

## RESEARCH NOTE

# Determination of Copper in Soymilk and Soy-paneer with Indirect Micellar Spectrophotometric using Green Chemistry

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### Abstract

Quantifying the contents of copper in various samples of soy-products by indirect micellar spectrophotometry using green chemistry approach. In the Green synthesis of ligand N-(2'-Thiazolyl)-2-hydroxybenzamide (N2T2HB) has been synthesized by the reaction of 2-aminothiazole with salicylic acid which is a weak acid and is found to be soluble in alcohol, acetone and in chlorinated hydrocarbons such as chloroform. The present investigations have revealed that N2T2HB is suitable for determination of copper, as spectrophotometric reagent. The method is quite sensitive, simple and rapid in Triton X-100 micellar medium. The developed methods has been applied successfully for the determination of copper in soya milk and soy-paneer. It was also observed that the market samples of both soya milk and tofu contained copper significantly lower than the products prepared at Lab while all were within permissible limit.

**Keywords:** Metals, Copper, Micellar spectrophotometric, soymilk and soy-paneer

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Copper is one of the important trace element considered essential for the growth and development of biological organisms. It has been found to occur in all tissues, enzymes and colored proteins of man also (WHO/FAO/IAEA, 1996). Copper is carried mostly in the bloodstream on a plasma protein called ceruloplasmin and first absorbed in the gut then is transported to the liver bound to albumin. Copper is also found in a variety of enzymes, including the copper centers of cytochrome c oxidase and the enzyme superoxide dismutase containing copper and zinc (Osredkar and Sustar, 2011). In addition to its enzymatic roles, copper is used for biological electron transport. The blue copper proteins that participate in electron transport include azurin and plastocyanin (Shleeva, *et al.* 2005).

All copper compounds, unless otherwise known, are considered as toxic. Thirty grams of copper sulfate is potentially lethal in humans. The World Health Organization recommends a minimal acceptable intake of approximately 1.3 mg/day. In toxicity, copper can inhibit the enzyme dihydrophil hydratase, an enzyme involved in haemopoiesis. Symptoms of copper poisoning are very similar to those produced by arsenic. Fatal cases are generally characterized by convulsions, palsy and insensibility.

Copper excess is a subject of much current research. Distinctions have emerged from studies that copper excess factors are different in normal populations versus those with increased susceptibility to adverse effects and those with rare genetic diseases.

(Turnlund, 1988). Either the case may be - Excess or Deficiency - but it is pertinent to identify the level of copper in the food we eat and the water we drink.

Numerous reagents known for the spectrophotometric determination of metal have been reviewed. (Prasad, 2012; Gupta, 2013. Hazra, 2014; Rajesh and Manikandan, 2007). Dithizone, dithiocarbamates and cuproine are well known and highly sensitive reagents for copper. Triton X-100 as suitable surfactant for determination of metal was also reviewed (Ojha *et al.* 2015). In the present investigation, the author proposed a novel green, simple, inexpensive and selective methods for determination of copper in soy products – soymilk and soy-paneer using micellar spectrophotometry.

## **MATERIALS AND METHODS**

Soybean seeds variety JS-335 supplied from Vasant Rao Naik Marathwada Krishi Vidyapeeth, Parbhani, was used in the study. All beans were stored in a cool dry storage room until used for preparation of soymilk and further soy-paneer. Coagulant (Calcium Chloride) food grade quality was procured from Scientific Trading, Mehsana. The non ionic surfactant Triton X-100 (Polyoxyethylenetert-octylphenylether) with a molecular weight of 624 (product of CDH) was used as such. EC Double Beam UV-VIS Spectrophotometer, with quartz cell of 10 mm light path was used for Electronic spectral measurement at GCRC (Green Chemistry Research Center) Bikaner, (Raj.). The Elico model pH meter was used to adjust the pH values of various solutions.

