

Synthesis and Analytical Studies of a New Bis-azo Dye: 2,6-Bis(9-hydroxyphenanthryl-10-azo) pyridine

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A new pyridyl bis-azo dye, 2,6-bis(9-hydroxyphenanthryl-10-azo)pyridine has been synthesized and proposed as a sensitive chromogenic reagent for the determination of copper, cobalt, zinc and cadmium. The molar absorptivities are $5.8 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ at 556 nm for copper, $4.7 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ at 540 nm for cobalt, $6.4 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ at 556 nm for zinc and $6.0 \times 10^4 \text{ L mole}^{-1} \text{ cm}^{-1}$ at 560 nm for cadmium. Other physico-chemical characteristics have been established spectrophotometrically. Trace levels of copper in some foodstuffs have been determined with the reagent.

Key words: Synthesis, Analytical, Bis-azo dye.

INTRODUCTION

Heterocyclic azo dyes are widely used as highly sensitive chromogenic reagents for a number of metal ions¹, PAN and PAR being very important reagents of this class. We synthesized a new bis-azo dye, 2,6-bis(9-hydroxyphenanthryl-10-azo)-pyridine using the Anderson and Nickless method^{2,3}. The synthesized azo dye showed a good sensitivity and high selectivity for copper(II), cobalt(II), zinc(II) and cadmium(II) ions.

As is well known, copper is recognized as an essential micronutrient. As a part of a balanced diet, an adequate amount of copper is vital to ensure a wide range of health benefits. In copper deficiency induced anemia in spite of elevated levels of iron in liver, the rate of hemoglobin synthesis is significantly reduced. The deficiency affects the cardiovascular system and causes extensive damage to heart and arteries^{4,5}. Food and Nutrition Board⁶ has proposed an allowance of *ca.* 2 mg of copper per day, for an adult, to prevent any symptoms of deficit. The maximum tolerable daily intake for copper is 0.5 mg/kg body weight⁷. Therefore copper is an essential bioelement for human life. Though deficiency of this element is very rare in human beings but its excess intake poses threat to human health. Keeping in view the biological importance of copper it was thought worth

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while to determine copper levels in some foodstuffs which are usually consumed by the local gentry of this area using the newly synthesized reagent.

EXPERIMENTAL

Synthesis of 2,6-bis(9-hydroxyphenanthryl-10-azo)pyridine (PBP)

0.01 Mole of 2,6-hydrazinopyridine was dissolved in minimum amount of dilute acetic acid. Separately, 0.02 mole of 9,10-phenanthroquinone was dissolved in ethanol. The two solutions were then mixed and kept for some time. The mixture solution was neutralized with ammonia. A yellow coloured solid so obtained was filtered, washed 2–3 times with 50% ethanol and dried at 60–70°C. The purity of the compound was checked by thin-layer chromatography. The coupling did not give a hydrazone but the azo dye, which was confirmed by infra-red spectrum of the compound. The spectrum showed the complete absence of $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ frequencies with the appearance of νOH frequency showing thereby the enol form of the compound.

A 2×10^{-4} M solution of PBP was prepared by dissolving 0.1038 g in 1 L of ethanol.

Metal ion solutions: 0.01 M stock solutions of copper(II), cobalt(II), zinc(II) and cadmium(II) were prepared by dissolving appropriate amounts of analytical grade copper sulfate pentahydrate, cobalt sulfate heptahydrate, zinc sulfate heptahydrate and cadmium acetate dihydrate respectively, in doubly distilled water. Solutions were standardized complexometrically with EDTA and diluted further as required for working standards.

Buffer solutions⁸

Phosphate buffer, pH 6.0: This buffer was prepared by diluting 250 mL of 0.2 M potassium dihydrogen phosphate and 28.2 mL of sodium hydroxide solution to 1 L with distilled water.

Borate buffers, pH 8.5 and 9.5: Buffers of pH 8.5 and 9.5 were prepared by diluting 250 mL of a solution containing 12.369 g of boric acid and 14.911 g of potassium chloride per litre and 105 mL and 185 mL of 0.2 M sodium hydroxide solution, respectively to 1 L with water.

Sodium acetate solution: 0.1 M Sodium acetate solution was prepared and was used for the studies of zinc(II) complex.

Potassium iodide solution: 1% Potassium iodide solution was prepared.

Foreign ion solutions: Solutions of various ions of suitable concentration were prepared using analytical grade reagents. Metal ion solutions were prepared from their acetate or sulfate salts and anions from their sodium or potassium salts.

