **Analytical Procedure for Soil samples**
The sample was homogenized in the laboratory using a pestle and mortar and air-dried for approximately 24 h before analysis. An aliquot of 500 mg of finely pulverized soil was digested with 5 mL of nitric acid (65%) in a Teflon vessel. The sample was digested for about 3 h at 80°C and again redigested at 160°C for three quarters of an hour. After treating with double distilled water the supernatant liquid was made up to the mark in a 25 mL standard flask.

2.8. **Analytical Procedure for alloy samples**
The present method was also applied for the determination of cobalt (II) in alloy samples such as high-speed tool samples (BCS 484 and 485) and alloy steel samples (BCS 233 AND 266). About 0.1 g each of oven dried (110°C) alloy sample was dissolved in 15 mL of aquaregia. They were heated to near dryness and the nitrate expelled from the residue using 5 mL of concentrated hydrochloric acid. Each residue was extracted into double distilled water, separately, and made up to 25 mL calibrated flask.

2.9. **Recommended procedure**
To an aliquot of solution containing 10.0-150 µg of copper (II) were added buffer of pH 6.0 and 1 x 10^{-2} M reagent solutions (0.4 mL); the mixture was made up to 25 ml volumetric flasks. And its absorbance was measured at 430 nm against to the reagent blank.

3. **RESULTS AND DISCUSSION**
3-methylthiophene-2-carboxaldehyde-3-thiosemicarbazone (3-MTAT) forms a 1:2 (M:L) complex with copper (II), which is determined from acetic acid sodium acetate (pH 6.0) buffer. The light yellow Cu (II)-3-MTAT complex has a maximum absorbance at 430 nm and is stable for 46 hours. The conditions for effective and established after studying the effects of various factors, such
as pH, choice of the solvent, reagent concentration and influence of diverse ions, in order to develop a rapid and sensitive selective spectrophotometric method for the determination of copper (II) in micro levels.

3.1. Absorption spectra of Cu (II)-3-MTAT complex

The absorption spectra of the reagent solution against the corresponding solvent as a blank and that of the solution containing cooper (II) complex against the reagent as a blank are recorded in the wavelength range 430 nm. The absorption spectra of the reagent and complex are shown in Figure 1. The spectra reveal that Cu (II)-3-MTAT complex shows maximum absorbance at 430 nm and the reagent shows minimum absorbance at 430 nm. Hence, further absorbance measurements of the Cu(II)-3-MTAT complex are recorded at 430 nm against the reagent blank.

3.2. Effect of pH

To arrive at the optimum pH required for full color development, the effect of pH on the color intensity is studied. In each case, a mixture containing 1.0 mL of 4X10^{-5} M cooper (II), 10.0 mL of suitable buffer, 1.0 mL of 4X10^{-4} M 3-MTAT solution is taken, and it was made up to the mark with distilled water for 25 mL of standard flask. The same procedure is applied for buffers of different pH values, ranging from 1.0 to 10.0. The absorbances are measured at 430 nm, using their corresponding reagent blanks. A plot is executed between the pH and the absorbance, and the same is represented in Figure 2. The plot shows that there is maximum absorbance and constancy in the pH range 5.0-7.0. Hence, pH 6.0 is chosen for further studies, considering this as an optimum pH.

3.3. Effect of reagent concentration

The effect of reagent concentration is studied using different aliquots containing constant volumes of 1.0X10^{-4} M cooper (II) solution, 10.0 mL of pH 6.0 buffer solution, 1.0 mL of 1 x 10^{-2} M of 3-MTAT solution containing different concentrations ranging from 1X10^{-4} to 20 X10^{-4} M, in order to obtain the maximum color formation and it was made up to the mark with double distilled water. The absorbances of the solutions are measured at 430 nm against their corresponding reagent blanks. It is clearly observed from the absorbance values that a maximum fifteen fold molar excess of the reagent is sufficient to get a maximum color formation of the complex.