### **Preparation of soymilk**

Soymilk was prepared as per the method described by Ojha *et al.* (2015). Approximately 200 gm soybean split was prepared from cleaned beans by soaking in water for half an hour and then, dried in sunlight for 2 days. The soybean split was prepared by breaking in stone miller. This soybean split was then cleaned. The purpose of soybean split making was to dehull the beans (Kulkarni, *et al.* 1985).

The dehulled soybean split were soaked in water at room temperature for a period of 16 hours and

ground with water at a bean: water ratio 1:9. The resultant slurry was strained through 3 layered muslin cloth to remove soy residue (okara), to obtain soy milk of 9°Brix. The brix of soy milk was measured using digital refractometer. Two market samples of UHT packed soymilk samples were also procured for simultaneous study.

### **Preparation of Soy-paneer toffu**

A 500gm portion of soymilk was heated to a temperature of 80°C under stirring. The time period of heating was kept constant for every soy-paneer preparations. Soy-paneer was prepared by coagulating the soymilk using coagulant calcium chloride @ 0.5% (Murdia *et al.* 2010).

Coagulant dissolved in 20 ml of cold water and was used immediately. The hot soymilk and coagulant solution was poured simultaneously into a glass container ensuring good mixing. The coagulant soymilk suspension was allowed to stand undisturbed for a period of 20 minutes to ensure coagulation. The curd formed was broken thoroughly and pressed in muslin cloth. The whey was drained off for 10 minutes and then pressed uniformly (Gangopadhyay *et al.* 1992). The Soy-paneer in muslin cloth was transferred to plastic bag and stored under refrigeration. Simultaneously two more ready to use market samples of tofu were procured from market. The products obtained were evaluated for copper contents.

### **Reagents**

In the Green synthesis of N-(2'-Thiazolyl)-2-hydroxybenzamide (N2T2HB) has been synthesized by the reaction of 2-aminothiazole with salicylic acid. It is a weak acid and is found to be soluble in alcohol, acetone and in chlorinated hydrocarbons such as chloroform. It also dissolves in alkaline aqueous media but is practically insoluble in water at pH less than 7. In the presence of surfactants its solubility has been found to be increased significantly. On addition of solution of N2T2HB in presence of surfactants to copper (II) solution, a yellowish green colored complex formed immediately. The local nonpolar

microenvironment of the micelles incorporates the non-polar colored complex. Secondly a marked increase in absorption intensity has also been observed. This phenomenon of micellar solubilization with increasing absorption intensity has been used to develop a novel and selective spectrophotometric method for determination of copper (II) in various samples of soy products.

Stock solution of N-(2'-thiazolyl)-2-hydroxybenzamide (N2T2HB) (0.1 M) was prepared by dissolving requisite amount of it in ethanol. Stock solutions of Triton X-100 solution ( $1.8 \times 10^{-2}$  M, 100 CMC). Acetate buffers were prepared by mixing 0.2 M sodium acetate and 0.2 M acetic acid and were used to maintain the pH in acidic medium. Borate buffers were prepared by mixing boric acid and sodium hydroxide and were used to maintain the pH in alkaline medium. Stock solution of copper(II) was prepared by dissolving appropriate amount of hydrated copper sulfate in double distilled water and was standardized according to reported procedure (31).