All the reagents used were of analytical grade and doubly distilled water was used throughout.

A Bausch and Lomb spectronic 2000 spectrophotometer with 10 mm matched glass cells was used for absorbance measurements. Beckman pH-meter model Φ 60 and a digital pH metre Nig 333 model with glass electrodes of 0.0–14 pH ranges were used for pH adjustments. Pre-calibrated pipettes, burettes and measuring flasks (pyrex or corning) were employed for volume measurements.

Procedures

For copper(II): To an aliquot containing 3.5 to 12.5 μg of copper(II) ions, add 5 mL of 2×10^{-4} M reagent solution, 2 mL of phosphate buffer (pH 6.5), 1 mL of 1% potassium iodide solution and 10 mL of water. Extract the complex formed in 10 mL chloroform.

For cobalt(II): To an aliquot containing 1.5 to 12.0 μg of cobalt(II) ions, add 5 mL of 2×10^{-4} M reagent solution, 2 mL of borate buffer (pH 8.5), 1 mL of 1% potassium iodide solution and 10 mL of water. Extract the complex formed in 10 mL of chloroform.

For zinc(II): To an aliquot containing 4.0 to 11.0 μg of zinc(II) ions, add 5 mL of 2×10^{-4} M reagent solution, 2 mL of sodium acetate buffer (pH 8.5), 1 mL of 1% potassium iodide solution and 10 mL of water. Extract the complex formed in 10 mL of chloroform.

For cadmium(II): To an aliquot containing 5.5 to 22.5 μg of cadmium(II) ions, add 5 mL of 2×10^{-4} M reagent solution, 2 mL of borate buffer (pH 9.5), 1 mL of 1% potassium iodide solution and 10 mL of water. Extract the complex formed in 10 mL of chloroform.

Record the absorbance at the respective λ_{max} against a corresponding reagent blank prepared under similar conditions.

RESULTS AND DISCUSSION

PBP is insoluble in water, slightly soluble in ethanol and methanol and highly soluble in water immiscible solvents, viz., chloroform, carbon tetrachloride, benzene etc. It shows a slight yellow colour on protonation in highly acidic media and slightly intense yellow in neutral and orange-red colour in highly alkaline media due to ionisation. The visible/UV spectra at neutral and high pH in 30% dioxane-water medium and after extraction in chloroform in the pH range 4–11 showed no shift in absorbance peak on protonation at low pH and thus the pK_{NH} could not be calculated. However, pK_{OH} determined in 30% dioxane-water medium comes out to be 10.73.

Ethanolic solution of PBP gives color reactions only with four metal ions, i.e., with copper, cobalt, zinc and cadmium. The complexes are precipitated if the ethanolic concentration is kept below 50%. However the precipitates are extractable in water-immiscible solvents, but colour fading continues in most of the cases. Colour stability is maximum in chloroform and when 1% potassium iodide solution is used as a stabilizer for colours of metal complexes. The colours of the complexes retained in chloroform after extraction from alkaline solutions are as follows: copper(II) gives a violet, cobalt(II) a red, while zinc(II) and cadmium(II) give pink coloured complexes with PBP in neutral to slightly alkaline media. In phosphate buffered solutions no colour reaction except copper(II) is given by PBP, thus making the method highly selective for the determination of copper, selecting properly conditioned phosphate buffer. Borate buffers are found to be suitable for cobalt and cadmium ions, while 0.01–0.05% sodium acetate concentration gives appropriate conditions for the development of maximum colour for

zinc ions. EDTA is tolerated in two cases, *i.e.*, in cobalt(II) and zinc(II) and is therefore used to mask cadmium up to a certain level. In the determination of metals other than copper, colour is suppressed using thiosemicarbazide as masking agent. However, attempts to mask zinc in the determination of cobalt and cobalt in zinc failed.

Various physico-chemical and analytical characteristics of the complexes are summarized in Table-1.