Figure 1. Absorption Spectra of: a: 3-MTAT Vs Water blank, b: Cu (II) –3-MTAT complex Vs 3-MTAT solution, Cu (II): 8 x10^{-5} M, 3-MTAT: 4 x10^{-4} M, pH: 6.0.
3.4. Validity of Beer’s law, Molar Absorptivity, Sandells’ Sensitivity and Correlation Coefficient for Cu(II)-3-MTAT complex

The Cu (II)-3-MTAT complex followed Beer’s law in the range of 0.35-3.53 ppm. The molar absorptivity of the complex was calculated to be $1.6 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$, and Sandell’s sensitivity of complex obtained from Beer’s law data for absorbance, 0.001, was found to be $0.0036 \mu g \text{ cm}^2$. The correlation coefficient value of the Cu (II)-3-MTAT complex, with an independent variable as concentration in $\mu g \text{ mL}^{-1}$ and dependable variable as absorbance, was found to be 0.999. This indicated an excellent linearity between the two variables.

![Figure 2](image-url)  
**Figure 2.** Effect of pH on the absorbance of Cu (II) –3-MTAT complex; Cu (II):$4 \times 10^{-5}$ M, 3-MTAT: $4 \times 10^{-4}$ M, $\lambda_{\text{max}}$: 430 nm.

3.5. Precision, accuracy and detection limit of the method

To assess the precision and accuracy of the method, determinations are carried out at different concentrations of cooper (II) under optimum conditions. The standard deviation of the method is found to be not more than 0.0038, and the relative standard deviation was less than 1.7 percent. It is evident from these results that the method is precise, besides being accurate. The detection limit $C_{\text{min}}$ is determined as the amount of copper (II) corresponding to five times the standard deviation of the blank values, and a value of $0.021 \mu g \text{ mL}^{-1}$ is obtained.

3.6. Determination of The Composition of Co(II)–3-MTAT complex

The composition of the Cu (II) complex with 3-MTAT was studied using Job’s method of continuous variation, and the mole ratio method [16]. Spectrophotometric investigation of the metal complex was conducted to obtain the composition of the complex. The composition of the complex was established by Job’s method of continuous variation. Equimolar solutions of Cu (II) and 3-MTAT ($2 \times 10^{-4}$ M) were prepared. The metal and reagent solutions were mixed in different proportions, keeping the total volume constant at 10.0 ml. To each solution, 10.0 ml of buffer (pH 6.0) solution was added and it was made up to the mark with distilled water. The absorbance values of the Cu (II)-3-MTAT were recorded at 430 nm, against their respective reagent blanks. From the above experimental results, it is evident that one mole of Copper (II) reacts with two mole of 3-MTAT, showing the composition of the complex to be 1:2. This composition was verified using the molar ratio method. From jobs continuous variation method the stability constant of the complex found to be $1.74 \times 10^{11}$.
3.7. Effect of foreign ions
The tolerance limit means the limit within which the foreign ion cannot be interfered with for the determination of Cu (II). In order to assess the possible analytical applications of this color reaction, the effect of some foreign ions are also examined by carrying out determinations of 75 µg of copper (II) with a known amount of the ion in question using the recommended general procedure. The criterion for interference is a variation of more than ±2 percent in absorbance from the expected value for copper (II) alone. In the case of some interfering ions, an increased tolerance limit is achieved by the addition of masking agents, such as thiosulfate, fluoride, tartrate or thiocyanate. Increasing the amount of masking agents proportionately could mask a higher amount of interfering ions. In this study, cations like As(III), As(V), Mg(II), Mn(II), Zr(IV), Sb(III), Ca(II), Sr(II), Ba(II), and Tl(III) do not interfere, when present up to 5500 µg and cations like Bi(III), Hg(II), Be(II), Th(VI), U(VI), Al(III), and V(V) are tolerated up to 3000 µg, but Cd(II), Co(II), Ni(II), Zn(II), Fe(III), Mo(VI) and Pd(II) do interfere with the determination of copper (II) when present in more than 2000 µg. The interference of Co (II) can be eliminated by using 1.0 mL 0.2% oxalate as a masking agent. Fe (II) & Fe (III) are masked with 1.0 mL of 3% of sodium fluoride. The interference of Zn (II), Cd (II) can be eliminated by using 1.0 mL of 0.5% of thiosulphate solution. Anions like fluoridde, thiocyanate, thiosulfate and thiourea do not interfere when present up to 3000 µg with the determination in the method. Citrate and borate are tolerated up to 1500 µg. Oxalate and phosphate interferes, even when present in trace amounts. EDTA masks copper (II) completely in the present determination.

