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DIRECT SPECTROPHOTOMETRIC DETERMINATION OF FERROUS IRON USING DIACETYLMONOXIME ISONICOTINOYL HYDRAZONE

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ABSTRACT

Diacetylmonoxime Isonicotinoyl Hydrazone is new chromogenic reagent for the determination of Ferrous Iron in trace quantities by direct spectrophotometric method . Iron (II) give yellow colour with Diacetylmonoxime IsonicotinoylHydrazone in ammonium hydroxide-ammonium chloride buffer medium P^H 9 and λ_{max} 355nm. The molar absorptivity and sandell's sensitivities are 3.83 X 10^4 L.mol^{-1}.cm^{-1} And 0.002929 µg.cm $^{-2}$. Ferrous iron forms coplex with the the reagent in 1:1 ratio. Microgram quantities of iron (ii) is determined in biological samples and cement sample.

Key words: Ferrous Iron Diacetylmonoxime IsonicotinoylHydrazone, biologicalsamples, Cementsamples, Computed.

INTRODUCTION

Hydrazones are very good analytical reagents for the determination of metal ions as they form complexes with metal ions. Isonicotinoyl hydrazones are of great importance in medicinal chemistry. Many of physically active hydrazones find application[1] in the treatment of diseases like tuberculosis, leprosy and mental disorders. Hydrazones also act as herbicides, nematocides, rodenticides, plant growth regulators, anti microbial, anti tuberculosis and anti tumor activities[2, 3]. Hydrazones act as chelating agents by forming colored complexes with metal ions. The potential applications of hydrazones derivatives for the spectrophotometric determination of metal ions have been reviewed by Singh et.al[4]. The great interest towards derivative spectrophotometry is due to the increased resolution of spectral bands that means it is resolving two overlapping spectra and eliminating matrix interferences in the assay of two component mixtures using zero-crossing technique[5, 6]. In the absence of zero-crossing point, two simultaneous equations can be solved to determine the components in such a mixture[7, 8]. Derivative spectrophotometric analysis of two-component mixtures is also carried out without need to solve simultaneous equations[9,10]. Hydrazones reagents are widely used in our laboratories for the derivative spectrophotometric determination of metal ions[8-11].

Iron is the most used of all the metals. Thanks to the combination of low cost and high strength and hence it is indispensable. Its applications go from food containers to family cars, from screwdrivers to washing machines, from cargo ships to paper staples. Steel is the best known alloy of iron, and some of the forms that iron takes include; pig iron, cast iron, carbon steel, wrought iron, alloy steels, iron oxides.

Iron is one of the earliest known metals to the mankind. Besides its industrial importance, it is now clearly established that the element is essential in biological systems as well. Iron can be found in meat, whole meat products, potatoes and vegetables. The human body absorbs iron from animal products at a faster rate than from plant products. Iron is an essential part of hemoglobin, the red coloring agent of the blood that transports oxygen in our bodies. It is also an important constituent in plant enzymes, peroxidase, catalase and cytochrome oxidase. Its presence in soils and plant tissue is well known. A more common problem for humans is iron deficiency, which leads to amaemia. A man needs on an average daily intake of 7 mg of iron and a woman 11mg; normal diet will generally provide all that is needed.

Excessive iron can be toxic because free ferrous iron reacts with peroxides to produce free radicals, which are highly reactive and can damage DNA, proteins, lipids and other cellular components. Hence, owing to the significance of iron, it’s determination from associated elements by extractive spectrophotometry has been of considerable importance. A wide variety of reagents have been reported for the spectrophotometric determination of iron. A number of reagents such as hydrazone [12, 13], thiosemicarbazone [14], oxime [15-19], etc. have been used for the determination of Fe (II). However, these methods suffer from limitations such as interference of ions[20-26], requirement of masking agent[27, 28] etc.

MATERIALS AND METHODS

Apparatus

Shimadzu 160A microcomputer based U.V - Visible spectrophotometer equipped with 1.0 cm quartz cells used for all absorbance studies and amplitude measurements in derivative spectrophotometery. An ELICO LI-120 digital pH mater was used in pH adjustments.

