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Analytical Methods

Analytical applications of 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone and determination of Cu(II) in food samples

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Abstract

New synthesized reagent 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone (2,6-DAPBPTSC) is proposed as a sensitive and selective analytical reagent for the spectrophotometric determination of copper(II) at pH 3.0 to form a yellowish orange colored 1:1 chelate complex. The maximum absorbance was measured at 370 nm. This method obeys Beer's law in the concentration range 0.63–6.30 g ml^{-1} and the correlation coefficient of Cu(II)–2,6-DAPBPTSC complex is 0.942, which indicates an adequate linearity between the two variables with good molar absorptivity and Sandell's sensitivity, 0.847×10^4 l mol^{-1} cm^{-1} and 0.0075 g cm^{-2}, respectively. The instability constant of complex calculated from Asmus' method is 1.415×10^{-4} at room temperature. The precision and accuracy of the method is checked with calculation of relative standard deviation ($n = 5$), 0.777% and the detection limit value is 0.0056 g ml^{-1}. The interfering effect of various cations and anions has also been studied. The method was successfully applied for the determination of Cu(II) in food samples. The performance of present method was evaluated in terms of Student '*t*' test and Variance '*f*' test, which is indicates the significance of present method is an inter comparison of the experimental values, using atomic absorption spectrometer (AAS).

Keywords: Copper(II); 2,6-Diacetylpyridine bis-4-phenyl-3-thiosemicarbazone (2,6-DAPBPTSC); Food samples

1. Introduction

Copper is both micro-nutrient as well as toxic element for living beings, depending up on the concentration level (Sharma, 1997). Inhalation of dusts, fumes and mists of Cu-salts results in congestion of nasal mucous membranes. Ulceration with perforation of the nasal septum on occasion and some times, pharyngeal congestion (Raymond & Donahue, 1997). It is also a gastrointestinal tract irritant (Judith, Peter, & Thomas, 1998). The study of copper in food items is of great concern, since it plays a definitive role in the intrinsic mechanisms regulating vital biological processes. Copper is also a essential element and the its deficiency causes the ischemic heart disease, anemia, abnormal wool growth and bone disorders (Eichhorn, 1975 & Underwood, 1979). Excess of copper enters through into the body as a pollutant present in water, food contamination and some other plant foods rich in copper.

Cu(II) is used to control fungal diseases in vineyard plants in France, South Africa (Schlotfeldt, 1992) and orange orchard in Thailand. High concentrations of copper were detected in some aquatic ecosystems collecting vineyard runoff water (GERBE, 1996). Copper is also a widely used metal industrially (Purnima & Vijay, 1991). In addition to this, it is an important pollutant in the environment resulting from the industrial discharge in the form of particulate (or) soluble copper waste from electroplating, chemical and textile industries. In view of this, separation and determination of copper from associated elements it indispensable.

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For the determination of copper at micro amount levels, there are several frequently adopted methods using analytical techniques (Goyal, Purohit, Page, & Sastry, 1992; Lobinski, Van Borm, Broekert, Tschopel, & Tolg, 1992) such as AAS, ICP-AES, ICP-MS, X-ray fluorescence spectroscopy, spectrophotometry, spectrofluorimetry and such other techniques. Among these, spectrophotometric methods are preferred because they are cheaper and easy to handle, with comparable sensitivity and accuracy.

Several organic reagents are used for determining copper in microgram quantities, but only a limited number of reagents are used for the separation and determination of it. Thio-, Phenylthio-semicarbazones are important sulphur-and nitrogen containing organic reagents, which are coordinated with copper to give more stable complexes. These stable Cu(II)–phenyl thiosemicarbazone complexes are extractable into organic solvents such as chloroform, *n*-butanol, etc.

The review of the literature revealed that only a few thio and phenyl semicarbazones were employed for the determination of copper(II) (Archana & Thakkar 2004; Desai & Desai, 1999; Jadhav & Kulkarni 1992; Jadhav & Vandre 1992, 1995; Lokande & Jaywant 1999; Lokande, Nirupa, & Chaudhary, 2002; Lokande, Poman, & Kapadi, 2001; Patel & Patel, 2000). A brief review of the methods presented for the determination of Cu(II) indicated that only a limited number of procedures were established for the spectrophotometric determination of Cu(II). Hence, the authors are prompted to introduce a new reagent 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone for the sensitive, selective and rapid spectrophotometric determination of Cu(II) in food and leafy vegetable samples. The proposed method, when compared with other spectrophotometric methods (Table 1), is found to more reliable and sensitive. It also has advantages like profundity and propagative properties in addition to its simplicity, instant color development and less interference.