3.8. Applications
The developed spectrophotometric method for copper (II) is applied for its determination in real samples such as leafy vegetable, soil, Pharmaceutical and standard alloy samples.

3.9. Determination of copper (II) in leafy vegetable samples
The present method was applied for the determination of copper (II) in leafy vegetables samples. Each aliquot was analyzed for copper (II) by the recommended procedure which was given in the materials and methods section. Copper (II) present in vegetable samples was determined from the calibrated plot (Beer’s law plot) using 3-MTAT and the results checked by atomic absorption spectrometry Table 1.

| Table 1. Determination of Copper(II) in leafy vegetables |
|----------------------------------|------------------|------------------|--------|---------|
| Leafy Vegetables                 | Amount added (µg/g) | Amount of Cu (II) found (µg/g) | SD     | % RSD   |
|                                  | AAS Method | Present Method  |        |         |
| cucumber (Cucumis sitivas)      | 10.00      | 10.52           | 10.51  | 0.03    | 0.28   |
| green peas (Pisum sativum)      | 10.00      | 11.15           | 11.14  | 0.05    | 0.43   |
| fresh bean (Dolichos lablab)    | 10.00      | 10.88           | 10.87  | 0.04    | 0.37   |
| white radish (Raphanus sativus) | 10.00      | 14.21           | 14.20  | 0.06    | 0.42   |

a Average of four determinations

3.10. Determination of copper (II) in soil samples
The present method was applied for the determination of copper (II) in soil samples. Each aliquot was analyzed for copper (II) by the recommended procedure which was given in the experimental section. Copper (II) present in soil samples was determined from the calibrated plot (Beer’s law plot) using 3-MTAT and the results checked by atomic absorption spectrometry Table 2.
3.11. Determination of Copper (II) in pharmaceutical samples

The present method was applied for the determination of copper (II) in Pharmaceutical samples. Each aliquot was analyzed for copper (II) by the recommended procedure which was given in the experimental section. Copper (II) present in soil samples was determined from the calibrated plot (Beer’s law plot) using 3-MTAT and the results checked by atomic absorption spectrometry Table 3.

<table>
<thead>
<tr>
<th>Soil Sample Site</th>
<th>Amount of Cu (II) founda (µg/g)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAS Method</td>
<td>Present Method</td>
</tr>
<tr>
<td>Kadapa</td>
<td>23.95</td>
<td>23.68</td>
</tr>
<tr>
<td>Mangampeta</td>
<td>23.24</td>
<td>22.93</td>
</tr>
<tr>
<td>Produtur</td>
<td>21.06</td>
<td>20.85</td>
</tr>
<tr>
<td>Yeeraguntla</td>
<td>22.32</td>
<td>21.99</td>
</tr>
<tr>
<td>Mydukur</td>
<td>21.43</td>
<td>21.39</td>
</tr>
</tbody>
</table>

a Average of four determinations

Table 3. Determination of Copper (II) in Pharmaceutical samples

<table>
<thead>
<tr>
<th>Pharmaceutical samples</th>
<th>Amount of Cu (II) founda (µg/g)</th>
<th>SD</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AAS Method</td>
<td>Present Method</td>
<td></td>
</tr>
<tr>
<td>Supradyn (Roche Chemicals, India)</td>
<td>0.86</td>
<td>0.85</td>
<td>0.011</td>
</tr>
<tr>
<td>Theragran-M (Sarabhai Chemicals, India)</td>
<td>2.00</td>
<td>1.99</td>
<td>0.013</td>
</tr>
<tr>
<td>Vimgram (Sarabhai Chemicals, India)</td>
<td>1.00</td>
<td>0.99</td>
<td>0.012</td>
</tr>
<tr>
<td>Fersolate (Glaxo, India)</td>
<td>0.66</td>
<td>0.65</td>
<td>0.011</td>
</tr>
</tbody>
</table>