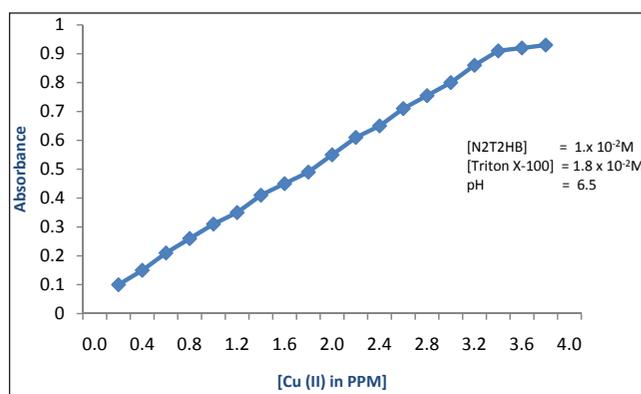
## RESULTS AND DISCUSSION

A series of solutions, containing 2.5 ml buffer solution of different pH values, 2.5 ml of  $1.1 \times 10^{-4}$  M copper (II) solution, 2.5 ml of  $1.8 \times 10^{-3}$  M Triton X-100 solution and 2.5 ml of  $1 \times 10^{-3}$  M N2T2HB. Their pH values were adjusted at different levels in a range 2.5 to 10 with a total volume of 25 ml. A plot of pH versus absorbance at  $\lambda_{\max}$  388 nm (Fig. 1) found that absorbance increases with increase in pH up to 6.5 and remained constant up to pH 7.2, thereafter it started decreasing. For further studies acetate buffer of pH 6.5 was selected.

To study the effect of varying concentration of N2T2HB, set of solutions containing increasing concentration of N2T2HB, at a fixed metal ion and Triton X-100 concentration (2.5 ml of  $1.1 \times 10^{-4}$  M and 2.5 ml of  $1.8 \times 10^{-3}$  M respectively) and at pH 6.5, were prepared and total volume was made up-to 25 ml and their absorbance were measured at 388 nm. For maximum complexation, 5 times molar excess of N2T2HB is required. However, in subsequent

studies, 10 times molar excess of N2T2HB has been maintained.

In order to investigate the effect of varying concentration of Triton X-100, set of solutions having increasing concentration Triton X-100 with fixed metal ion concentration 2.5 ml of  $1.1 \times 10^{-4}$  M, and fixed N2T2HB concentration 2.5 ml  $1 \times 10^{-3}$  M and 2.5 ml of buffer of pH 6.5, were prepared and total volume was made upto 25 ml and absorbance were measured at 388 nm. The absorbance initially increased but remain constant after  $1.8 \times 10^{-3}$  M, however in subsequent studies  $1.8 \times 10^{-2}$  M was maintained.

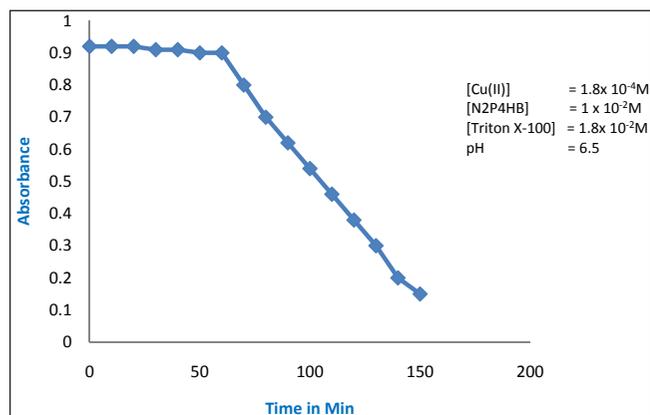


**Fig. 1:** Effect of concentration of on Cu-N2T2HB complex (Beer's Law)

To identify the effect of copper concentration on the absorbance -Solution containing increasing amounts of metal ion at a fixed N2T2HB concentration of 2.5 ml of  $1 \times 10^{-2}$  M, Triton X-100 concentration 2.5 ml of  $1.8 \times 10^{-2}$  M and 2.5 ml of buffer of pH 6.5 were prepared and total volume was made up to 25 ml and absorbance were measured at 388 nm. A plot of Cu(II) concentration (in ppm) versus absorption intensities at  $\lambda_{\max}$  388 nm has been represented in Fig. 1. A linear curve was obtained in the range 0.2 to 3.4 ppm of Cu(II) ions and the optimum range for the accurate determination of Cu(II) is recommended as 0.2 to 3.2 ppm.

In Fig. 2. a plot of absorption intensity versus time has been represented for copper- N2T2HB complex in the presence of Triton X-100. The complex is found to be reasonably stable over a period of approximately 60 minutes during which the analysis can easily

be completed. The complex solution however, is unstable when stored for prolonged periods of time and should be prepared fresh before analysis.