2. Materials and methods

2.1. Apparatus

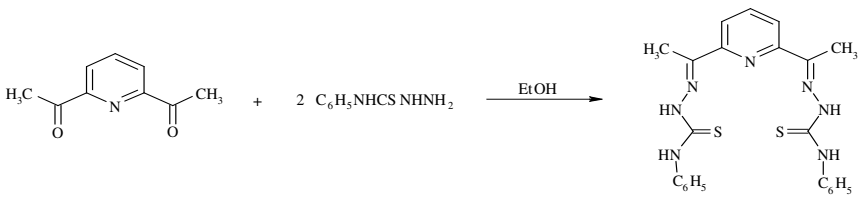
A Shimadzu 2450 UV–vis spectrophotometer with 1.0 cm quartz cell is used for absorbance studies. An Elico LI-120 digital pH meter is used for pH adjustment. A Perkin–Elmer 2380 atomic absorption spectrometer is used for the comparison of results. A Nicolet FT-IR 560 Magna spectrometer using KBr (neat) was used to obtain the infrared spectrum of the compound (2,6-DAPBPTSC). The Bruker 300 MHz NMR spectrometer was used to obtain the ^{1}H NMR spectrum of the ligand.

2.2. Reagents

2,6-Diacetylpyridine bis-4-phenyl-3-thiosemicarbazone (2,6-DAPBPTSC) is prepared as per the procedure: 1.60 g of 2,6-diacetylpyridine is dissolved in 30 ml of absolute ethanol and mixed in a round bottomed flask with 2.20 g of 4-phenyl-3-thiosemicarbazide is dissolved in 30 ml ethanol. The mixture was heated under reflux for 3 h and then allowed to cool to room temperature and kept for 12 h. The crystals obtained are filtered and washed with cold ethanol and then recrystallized from ethanol (Scheme 1). The melting point was 152 °C. 2,6-DAPBPTSC dissolves in *N*,*N*-dimethylformamide (DMF), acetone and dimethyl sulphoxide. The characterization of 2,6-DAPBPTSC was carried out by IR and ^{1}H NMR spectroscopy. The IR spectrum of 2,6-DAPBPTSC shows absorption bands around 1485 cm^{-1} (C=S) 1540 cm^{-1} (C=N) and

Table 1
Comparison of the present method with other reported spectrophotometric methods for determination of copper(II)

Reagent	pH	λ_{max} (nm)	Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	*M*:*L*	Beer's law range ($\mu g\ ml^{-1}$)	References
2,7-Dichloroquinoline 3-carbaldehydethiosemicarbazone	6.0	406	1843	–	0.03	Jadhav and Vandre (1992)
7-Methoxyl-2-chloroquinoline-3-carbaldehyde thiosemicarbazone	4.0	400	343	–	5.0	Jadhav and Kulkarni (1992)
2,4-Dihydroxy-5-bromoacetophenone thiosemicarbazone	6.0	400	1450	1:1	12.7	Desai and Desai (1999)
4-Chloroisonitrosoaceto phenon thiosemicarbzone	7.5–8.5	400	2518	1:2	0.2–2.0	Lokande et al. (2001)
2H-Benzopran-2-one-3-acetylthiosemicarbazone	8.8–9.2	410	0.702	1:2	2.0–2.5	Lokande et al. (2002)
8-Methoxyl-2-chloro quinoline-3-carbaldehyde thiosemicarbazone	5.0	410	0.00267	1:1	3.0	Jadhav and Vandre (1995)
P-Methylisonitroso acetophenonehydrazone	7.0	510	6280	–	0.1–1.0	Lokande and Jaywant (1999)
Benzylaldehyde-4-(2-hydroxy-5-sulforphenyl)-3-thiosemicarbazone	4.5	325	0.744	1:2	7.62	Patel and Patel (2000)
Isonitrosoprophenone thiosemicarbazone	10.0	390	5830	1:2	0.5–6.0	Archana and Thakkar (2004)
2,6-Diacetylpyridine bis-4-phenyl-3-thiosemicarbazone	3.0	370	8475	1:1	0.6–6.3	Present method



2,6-diacetylpyridine Bis 4-phenyl-3-thiosemicarbazide 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone

Scheme 1. 2,6-Diacetylpyridine bis 4-phenyl-3-thiosemicarbazide 2,6-diacetylpyridine bis-4-phenyl-3-thiosemicarbazone.