Reagents

All the reagents used were A.R grade unless and otherwise stated. All the solutions were prepared with doubly distilled water. Stock solutions (0.01 M) of Iron(II) were prepared by dissolving requisite quantity of gallium chloride (AR GSC) and Ferrous sulphate (AR GSC) in 250 mL doubly distilled water respectively. The stock solutions were standardized and suitably diluted to obtain working solutions of metal ions.

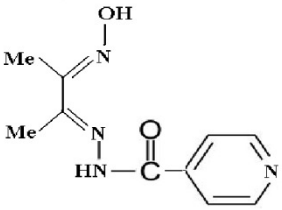

Figure 1:- Structure of Diacetyl monoxime Isonicotinoyl Hydrazone (DAMINH)

The reagent was prepared by simple condensation of Diacetylmonoxime with IsonicotinoylHydrazide in 1:1 mole ratio and its structure is given above in figure 1.The reagent solution (0.01 M) was prepared by dissolving 0.22 mg of compound in 100 mL of dimethylformamide (DMF) and the solution was found to be stable for more than three hours.

Triton X-100 solution

A 5% solution was prepared by diluting 5.0 mL of Triton X-100 (AR Merk) to 100 mL with doubly distilled water.

Buffer solutions

Buffer solutions prepared by Hydrochloric acid (1.0 M) – Sodium acetate (1.0 M) (pH 0.5 - 3.5); Acetic acid (0.2 M) - Sodium acetate (0.2 M) (pH 4.0 - 6.5); Acetic acid (0.2 M) - Sodium acetate (1.0 M) (pH 7.0); Sodium tetraborate decahydrate (0.25 M) - Hydrochloric acid (0.1 M) (pH 8.0 – 9.1); Sodium tetraborate decahydrate (0.2 5 M) - sodium hydroxide (0.1 M) (pH 9.2 - 10.8) were used in the present study.

Table-1: **Analytical Characteristics of Diacetyle Monoxime Isonicotinoyl Hydrazone**

Metal ion	**Ph**	**Color**	**λ_{max} (nm)**	**Composition of the complex**	**Stability Constant**	**Molar Absorptivity**	**Sandell's sensitivity**	**Range of determination (µg/ml)**	**Remarks Statistical data**	
									S.D.	**R.S.D (%)**
Fe(II)	9	Yellow	355	1 : 1	2.343 x 10^{5}	3.83 x 10^{4} lit $mol^{-1}cm^{-1}$	0.002929	0.540 – 5.396	0.001194	0.138
V(V)	7	Greenish Yellow	356	1 : 1	2.412 x 10^{7}	2.65 x 10^{4} lit $mol^{-1}cm^{-1}$	0.008262	0.508 – 5.084	0.002649	0.579
Ga(III)	6	Greenish Yellow	362	1 : 1	6.204 x 10^{6}	3.17 x 10^{4} lit $mol^{-1}cm^{-1}$	0.002002	0.203 – 2.033	0.0149	2.899
Ni (II)	9	Brown	365	1 : 1	3.21 x 10^{6}	4.08 x 10^{4} lit $mol^{-1}cm^{-1}$	0.00219	0.188 – 1.886	0.478	0.00182
Ru (III)	10	Yellow	363	1 : 2	8.620 x 10^{6}	3.45 x 10^{4} lit $mol^{-1}cm^{-1}$	0.005024	0.470 – 4.695	0.003349	0.589
Al (III)	8	Golden Yellow	355	1 : 2	1.01 x 10^{5}	2.69 x 10^{4} lit $mol^{-1}cm^{-1}$	0.007526	0.785 – 7.588	0.00332	0.348
Hg (II)	6	Pale Yellow	364	1 : 1	6.24 x 10^{7}	5.06 x 10^{4} lit $mol^{-1}cm^{-1}$	0.003240	0.942 -9.426	0.00174	0.302

Reaction with metal ions

At different PH values some important metal ions were tested with DAMINH reagent. The samples were prepared in 25 ml volumetric flasks by adding 10ml of buffer, 1 ml of metal ion, 1 ml of 0.01 M DAMINH, 3 ml of DMF. The reaction mixture was diluted to the mark with distilled water. The absorbance was measured in 250 - 600 nm range against reagent blank. The results are computed in table 1 which indicates that the reagent is potential for the spectrophotometric determination of various metal ions.

