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MICELLAR SPECTROPHOTOMETRIC DETERMINATION OF COPPER IN THE VARIOUS SAMPLES OF MILK

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ABSTRACT: Milk is a complex system where analysis of metal ions is a difficult task due to the presence of interfering materials. The formation of a coloured complex between the metal and a coloured or colourless ligand is the basis for the development of spectro-photometric methods. The aim of this study is to analyze and estimate copper present in various samples of milk (*i.e*., cow, buffalo, goat, camel, soya, and powdered milk) by micellar spectrophotometry method. For this purpose, the ligand N-(2'-Pyridyl)-2-hydroxy-benzamide (NPHB-2) was synthesized by the reaction of 2-aminopyridine with salicylic acid, which is a weak acid and is found to be soluble in alcohol, acetone, and chloroform. It is insoluble in water at a pH less than 7. In addition to the solution of this ligands to copper (II) solution in the presence of micellar medium, a yellowish-green or light green coloured complex is formed immediately. It has been observed that in the presence of a micellar medium, solubility, as well as absorption intensity, has been found to be increased. This phenomenon has been used to develop a novel and selective micellar spectrophotometric method for the determination of metal ions in various samples of milk.

INTRODUCTION: Milk is the most valuable and nutritious product for human consumption because it contains vital nutrients, including proteins, essential fatty acids, lactose, vitamins, and minerals in balanced proportions [1, 2]. Milk is also an important source of metallic elements, including trace elements such as alumi-nium, copper, iron, manganese, silicon, zinc *etc*. for human nutrition [3].

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Copper is an important trace element believed to be essential for living beings. However, excess copper catalyzes oxidative changes, which have an adverse effect on flavour of the products, *e.g*., rancidity is caused in milk by oxidation of ascorbic acid, and excess also impairs the taste of beer [4].

For the determination of copper at micro levels there are several analytical techniques have been used including atomic absorption spectrometry [5-8], atomic emission spectrometry [9], electroanalytical techniques [10], spectrophotometry [11-16], inductive coupled plasma-emission spectrometry, inductive coupled plasma-mass spectrometry [17], Voltammetry [18], flow injection diode array spectro-photometry and X-ray fluorescence spectrometry [19-22].

Among these, the spectrophotometric methods are preferred as they are cheaper, easier to handle comparable sensitivity, and involve less expensive instrumentation. The aim of this study was to develop a novel and selective micellar spectrophotometric method for the determination of metal ions in various samples of milk because milk is a complex system where an accurate estimation of metal ions is a difficult task due to the presence of interfering materials. The formation of a coloured complex between the metal and a coloured or colourless ligand is the basis for the development of spectrophotometric methods. In order to develop such methods, we have explored the potential use of amide group-containing ligands in the presence of micellar medium, which has been found to increase the solubility as well as absorbance or fluorescence intensity resulting a selective and sensitive micellar spectrophotometric method for the determination of metal ions in various samples of milk knowingly or unknowingly.

EXPERIMENTAL:

Preparation of Solutions: Stock solution of N-(2'-Pyridyl)-2-hydroxybenzamide (NPHB-2) (0.1 M) was prepared by dissolving requisite amount of it in ethanol.

Surfactant Solution: Stock solutions of the following surfactants were prepared, Triton X-100 solution (1.8×10^{-2} M, 100 CMC), sodium dodecyl sulfate SDS (0.50 M, 75 CMC), hexadecyltrimethyl ammonium bromide HTAB (9.2×10^{-2} M, 100 CMC).

Buffer Solution: 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. A stock solution of copper (II) was prepared by dissolving an appropriate amount of hydrated copper sulfate in double-distilled water and was standardized according to reported procedure [23].

Determination of Copper in Samples of Milk: Pipetted out 25 ml of milk slowly at the rate of approximately one drop per second 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. At this temperature, ignition was continued until grey ash was obtained. The crucible was allowed to cool.

The ash was dissolved in a 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 a minimum amount of dilute nitric acid, and the amount of copper was determined by following the recommended procedure.

Recommended Procedure: Into 25 ml standard flask, add 2.5 ml of buffer solution of pH 6.5, a suitable aliquot of copper, 2.5 ml of 1.8×10^{-2} M Triton-X-100 solution, and 0.5 ml of 5×10^{-3} M NPHB-2 solution. Dilute the solution to volume with double distilled water and then record the absorbance at λ_{max} 388 nm. For finding the precision and accuracy, experiments were repeated five times each.

RESULTS AND DISCUSSION: N-(2'-Pyridyl)-2-hydroxybenzamide (NPHB-2) has been used for the estimation of copper metal. Various effects *i.e*., pH, micellar medium, ligand concentrations, copper concentration on absorbance, the stability of complexes, and effect of diverse ions *etc.*, have been observed at λ_{max} 388 nm and pH at 6.5.