TABLE-1
PHYSICO-CHEMICAL AND ANALYTICAL CHARACTERISTICS OF VARIOUS METAL ION COMPLEXES

Characteristics	Copper(II) complex	Cobalt(II) complex	Zinc(II) complex	Cadmium(II) complex
λ_{\max} , nm	556	540	556	560
pH range	6.0-7.5	7.5-9.5	6.6-8.0	8.0-10.5
Reagent required for maximum complexation/ mole of metal ion	6	8	6	8
Beer's law validity range, ppm	0.0-2.20	0.0-1.95	0.0-1.95	0.0-3.5
Optimum concentration range, ppm.	0.35-1.25	0.15-1.20	0.4-1.10	0.55-2.55
Sandell's sensitivity ($\mu\text{g}/\text{cm}^2$)	0.0011	0.00125	0.001	0.0019
Molar absorptivity ($\text{L mol}^{-1} \text{cm}^{-1}$)	5.8×10^4	4.7×10^4	6.4×10^4	6.0×10^4
Composition (M : L) by Job's method	1 : 1	1 : 2	1 : 2	1 : 2

Effect of diverse ions: In the determination of copper(II), cobalt(II), zinc(II) and cadmium(II) at the 1.27 $\mu\text{g}/\text{mL}$, 1.30 $\mu\text{g}/\text{mL}$, 1.17 $\mu\text{g}/\text{mL}$ and 2.25 $\mu\text{g}/\text{mL}$ respectively, fluoride, chloride, bromide, iodide, nitrate, acetate, sulfate, sulfide, sulfite, tartarate, borate (up to 1000-fold), calcium, strontium, barium, titanium, vanadium, niobium, tantalum, aluminium, lanthanides (upto 100-fold) do not interfere.

The tolerance of other anions and cations which do not cause deviation of $\pm 2\%$ in absorbance, which interfere seriously, in addition to masking possibilities are listed in Table-2.

Determination of copper in foodstuffs: Wet-ash 2 to 5 g of food (dried for 24 h at 70°C in an oven) with nitric and perchloric acids. Take up the ash with 5 mL of hydrochloric acid (1 + 9) and evaporate to dryness; repeat this step. Dissolve the dry residue in water, filter the solution into a 25 mL standard flask, add 1 or 2 drops of concentrated hydrochloric acid and make up to volume and determine copper as described already.

The copper levels determined in various foodstuffs are given in Table-3.

TABLE-2: EFFECT OF DIVERSE IONS

Foreign ions	Copper(II)	Cobalt(II)	Zinc(II)	Cadmium(II)
EDTA	SI	20 fold	20 fold	SI
Nitrite	50 fold	NI	NI	NI
Thiosulfate	50 fold	NI	NI	NI
Thiosemicarbazide	SI	NI	NI	NI
Oxalate	SI	NI	NI	NI
Thiocyanate	SI	NI	NI	NI
Cyanide	200 fold	NI	NI	250 fold
Phosphate	NI	SI	SI	SI
Silver(I)	8	8	10	10
Manganese(II)	25	20	25	25
Iron(II)	12	10	12	12
Cobalt(II)	3	–	SI	SI
Nickel(II)	5	5	8	7
Copper(II)	–	4 ^a	5 ^a	5 ^a
Zinc(II)	5	SI	–	SI
Cadmium(II)	5	5 ^b	5 ^b	–
Mercury(II)	8	10	5	5
Palladium(II)	5	5	8	10
Lead(II)	50	30	30	25
Chromium(II)	50	50	50	50
Gold(II)	12	15	15	15

SI indicates serious interferenc; NI indicates no interference (1000–fold).

^a masked by thiosemicarbazide; ^b masked by EDTA

TABLE-3: CONTENTS OF COPPER IN VARIOUS FOODSTUFFS

Food Sample	No. of analysis	Sample ashed (g)	Cu found in whole sample µg	Range of Cu Levels, mg per 100 g
<i>Zea mays</i> (Maize)	4	4	51.55, 50.08 53.39, 50.81	1.25–1.34
<i>Triticum aestivum</i> (Wheat flour)	4	5	15.46, 15.46 14.73, 16.50	0.295–0.330
<i>Pennisetum typhoideum</i> (Bajara)	4	5	34.61, 35.35 38.66, 37.56	0.69–0.77
<i>Lens culinaris</i> (Masur)	4	4	24.30, 22.83 24.19, 23.55	0.56–0.61
<i>Oryza sativa</i> (Rice bran)	4	2	11.02, 10.67 11.77, 11.77	0.53–0.59
Milk powder	4	5	14.25, 15.25 14.0, 14.0	0.28–0.30
Tea samples	6	2	9.20, 10.31 11.05, 13.99 11.81, 14.76	0.46–0.74

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DALTON DISCUSSION 5: LIGAND DESIGN FOR FUNCTIONAL COMPLEXES

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