**Fig. 2:** Effect of time on absorbance of Cu- N2P4HB complex

Reagent, surfactant, metal ion and buffer solution were mixed and had no influence on absorbance intensity. However, in some cases a precipitate appeared, particularly when lead and nickel ions are found in matrices above 10 ppm. It dissolved by the addition of surfactant solution. In order to avoid any complication following order i.e. buffer solution, metal solution, surfactant solution and reagent solution has been preferred.

**Recommended procedure**

Into 25 ml standard flask, add 2.5 ml of buffer solution of pH 6.5, a 2.5 ml of suitable aliquot of solution containing 0.2 to 3.2 PPM of copper, 2.5 ml  $1.8 \times 10^{-2}M$  Triton X-100 solution and 2.5 ml  $1 \times 10^{-2}M$  N2T2HB solution. Dilute the solution to volume with double distilled water and then record the absorbance at 388 nm. For finding the precision and accuracy, experiments were repeated three times each.

**Effect of diverse ions**

The selectivity of the method was investigated for the determination of 1 ppm of copper in the presence of other ions. An ion was considered to be interfering, if the absorbance obtained differed more than  $\pm 5\%$  from that of copper (II) alone. Most of the cations and

several anions did not interfere. Only lead and nickel ions interfered.

**Analytical Application**

The most selected analytical conditions and characteristics for the determination of copper with N2T2HB in triton X-100 micellar medium are given in Table 1.

**Table 1:** Optimum analytical conditions and analytical characteristics

$\lambda_{max}$	-	388 nm
pH	-	6.5 to 7.2
[N2T2HB]	-	$> 1 \times 10^{-3} M$
[Triton X-100]	-	$> 1.8 \times 10^{-3} M$
Beer's law (in ppm) optimum concentration of Copper	-	0.2 to 3.2
Sandell's sensitivity [ $\mu g Cu(II)/cm^2$ ]	-	0.001

The proposed method was applied for the determination of copper in soy-products. After pretreatment 15 gm of soymilk and soy-paneer samples were taken into a slowly heated silica crucible when all the moisture was removed the temperature was raised to approximately 450-500°C avoiding loss of sample by foaming and swelling.

**Table 2:** Copper content in different Samples of Soymilk

Sl. No.	Sample	Amount of Copper Present ( $\mu g/g$ )
1	Soya milk (Market sample – 1)	1.32 <sup>a</sup> $\pm$ 0.01
2	Soya milk (Market sample – 2)	1.28 <sup>a</sup> $\pm$ 0.03
3	Soya milk prepared at lab	1.60 <sup>b</sup> $\pm$ 0.02
4	Tofu (Market sample – 1)	1.90 <sup>aa</sup> $\pm$ 0.02
5	Tofu (Market sample – 2)	1.96 <sup>aa</sup> $\pm$ 0.01
6	Soy-paneer prepared at lab	2.35 <sup>ab</sup> $\pm$ 0.06

$\pm$  means standard error. Each value is mean of value in triplicate ( $n=3$ ). Values with different superscript shows significant difference ( $p<0.05$ ).

At this temperature, ignition was continued until grey ash was obtained. The crucible was allowed to cool. The ash was dissolved in minimum amount of

concentrated nitric acid and evaporated to dryness and ignited again at 450-500°C for 1h. Dissolved the resulting white ash in minimum amount of dilute nitric acid and the amount of copper was determined by following the recommended procedure. The results for the estimations of are presented in Table 2.

## CONCLUSION

The present investigations have revealed that N2T2HB is suitable for determination of copper, as spectrophotometric reagent. The method is quite sensitive, simple and rapid in Triton X-100 micellar medium. The developed methods have been applied successfully for the determination of copper in soya milk and soy-paneer. It was also concluded that the market samples of both soya milk and tofu contains copper significantly lower than the products prepared at Lab while all are within permissible limit. This may be due to the change in variety of soybean, or source of water or because difference in unit operations compared to commercial production.

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