Effect of pH on Absorbance: Absorption spectra of a solution containing copper- NPHB-2 complex in the micellar medium was recorded against reagent blanks by adjusting the pH in the range of 2.5 to 10.0 at λ_{max} 388 nm.

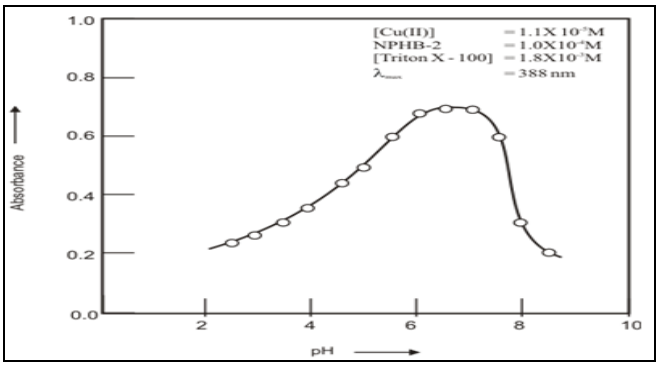

FIG. 1: EFFECT OF pH ON CU-NPHB-2 COMPLEX

The effect of pH was investigated on copper-NPHB-2 complex. The absorbance has been found to increase with the 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 with NPHB-2. Absorption spectra of solution containing copper- NPHB-2 complex in micellar medium is represented in **Fig. 1.**

Effect of Micellar Medium: The absorption spectra of a solution containing copper- NPHB-2 complex have been recorded in the presence of different surfactants, nonionic Triton-X-100, cationic - HTAB, and anionic-SDS for comparison purpose at pH 6.5 and λ_{max} 388 nm. In Triton-X-100 the absorbance was found to be maximum. The absorption spectra are given in **Fig. 2.**

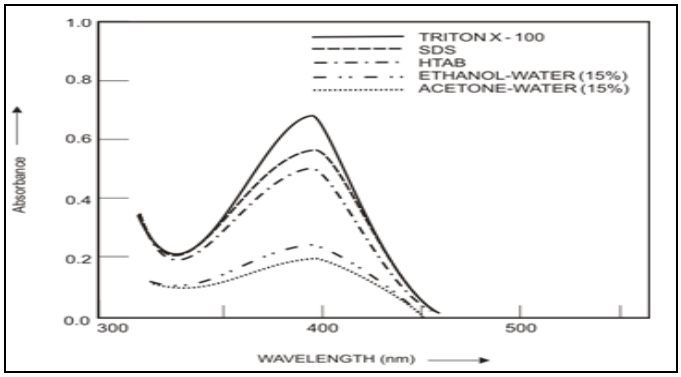

FIG. 2: EFFECT OF MICELLAR MEDIUM AND SOLVENTS ON CU-NPHB-2 COMPLEX

Effect of Ligand Concentration: For maximum complexation, 5 times molar excess of NPHB-2is required. However, in subsequent studies, 10 times molar excess of NPHB-2 has been maintained. A plot of absorbance versus mole ratio of NPHB-2/ NPHB-3/ NPHB-4 is represented in **Fig. 3.**

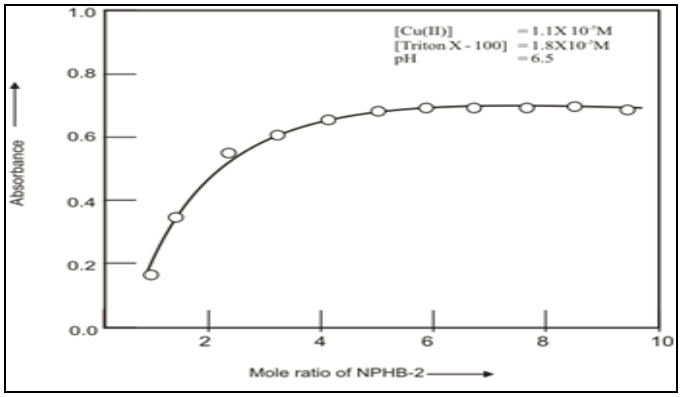

FIG. 3: EFFECT OF NPHB-2 ON CU-NPHB-2 COMPLEX

Effect of Copper Concentration on the Absorbance: The effect of copper concentration was investigated on Cu-NPHB-2 Complex. A linear curve was obtained in the range 0.11 ton 0.95 ppm of Cu (II) ions, and the optimum range for the accurate determination of Cu (II) is recommended as 0.11 to 0.85 ppm.