3300 cm^{-1} (–NH). The ^1H NMR (DMSO, ppm): 9.85 (–NH), 2.6 ($-CH_3$) and 7.2–7.8 (Ph).

2.3. Preparation of standard solution of copper(II)

The stock solution was prepared by dissolving 3.93 g of copper sulphate pentahydrate ($CuSO_4 \cdot 5H_2O$) in double distilled water containing a few drops of concentrated sulphuric acid. The solution was made up to the mark and standardized by iodometry (Vogel, 1961). This stock solution was diluted further, whenever necessary, with double distilled water.

2.4. Buffer solutions

Sodium acetate 1.0 mol/l and acetic acid 1.0 mol/l solution were prepared in double distilled water and suitable portions of these solutions were mixed to obtain the desired pH.

2.5. Collection of food samples, preparation of solutions and analytical procedure

The food samples analyzed have been brought from the city grocery stores. The samples are cleaned and dried in open air covering by net, protecting them from mineral contamination. The dried samples are pulverized to fine particles in a mortar for the analysis of Cu(II). Four grams of each powdered sample is taken into a silica crucible, heated to oxidize the organic matter and brought to ashes at 550 °C in a muffle furnace over a period of 4–5 h. The ash is dissolved by heating with 4 ml of 2 N hydrochloric acid, filtered through an acid washed filter paper (Whatmann no. 41) and then the residue is washed with hot water. The filtrate and washings are collected in a 10.0 ml volumetric flask and finally made up to the mark with double distilled water. An appropriate aliquot of the diluted solution is taken and its added pH 3.0, determination of copper(II) in food and leafy vegetable at 370 nm. The process is repeated four times for each sample and the results obtained are confirmed by direct atomic absorption spectrometer.

2.6. General procedure

To an aliquot of a working standard solution of 1.0×10^{-5}–20.0×10^{-5} mol/l copper(II) in 10.0 ml standard flask were added pH 3.0 buffer (2.0 ml) solution and reagent solution (1.0 ml) and the contents were made up to the mark with double distilled water. The absorbances of the solutions were recorded at 370 nm, against the reagent blank. Standard and relative standard deviations were evaluated with following formulae.

$$\text{Standard deviation} = \sqrt{\sum}(X - M)^2/(N - 1)$$

$$\text{Relative standard deviation} = \text{SD}/M \times 100$$

where $\sum$ = sum of, X = individual score, M = mean of all scores, N = sample size (number of scores).

3. Results and discussion

2,6-Diacetylpyridine bis-4-phenyl-3-thiosemicarbazone (2,6-DAPBPTSC) forms a 1:1 (M:L) complex with copper(II), from sodium acetate to acetic acid (pH 3.0) buffer. The yellowish orange Cu(II)–2,6-DAPBPTSC complex has a maximum absorbance at 370 nm and is stable for 48 h. The conditions for effective determination are established after studying the effects of various factors, such as pH, reagent concentration, metal ion concentration, salting-out agent and influence of diverse ions, in order to develop a sensitive, selective and rapid spectrophotometric method for the determination of copper(II) at micro gram levels.

3.1. Absorption spectra of reagent and Cu(II)–2, 6-DAPBPTSC complex

The absorption spectrum of Cu(II)–2,6-DAPBPTSC complex was recorded against the reagent blank. Similarly the absorption spectrum of the reagent (2,6-DAPBPTSC) was recorded against the solvent blank. The absorption spectra of both the complex and reagent are shown in Fig. 1. From the absorption spectra it is clear that the complex and reagent have shown maximum absorptions at 370 nm and 310 nm, respectively. The reagent has minimum absorbance at the maximum absorbance of the complex. The reagent absorbance at the maximum absorbance of metal complex was further suppressed using suitable concentration of reagent as blank for further absorbance measurements at 370 nm.