Recommended procedure

Determination of Ferrous iron

An aliquot of the solution containing 0.54 –5.396 µg / mL of Fe(II),0.5ml of hydrazinium sulphate, 10 ml of NH4cl-NH4OH buffer solution (pH 9.0),1 ml of triton X solution, 3 ml of DMF and 1 ml o f 0.01 M DAMINH were combined in a 25 ml volumetric flasks and the resulting solution was diluted to the mark with distilled water. The absorbance of the solution was read at 355 nm against reagent blank. The measured absorbance is used to compute the amount of Ferrous Iron from predetermined calibration curve.

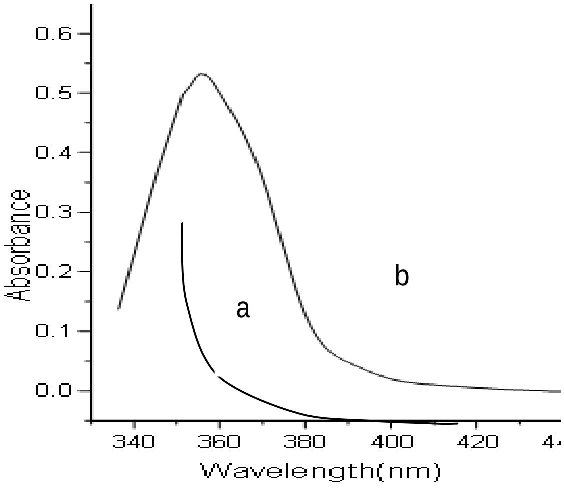

Fig: 1 Zero order Absorption Spectra, (a) Reagent DAMINH Vs water blank, (b) Fe (II) –DAMINH Complex vs Reagent blank

Absorption spectrum of Fe (II)-DAMINH complex

[Fe(II)] = 1.96×10^{-5}M

[DAMINH] = 1.96×10^{-4}M

pH = 9.0

RESULTS AND DISCUSSION

Diacetylmonoxime Isonicotinoyl Hydrazone is a new chromogenic reagent was not used for spectrophotometric simultaneous determination of Ferrous Iron so far. DAMINH reagent can easily prepared like any other Schiff base reagent. The reactions of some important metal ions with DAMINH are summarized in table1. The color reactions are mainly due to the complex formation of DAMINH with certain metal ions like Ni (II), Al (III), Ru(III), V(V), Hg(II), Ga(III) to give intense color complexes

The selectivity of the derivative methods is tested by studying the effect of foreign ions which brings about change the amplitude by ± 2 % was taken as the tolerance limit. Interference of diverse ions which often accompany with Fe(II) has been studied in the determination of 3.428 µg/mL of Fe(II). The results are computed in table3.

Table 2: Tolerance limit of foreign ions

Tolerance limit of foreign ions in the determination of 3.428 µg/ml of Fe(II)

pH = 9.0; λ_{max} = 355.0 nm

Foreign ion	Tolerance limit (µg/ml)	Foreign ion	Tolerance limit (µg/ml)
Fluoride	15	U (VI)	85
.Chloride	14.44	Al (III)	4.5
Iodide	22.15	W (VI)	7.87
Nitrate	18.38	Co (II)	6.55
Acetate	32.25	Se (IV)	12.45
Oxalate	36.68	Mn (II)	5.76
EDTA	41.24	Cr (VI)	0.41
thiosulphate	28.16	Cu (II)	0.81
		Ce (IV)	6.231
		Ni (II)	0.780
		Zr (IV)	1.890
		La (III)	31.75
		Pd (II)	0.35

		Sn (II)	4.480
		Sr (II)	5.39
		Th (IV)	9.055
		Mo (VI)	28.43
		Mg (II)	1.314
		Ca (II)	0.591

