

CHAPTER-3

THEORETICAL ANALYSIS

Hydrazones are prominent class of chromogenic organic reagents, which are used for the determination of metal ions by spectrophotometric method. Generally each type of chromogenic reagents has more than one functional groups. In this point of view the author has designed and synthesized new chromogenic reagent. This organic reagent show good analytical properties with various metal ions. According to the literature survey of the previous work shows that hardly any hydrazones are used as analytical reagents for the determination of metal ions by direct and derivative spectrophotometric method. In the literature previously Direct and derivative spectrophotometric determination of metal ions was reported.

Aims and objectives of present investigation

The present research work focus on the synthesis and characterisation of new organic reagent and determination of metal ions by employing new chromogenic organic reagent by direct and derivative spectrophotometry. In this context present investigations are as follows.

- i) Synthesis of new chromogenic organic reagent α -Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH).
- ii) To investigate the characterization and analytical properties of above synthesized reagent.
- iii) Aimed to develop sensitive and selective method for the determination of Hg (II) and Cd (II) by ACINH by spectrophotometry.
- iv) To study the applications of Novel reagent in the estimation of metal ions like Mercury (II) in Water samples and Cadmium (II) in Soil samples.

Materials and Methods

3.1 Preparation of solutions

The general procedures and chemicals used for the determination of metals by spectrophotometry is presented in this chapter.

3.1.1 Preparation of reagent solution

The reagent was synthesized by simple condensation of α -amyl Cinnamaldehyde and Isonicotinoyl hydrazide. Stock solution 0.01M of reagent was prepared in water by dissolving appropriate quantity of reagent substances.

α - Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH)

0.01M solution of the ACINH was prepared by the dissolving 3.214gm in 100 of water in 100 ml volumetric flask. For preparation of working solution stock solutions were diluted with distilled water.

3.1.2 Preparation of metal ion solutions

(i) Preparation of Mercuric chloride solution

The stock solution of Mercury (II) (1×10^{-2} M) was prepared by dissolving 0.2715gm of HgCl_2 (AR BDH) in distilled water and diluted using double distilled water in a 100ml volumetric flask. The stock solution was standardised.

(ii) Preparation of Cadmium nitrate solution

The stock solution of Cadmium (II) (1×10^{-2} M) was prepared by dissolving 0.30847 gm of $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (AR SMC) in minimum amount of dilute nitric acid and diluted using double distilled water in a 100 ml volumetric flask. The stock solution was standardised.

3.1.3 Preparation of Inorganic stock solutions

By dissolving sufficient amount of salts in required quantity of distilled water inorganic stock solutions were prepared. Where ever necessary to eliminate hydrolysis before dilution a few drops of suitable acid were added. By using standard

procedures prepared solutions were standardized. The formulae, quality, specific gravity or molecular weight and stock solution molarity are summarized in Table 3.1.

Table 3.1: Salt solutions used in the qualitative and quantitative studies

Ion/ Reagent	Formula	Quality and make	Sp.Gr/ Mol.Wt	Molarity of the stock solution
Silver(I)	AgNO ₃	AR S.d.Fine	169.87	0.01
Aluminium (III)	Al ₂ (SO ₄) ₃ .(NH ₃) ₂	AR BDH	453.33	0.01
As(III)	As ₂ O ₃	AR Hopkin& William Ltd	197.82	0.01
Barium (II)	BaCl ₂ .2H ₂ O	AR S.d.Fine	244.27	0.01
Bismuth (III)	Bi(NO ₃) ₃ .5H ₂ O	AR GSC	485.07	0.01
Calcium(II)	CaCl ₂ .2H ₂ O	AR SMC	147.02	0.01
Caesium (IV)	((NH ₄) ₂ Ce(NO ₃) ₆)	AR LOBA	548.23	0.01
Cadmium (II)	Cd(CH ₃ COO) ₂	AR BDH	266.52	0.01
Cobalt (II)	Co(NO ₃) ₄ .6H ₂ O	AR BDH	291.04	0.01
Chromium (VI)	K ₂ Cr ₂ O ₇	AR BDH	294.19	0.01
Copper (II)	CuSO ₄ .5H ₂ O	AR GSC	249.68	0.01
Fe(II)	(NH ₄) ₂ FeSO ₄ .6H ₂ O	AR S.d.Fine	392.14	0.01
Fe(III)	NH ₄ Fe(SO ₄)12H ₂ O	AR BDH	482.19	0.01
Mercury (II)	HgCl ₂	AR BDH	271.50	0.01
Lanthanum (III)	La(NO ₃) ₃ .6H ₂ O	GR LOBA	433.02	0.01
Lithium (I)	Li ₂ SO ₄ .H ₂ O	AR LOBA	127.76	0.01
Manganese (II)	MnSO ₂ .4H ₂ O	GR SM	223.06	0.01
Molybdenum (VI)	Na ₂ MoO ₄ .2H ₂ O	E.Merck	241.95	0.01
Sodium(I)	NaNO ₃	AR BCP Ltd.	85.00	0.01
Nickel (II)	NiSO ₄ .7H ₂ O	ARBDH	280.80	0.01
Lead (II)	Pb(NO ₃) ₂	AR BDH	331.20	0.01
Lead (II)	PdCl ₂	AR J.M & Co.Ltd	177.33	0.01
Ruthenium (III)	RuCl ₃ .3H ₂ O	AR LOBA	225.42	0.01
Selenium (IV)	Na ₂ SeO ₃	AR LOBA	172.94	0.01