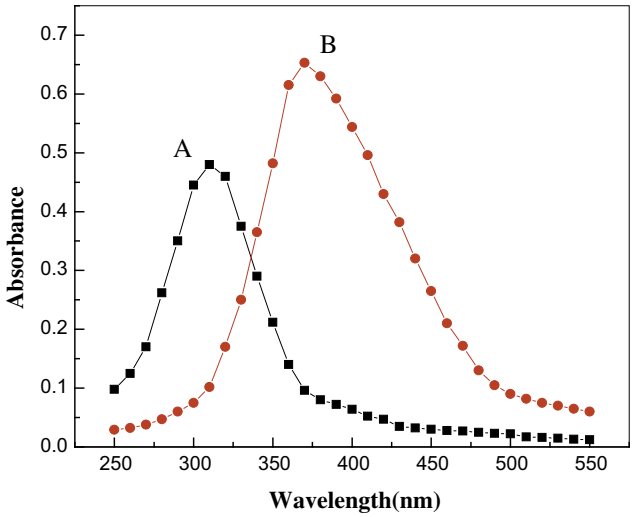


Fig. 1. (A) Absorption spectrum of 2,6-DAPBPTSC vs blank. (B) Absorption spectrum of Cu(II)–2,6-DAPBPTSC complex: Cu(II): 1 ml of 1.0×10^{-4} mol/l; 2,6-DAPBPTSC: 1.0 ml of 1.0×10^{-3} mol/l and pH 3.0.

3.2. Effect of pH

To arrive at the optimum pH required for maximum color development, the influence of pH on the color intensity was studied by using different buffers in pH range 1.0–6.5. The absorbance of the Cu(II)–2,6-DAPBPTSC complex increases as the pH increases from 1.0 to 2.5 and remains constant in the pH range 2.5–3.5. However, it has decreased beyond 4.0. Hence, acetate buffer is used for further studies, considering 3.0 as the optimum pH.

3.3. Effect of reagent concentration

The absorbances of the complex solutions obtained from the solutions of pH 3.0, containing constant amount of copper(II) and varying amounts of reagent were measured at 370 nm by adopting the following procedure. Different aliquots containing 1.0 ml of 1.0×10^{-4} mol/l copper(II) solution, 2.0 ml of pH 3.0 buffer solutions and the reagent solution containing different concentrations ranging from 1.0×10^{-4} to 20.0×10^{-4} mol/l were taken into a set of 10.0 ml standard flasks. There colored solutions were made up to the mark with double distilled water. The absorbances of these solutions were measured at 370 nm against their corresponding blanks. The results clearly indicate that a fifteen fold molar (15.0×10^{-4} mol/l) excess of reagent to that of the metal ion is sufficient for maximum color development of the Cu(II)–2,6-DAPBPTSC complex. Hence a fifteen fold molar excess of the reagent was maintained for maximum color formation.

3.4. Applicability of Beer's law

Various aliquots containing different amounts of copper(II) 1.0×10^{-5}–20.0×10^{-5} mol/l, 2.0 ml of pH 3.0 buffer and 1 ml of reagent (2,6-DAPBPTSC) 15.0×10^{-4} mol/l were taken into 10.0 ml standard flasks and made up to the mark with double distilled water. The absorbances of all the solutions were recorded at 370 nm, against their corresponding reagent blanks. The obtained results revealed that the complex system obeys Beer's law in the concentration range 0.63–6.30 μg/ml of copper(II). The molar absorptivity of the complex was calculated and noted as 0.847×10^4 l mol^{-1} cm^{-1} and the Sandell's sensitivity of the complex was 0.0075 μg cm^{-2}. The correlation coefficient value of the Cu(II)–2,6-DAPBPTSC complex, with an independent variable as concentration in μg/ml and a dependent variable as absorbance, was found to be 0.942. This indicates a satisfactory linearity between the two variables. The values of the slope and intercept for the best fitted line were obtained as 1.521 and −0.706, respectively. Thus, the content of Cu(II) in real samples can be determined using the following straight line equation $Y = 1.521X - 0.706$.

3.5. Ringbom plot for Cu(II)–2,6-DAPBPTSC complex

Ringbom plot is the standard adopted to know the optimum range of the concentration for a system, which emaciates Beer's law. The plot is drawn between log C of Cu(II) and $(1 - T)$ [where T is the transmittance]. The plot has a sigmoid shape with a linear segment at intermediate concentration values ranging from 3.10 to 3.87 g/l, which indicates that copper(II) is precisely determined in the range 3.10–3.75 g/l. The slope of the plot from Fig. 2 is 0.635. Based on this value at 1% photometric error, the ratio between the relative error in concentration and the photometric error is 3.626. Hence, the relative error in concentration is 0.0362.