A plot of Cu (II) concentration (in ppm) versus absorption intensities at λ_{max} 388 nm has been represented in **Fig. 4.**

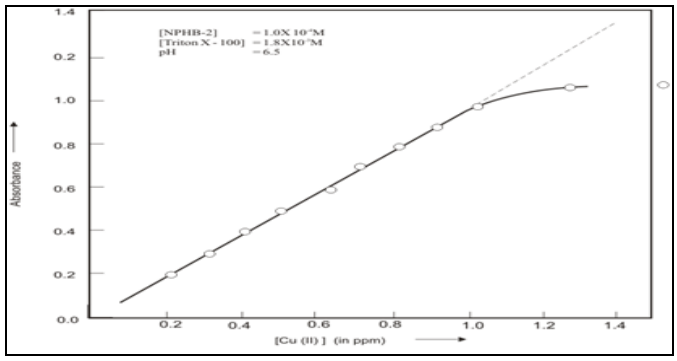

FIG. 4: EFFECT OF CONCENTRATION OF COPPER ON CU-NPHB-2 COMPLEX (BEER'S LAW)

Stability of the Complexes: The stability of copper-NPHB-2/NPHB-3/NPHB-4 complexes has been carried out in the presence of Triton-X-100. The complex is found to be reasonably stable over a period of approximately 90 min during which the analysis can easily be completed. The complex solution, however, is unstable when stored for prolonged periods of time. The complex solution should be prepared fresh before analysis. The present investigations have revealed that NPHB-2, like a spectrophotometric reagent for copper, compares well with other well-known methods. The method is highly sensitive, simple, and rapid in Triton-X-100 micellar medium. Although nickel and cobalt ions interfere and make it less selective, it ranks amongst the most sensitive reagents known so for the purpose. A plot of absorption intensity versus time has been represented for copper-NPHB-2 complex in the presence of Triton-X-10 in **Fig. 5.**

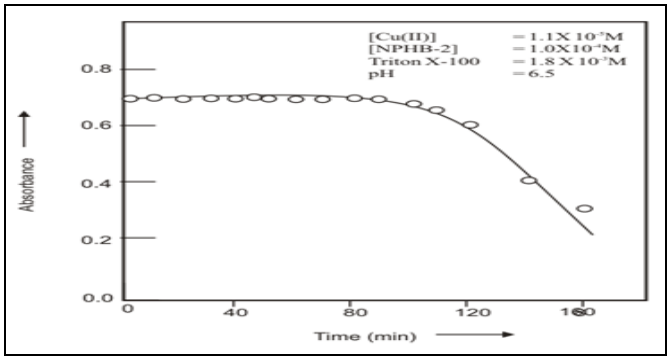

FIG. 5: EFFECT OF TIME ON ABSORBANCE OF Cu-NPHB-2 COMPLEX

Effect of Diverse Ions: The effect of various ions on the absorption intensity of the Cu- NPHB-2 complex has been investigated following the recommended procedure. The absorbance was measured at λ_{max} 388 nm.

An ion is considered to be interfering if its presence produces a change greater than ± 5% in absorption intensity. The investigated ions and the maximum concentration at which they do not interfere are given in **Table 1.**

TABLE 1: CONCENTRATION RATIO OF FOREIGN IONS THAT DO NOT PRODUCE A CHANGE GREATER THAN ± 5% IN THE ABSORPTION INTENSITY OF CU-NPHB-2 COMPLEX. [Cu^{2+}] = 0.7 ppm [Triton X - 100] = 1.8 x 10^{-3}M ($NPHB^{-2}$] = 1.0 x 10^{-4}M pH = 6.5 λ_{max} = 388 nm Absorbance (in the absence of foreign ion) = 0.697.

Ions	Tolerance Limit (ppm)
NH_4^+	1000
Ca^{2+}	2000
Li^+	900
Al^{3+}	200
Mg^{2+}	100
Pb^{2+}	80
Be^{2+}	80
Mn^{2+}	50
Ni^{2+}	5
Fe^{3+}	14
Co^{2+}	2
Zn^{2+}	8
Ag^+	2
Cd^{2+}	2
ClO_4^-	>20,000
Cl^-	>20,000
F^-	450
Citrate	5
Tartarate	15

Analytical Applications of the Method: A novel and selective method for the determination of copper in milk samples has been developed, which meets the criteria of Green Chemistry. This method was compared with the existing methods. The results for the determination of copper in various samples of milk are presented in **Table 2.**

TABLE 2: DETERMINATION OF COPPER IN MILK SAMPLES

Sample	Amount of Copper Found (µg/g)	
	From the Present Method	From AAS
Buffalo Milk	0.257*	0.253
Camel Milk	0.222*	0.218
Cow Milk	0.212*	0.200
Goat Milk	0.206*	0.208
Powdered Milk	4.25*	4.28
Soya Milk	0.285*	0.282

*Mean of five determinations

CONCLUSION: The present investigations have revealed that NPHB-2, as spectrophotometric reagent for copper, compares well with other well-known methods. The method is highly sensitive, simple, and rapid in Triton-X-100 micellar medium. Although nickel and cobalt ions interfere and make it less selective but it ranks amongst the most sensitive reagents known so for the purpose.

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CONFLICTS OF INTEREST: None Declared

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