Antimony (III)	$\text{KSb.C}_4\text{H}_4\text{O}_6$	AR BDH	324.93	0.01
Tin(II)	$\text{SnCl}_2.2\text{H}_2\text{O}$	LR Nice	225.63	0.01
Strontium (II)	$\text{Sr}(\text{NO}_3)_2$	AR GSC	211.63	0.01
Titanium(IV)	$\text{K}_2\text{TiO}_2(\text{C}_2\text{O}_4)2\text{H}_2\text{O}$	AR BDH	354.17	0.01
Thorium(IV)	$\text{Th}(\text{NO}_3)_4.6\text{H}_2\text{O}$	AR BDH	354.17	0.01
Uranium (VI)	$\text{UO}_2(\text{CH}_3\text{COO})_2.6\text{H}_2\text{O}$	AR William M.Ltd.	516.10	0.01
Vanadium (IV)	$(\text{VO})_4\text{SO}_4.4\text{H}_2\text{O}$	Merck	239.03	0.01
Tungsten (VI)	$\text{Na}_2\text{WO}_2.2\text{H}_2\text{O}$	AR BDH	329.86	0.01
Zink (II)	$\text{ZnSO}_4.7\text{H}_2\text{O}$	AR BDH	287.54	0.01
Zirconium (IV)	$\text{Zr}(\text{NO}_3)_4$	AR LOBA	570.13	0.01
Br^-	NaBr	AR BDH	102.91	0.1
I ⁻	KI	AR BDH	166.00	0.1
Cl^-	KCl	AR BDH	136.09	0.1
F^-	NaF	Beckers and AAdams, USA	42.00	0.1

3.1.4: Preparation of Buffer solution

By using standard procedures buffer solutions were prepared. The buffer solutions used for further studies are summarized in Table 3.2.

Table 3.2: Preparation of Buffer solutions

pH	Constituents
1.0-3.0	1 M Hydrochloric acid + 1 M Sodium acetate
3.0-6.0	1M Acetic acid + 0.2 M Sodium acetate
7.0	1M Sodium acetate + 0.2 Acetic acid
8.0-12.0	2.0 M ammonium chloride + 2.0 M Ammonia

According to literature survey the determination of metal ions at micro gram level there is need to develop simple, selective and sensitive spectrophotometric method. Keeping this in mind, the determination of various metal ions in different samples the cost effective, sensitive and simple spectrophotometric method was

developed. All the developed methods were applied for the determination of metal ions and the results are compared with certified results. Newly synthesised chromogenic organic reagent is used for spectrophotometric determination of Hg(II) and Cd(II). The following methods, materials and experimental procedures were adopted.

3.2 General experimental procedures

For the determination of different metal ions like Mercury (II) and Cadmium(II) in various samples at microgram levels by newly synthesized analytical chromogenic organic reagent α -Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH) the following procedure was employed.

The reagent α -Amyl Cinnamaldehyde Isonicotinoyl Hydrazone (ACINH) reacts with Hg (II) and Cd (II) in basic medium to give colour complexes and these reactions were studied systematically. In this the reaction conditions and experimental procedures employed for the determination of Mercury and Cadmium are given.

3.2.1 Absorption spectra of reagent solution and metal complexes

In a 10 ml volumetric flask appropriate reagent solution ($1 \times 10^{-2} \text{M}$) and 5 ml of buffer solution of required pH were taken and the mixture is diluted with distilled water. At suitable wavelength region the absorbance of the solution is measured against a blank consisting of 5 ml of buffer solution and diluted with distilled in a volumetric flask (10ml). A plot is drawn between wavelength and absorbance.

In a 10-ml volumetric flask, 5ml of buffer solution of desired pH, appropriate volume of reagent solution ($1 \times 10^{-2} \text{M}$) and metal ion solution ($1 \times 10^{-3} \text{M}$) were taken, mixture is diluted with distilled water. At suitable wavelength region the absorbance is measured against blank solution prepared under identical conditions. A plot between wavelength and absorbance is drawn from which the analytical wavelength is selected.

3.2.2 Effect of pH on the absorbance of the solution containing complex species

In a set of 10 ml of volumetric flasks a known aliquot of metal ion ($1 \times 10^{-3} \text{M}$) and appropriate volumes of reagent solutions ($1 \times 10^{-2} \text{M}$) are taken. Each flask containing 3ml of buffer solution (different pH values 1.0 to 11.0), mixture is diluted with distilled water and the absorbance of these solutions are measured against the corresponding reagent blank at the selected wavelength. A graph was plotted between pH and absorbance, from the graph the corking is chosen.