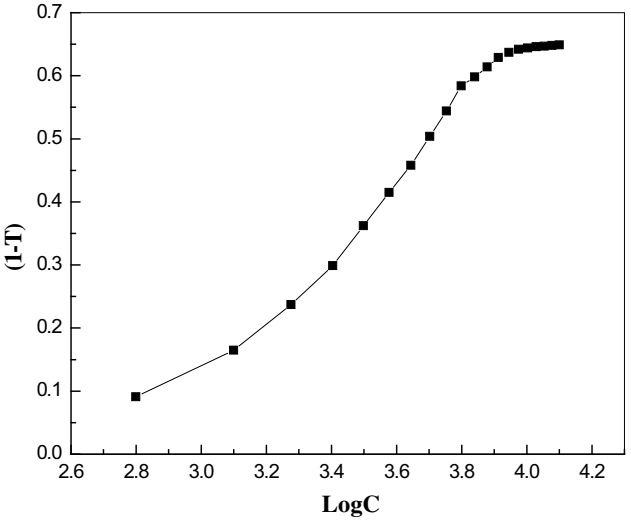


Fig. 2. Ringbom plot of Cu(II)–2,6-DAPBPTSC complex. Cu(II): 630–12,600 g/l; 2,6-DAPBPTSC: 1.0 ml of 15×10^{-4} mol/l; pH 3.0 and λ_{max}: 370 nm.

3.6. Precision, accuracy and detection limit of the method

To assess the precision and accuracy of the method, determinations were carried out for a set of five measurements, with different concentrations of Cu(II), under optimum conditions. Calculations reveal that the standard deviation of method was not more than 0.0012 and the relative standard deviation was less than 0.777. It is evident from these results, that the method is precise, besides being accurate. The detection limit, C_{min} is determined as the amount of Cu(II) corresponding to three times the standard deviation of the blank values and a value of 0.0056 g/l is obtained.

3.7. Determination of the composition of Cu(II)–2, 6-DAPBPTSC complex

Job's method of continuous variation, mole ratio methods were employed to elucidate the composition of the complex. Equimolar solutions of copper(II) and 2,6-DAPBPTSC (15×10^{-4} mol/l) were used to determine the metal to ligand ratio by Job's method of continuous variation. The absorbance values were recorded at 370 nm against the reagent blank. A plot was drawn between the absorbance and $V_M/V_L + V_M$, where V_L and V_M are the volumes of the reagent and the metal, respectively. The obtained curve indicates that 1:1 (M:L) stoichiometry in the extracted species. This was further confirmed by the mole ratio method by using the solution containing 1.0 ml of copper(II) (1.0×10^{-3} mol/l) 2.0 ml of buffer (pH 3.0) and 2,6-DAPBPTSC (0.25–2.0 ml of 1.0×10^{-3} mol/l) solution. The absorbance values were recorded at 370 nm against the reagent blanks. A plot was drawn between the absorbance and the volume of the reagent. From the curve it is noticed that one mole of the copper(II) reacts with one mole of 2,6-DAPBPTSC.

Finally, the composition of the complex was verified by Asmus′ method. The experimental part of Asmus′ method is similar to that of the mole ratio method. A liner plot was obtained between $1/m$ and $1/v$, where 'm' is the extraction modules, which is calculated by dividing the optical density with the cell width and 'v' is the volume of the reagent. This plot also confirms the metal to ligand ratio to be 1:1.

3.8. Determination of instability constant of Cu(II)–2, 6-DAPBPTSC complex

The instability constant of Cu(II)–2,6-DAPBPTSC complex was calculated using as Asmus′ method (Asmus′, 1960). The absorbance values were obtained at 370 nm for the solutions containing fixed volumes of copper(II) (1.0 ml of 1.0×10^{-3} mol/l) and 2.0 ml of pH 3.0 buffer with different known volumes of 0.25–2.0 ml of 1.0×10^{-3} mol/l of 2,6-DAPBPTSC. The instability constant of Cu(II)–2,6-DAPBPTSC complex was calculated to be 1.415×10^{-4} at room temperature.