a Average of four determinations

3.12. Determination of Copper (II) in standard alloy samples

The present method was also applied for the determination of copper (II) content in standard alloy samples. The amount of copper (II) present in each one of the sample solutions was determined from a calibrated plot and the results checked by direct Atomic Absorption Spectrometer Table 4.
Table. 4. Determination of Copper (II) in standard alloys

<table>
<thead>
<tr>
<th>Standard alloy</th>
<th>Composition</th>
<th>Amount of Cu (II) found$^a$ (µg/g)</th>
<th>% Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AAS Method</td>
<td>Present Method</td>
</tr>
<tr>
<td>NKK-1021-Alloy</td>
<td>Si, 5.56%; Cu, 2.72%; Zn, 1.76%; Fe 0.99%; Mg, 0.29%; Mn 0.20%; Ni, 0.14%; Cr 0.03%</td>
<td>1.02</td>
<td>1.00</td>
</tr>
<tr>
<td>NBS-SRM – 54 D</td>
<td>Sn 88.5%; Sb 7.04%; Cu 3.62%; Pb 0.62%; As 0.08%; Bi 0.04%; Fe 0.03%; Ag – 0.003%; Ni – 0.002%</td>
<td>1.02</td>
<td>1.01</td>
</tr>
<tr>
<td>Alloy steel (BCS 233)</td>
<td>Cu, 5.09; Co, 23.4; Ni 11.22; Sn, 7.95; Mn, 0.235</td>
<td>5.00</td>
<td>4.98</td>
</tr>
<tr>
<td>Alloy steel (BCS 266)</td>
<td>Cu, 3.33; Co, 23.4; Ni, 13.3; Al, 7.95</td>
<td>3.30</td>
<td>3.28</td>
</tr>
<tr>
<td>Aluminum base alloy (BCS 216/1)</td>
<td>Cu, 4.42; Mn, 0.73; Fe, 0.40; Zn, 0.11; Ti, 0.10</td>
<td>4.40</td>
<td>4.36</td>
</tr>
<tr>
<td>Copper base alloy (BCS 207)</td>
<td>Cu, 86.84; Sn, 9.8; Zn 2.53; Pb, 0.41</td>
<td>86.76</td>
<td>86.70</td>
</tr>
<tr>
<td>Copper base alloy (BCS 179)</td>
<td>Cu, 58.8; Zn, 33.9; Sn, 1.75; Al, 1.62; Mn, 1.03; Ni, 1.01; Fe, 0.91</td>
<td>58.72</td>
<td>58.65</td>
</tr>
<tr>
<td>BAS-20</td>
<td>Cu.4.10; Ni1.93; Fe0.43; Mn 19; Si 0.29; Mg 1.61; Rest Al</td>
<td>1.20</td>
<td>1.19</td>
</tr>
<tr>
<td>BAS-85</td>
<td>Cu0.90; Ni0.91; Feb1.15;Mn 0.02; Si 2.04; Mg 0.18; Zn 0.01; Rest Al</td>
<td>0.70</td>
<td>0.69</td>
</tr>
</tbody>
</table>

$^a$ Average of four determinations

4. CONCLUSION

A thorough survey of the literature reveals that many thiosemicarbazones are utilized for the determination of copper (II). Studies upon the use for 3-methylthiophene-2-carboxaldehyde thiosemicarbazone as an analytical reagent are however limited. The present investigations are carried out with a view to test the potential of 3-MTAT as a complexing agent for copper (II) and its subsequent determination by spectrophotometry. The selectivity of this method is enhanced by using masking agents for Ni (II), Cd (II), Co (II), Fe (III) and Zn (II). Finally, it is established that this method is suitable for the determination of copper (II) in vegetable, soil, pharmaceutical and standard alloy samples.

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References