Standard Deviation (S.D.) = 0.001194 for ten determinations

R.S.D. = 0.138 %

Table 3: Statistical analysis of the data

[Fe(II)] = 2.4×10^{-5}M

[DAMINH] = 3.6×10^{-4}M

λ_{max} = 355.0 nm

pH = 9.0

S. No.	**Volume of metal ion (1ml)**	**Volume of reagent (1 ml)**	**Absorbance (x)**	**d (X - M)**	**d^2 $(X - M)^2$**
1	1	1	0.956	0.0013	0.00000169
2	1	1	0.955	0.0003	0.00000009
3	1	1	0.953	-0.0017	0.00000289
4	1	1	0.953	-0.0017	0.00000289
5	1	1	0.954	-0.0007	0.00000049
6	1	1	0.955	0.0003	0.00000009
7	1	1	0.957	0.0023	0.00000529
8	1	1	0.953	-0.0017	0.00000289
9	1	1	0.956	0.0013	0.00000169
10	1	1	0.955	0.0003	0.00000009

Applications

The spectrophotometric method developed is applied for the determination of Fe(II) in plant, human blood and cement samples.

i. Andhra Pradesh Agricultural Research institute, Hyd, India, supplied the grape leaf sample solution.

ii. Determination of iron in Human blood

The blood sample is collected from Kurnool Medical College (KMC) Kurnool, AP, India. The blood sample is prepared by the procedure described in the literature. 10 ml of blood sample is taken in Kjeldahl flask and heated with 10 ml of the mixture of nitric, sulphuric and per chloric acids (taken in the ratio 3:1:1). The mixture is evaporated to dryness. The residue is dissolved in 5ml of slightly acidified water by heating. The solution is transferred quantitatively into a 100 ml volumetric flask and made up to the mark with distilled water. The solution is further diluted as required.

iii. Determination of iron in cement sample

The cement is brought into solution by adopting the following procedure.

Required amount of cement is taken, dissolved in concentrated hydrochloric acid and digested for half an hour by heating on a hot plate. The solution is cooled and filtered and the filtrate is collected quantitatively in a 50 ml standard flask and made upto the mark with double distilled water.

General procedure

A known aliquot of sample is taken in 10 ml volumetric flask containing 5 ml of buffer of pH 9; 0.5 ml of 0.25 M hydrazinium sulphate (to reduce Fe(III) to Fe(II)), 0.5 ml of 0.1 M thiourea solution (to mask copper) and 1 ml of reagent solution (1 x 10^{-2} M) were added to the flask and the contents are made upto the mark with distilled water. The absorbance of the solution was measured at 355 nm against reagent blank. The amount of iron present in the sample was determined from the pre-determined calibration plot. The results are presented in the following tables.

Table 4: Determination of iron in grape leaves

Sample	Amount of iron(II) (μg/ml)		Relative error %
	APARI value	Present method*	
Leaf sample of grape (Vitis Vinifera L)	32.80	32.32	-0.84

* Average of four determinations.

Table 5: Determination of iron in human blood

Sample	Amount of iron (µg/(milligram)		Relative error %
	AAS method	Present method*	
Human blood	773.2	772.9	-0.32

* Average of four determinations.

Table 6: Analysis of cement

Sample and composition %	**Amount of iron**		**Relative error %**
Portland cement	Taken (ppm)	found (ppm)	
Cao = 50.60, SiO_2 = 20.25	0.100	0.102	+1.4
Al_2O_3 = 5.10, MgO = 2.3	0.300	0.304	+1.20
Fe_2O_3 = 1.2, SO_3 = 1.2 Na_2O = 1, K_2O = 1	0.500	0.485	-3.15

CONCLUSIONS

Direct spectrophotometric determination of Ferrous Iron using DAMINH in blood , grape leaves and cement solution is not laborious and do not involve any extraction , separation or heating the contents. Ferrous iron is not oxidized to ferric iron by adding hydrazinium sulphate . Hence the present method of determination of ferrous iron is simple , rapid , selective and reasonably sensitive. In the present method diverse cations and anions do not interfere in the determination of iron . Hence ferrous can be accurately determined by the proposed method.

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