Table 2
Determination of Cu(II) in foods samples

Samples[b]	Amount of copper(II)[a] found					
	AAS method	Present method	Present method		*f*-test	*t*-test
			SD	RSD (%)		
Kakara (Momordica charantia)						
Sample location						
Srikalahasthi	25.3	25.2	0.205	0.82	1.86	1.54
Madanapalli	18.5	18.3	0.195	1.07	1.40	1.92
Tirumala	21.2	20.9	0.175	0.83	1.74	1.80
Srinivasamanga puram	24.6	24.3	0.172	0.70	2.95	1.58
Pakala	17.3	17.0	0.225	1.30	2.40	3.26
Mangalam	22.5	22.4	0.210	0.95	1.62	3.83
Nagari	22.2	21.9	0.198	0.90	2.97	5.47
Leafy vegetables[b]						
Name of the samples						
Chama (Colocasia esculenta)	14.28	14.15	0.20	1.43	1.56	1.18
Avalu (Brassica nigra)	16.14	16.10	0.245	1.53	1.18	0.88
Pala teege (Ichnocarpus frutesccens)	23.25	22.95	0.250	1.10	1.64	1.70
Drumstick (Moringa oleifera)	20.50	20.32	0.192	0.95	1.49	1.99
Thotakura (Amaranthus gangeticus)	15.45	14.90	0.197	1.362	1.47	1.45
Chilakamukkaku(Impotiens balsamina)	34.80	34.0	0.232	0.68	1.22	1.49
Milk samples[c]						
Raw milk	4.00	3.98	0.022	0.60	1.23	1.05
Choco milk	3.50	3.50	0.026	0.76	1.14	1.29
Cow	5.00	4.92	0.148	0.31	1.05	1.12
Dairy	4.25	4.20	0.017	0.44	1.41	1.74

[a] Average of five determinations.
[b] Concentration in μg/g.
[c] Concentration in μg/ml.

3.9. Effect of foreign ions on extraction of Cu(II)–2, 6-DAPBPTSC complex

Interference of a number of cations and anions is studied in the color absorbance of the Cu(II)–2,6-DAPBPTSC complex. A change in absorbance of ±0.0025 is taken as the tolerance limit for interference. Cations like Ca^{2+}, Mg^{2+}, Mn^{2+}, Bi^{3+} and Pb^{2+} and anions like fluoride, bromide, iodide, chloride, nitrate, sulphate, thiosulphate, citrate, acetate and tartate do not interfere, even when present up to 5000 μg/ml. Interference due to Al^{3+}, Cr^{3+}, Ag^{+} and Sb^{2+} can tolerated up to 2500 μg/ml. Determination copper(II) is not possible in the presence of Co^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Pd^{2+}, Cd^{2+}, Mo^{6+}, Se^{4+}, thiocyanate, oxalate and EDTA due to their interference, even when present in trace amounts. However, Interference of Fe^{2+} and Fe^{3+} is suppressed with 1.0 ml of 0.2 percent fluoride as a masking agent and of Co^{2+}, Ni^{2+}, Zn^{2+}, Pd^{2+}, Mo^{6+}, Se^{4+} and Cd^{2+} is suppressed, by adding 1.0 ml of 0.2% citrate solution. Increasing the amounts of their corresponding masking agents proportionately can mask higher amounts of interfering ions.

4. Applications of developed method

The developed sensitive spectrophotometric method for Cu(II) was successfully applied for its determination in food samples.

4.1. Determination of Cu(II) in food samples

The foods like kakara (Momordica charantia), leafy vegetables and milk samples were analyzed for copper(II) using the proposed method. The content of the copper(II) present in the solution was determined by using a calibrated plot and results obtained were conformed by direct atomic absorption spectrophotometer. The data obtained in the analysis of leafy vegetables and milk samples are given in Table 2.

5. Conclusions

The present investigations proved that 2,6-DAPBPTSC is a promising complexing agent for Cu(II) and its subsequent determination by spectrophotometry was rapid and precise. The method has good sensitivity when compared to other existing spectrophotometric determination methods. The selectivity of this method is improved by using masking agents for Co^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, Zn^{2+}, Pd^{2+}, Cd^{2+}, Mo^{6+} and Se^{4+}. It has been successfully applied for the determination of copper(II) in food, leafy vegetables and milk samples.

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