3.2.3 Effect of reagent concentration

The following procedure was used to find out optimum amount of reagent required for full colour development. A known and varying aliquot of reagent solution ($1 \times 10^{-2} \text{M}$) was taken in a set of 10ml volumetric flasks each containing fixed amount of metal ion solution and 3 ml of buffer solution mixture were diluted with distilled water. In each flask absorbance of the solution was measured at selected wavelength against suitable reagent blank prepared identically.

3.2.4 Effect of time on the absorbance of reaction mixture and stability

In a 10 ml volumetric flask 3ml buffer solution, 1 ml of reagent ($1 \times 10^{-2} \text{M}$) (usually 10-fold molar excess) and 1ml of metal ion ($1 \times 10^{-3} \text{M}$) (usually in the range Beer's law) and made up along with distilled water up to the mark. At a selected wavelength the absorbance of coloured complex solution was measured with different time intervals against reagent blank prepared similarly. Time interval required for full colour development and time stability of the complex was known from this experiment.

3.2.5 Adherence of the system to Beer's law

To ascertain the sensitivity of the colour reactions and to explore the possibility of determining micro amounts of metal ions the following method is adopted.

In a set of 10ml volumetric flasks varying known aliquots of metal ion solutions and each containing 3ml buffer solution of desired pH an excess of reagent solution ($1 \times 10^{-2} \text{M}$) are added and mixture were diluted with distilled water and the absorbance of the solution is measured at the analytical wavelength against the reagent blank. A plot is constructed between quantity of metal ion ($\mu\text{g/ml}$) and absorbance. The slope and the intercept of the plot are computed by the slope molar absorptivity is calculated.

3.2.6 Effect of foreign ions on the absorption of reaction mixture

In order to assess the applicability of the proposed method for the analysis of synthetic or real samples containing metal ions, the effect of the presence of various foreign ions which were generally found associated with the test metal ions on the absorbance of the complexes solution was studied by adopting the following method.

In a 10ml volumetric flasks 3ml of buffer solution of desired pH, fixed amount of metal ion solution were taken. Then appropriate amount of foreign ion was added to all the flasks except one and at the end the reagent solution added to all. The contents were diluted with distilled water. At a chosen wavelength against reagent blank, the absorbance of complex was measured. A tolerance limit of the foreign ion was determination from the absorbance. The amount of foreign ion which brings about a change in absorbance by $\pm 2\%$ was taken as its tolerance limit. By employing adequate quantity of suitable masking agents some of the cations interfering were masked.

3.2.7 Composition and Stability of the complexes

By using Job's continuous variation method and mole ratio methods the composition of the complex species was calculated, by employing data obtained from jobs' plot, stability constant of complexes were calculated.

3.2.7.1 Job's Continuous variation method

In a set of volumetric flask (10ml), equimolar solutions reagent solution, metal ion solution and 3ml of buffer solution of desired pH were added to each flask, in

such a proportion that the total volume of the solution was 10ml and contents were diluted with distilled water. In each flask at a selected wavelength, the absorbance of the coloured complex was measured against a corresponding reagent blank solution. A plot between absorbance and mole fraction of the metal ion (C_M/C_{M+C_L}) or (V_M/V_{M+V_L}) was made from which composition of the complex was computed.

3.2.7.2 Molar ratio method

In a 10ml volumetric flask known volume of metal ions solution ($1 \times 10^{-3}M$), 3ml of buffer solution and varying of the reagent solution ($1 \times 10^{-2}M$) were added. The contents of the each flask diluted with doubly distilled water. At the selected wavelength the absorbance of each solution is measured against the corresponding reagent blank solution prepared under identical conditions. From the plot between the volume of the reagent and absorbance, the composition of the complex is ascertained.

3.2.8 Determination of stability constant of the metal complexes

By using Job's method data stability constant of the complex species can be calculated under investigation. For the complex formation reaction, the stability constant β in terms of experimentally obtained absorbance value was given by the general equation $mM + nL \rightarrow M_mL_n$

$$\beta = \frac{A/A_m}{m^m n^n [(1 - A/A_m)]^{m+n} [C]^{m+n-1}}$$

where

A_m = Absorbance corresponding to the point of inter of the extrapolated lines

A = Observed absorbance at concentration 'C'

C = Concentration corresponding to the point of intersection

β = Stability constant

For 1:1 complex

$$\beta = \frac{1-\alpha}{\alpha^2 C}$$

For 1:2 complex

$$\beta = \frac{1-\alpha}{4\alpha^3 C^2}$$

For 1:3 complex

$$\beta = \frac{1-\alpha}{27\alpha^4 C^3}$$

$$\alpha = \frac{A_m - A}{A_m}$$

α = degree of dissociation

3.2.9 Applications

The determination of Hg(II) and Cd(II) in Water samples and soil samples respectively the present methods were applied. Synthetic samples were prepared where ever original samples are not available and used.

Recommended procedure for the estimation of real or synthetic samples

In 10ml volumetric flasks 3 ml buffer solution, a known aliquot of the sample solution and suitable aliquot of the reagent solution ($1 \times 10^{-2} \text{M}$) was added and contents of the flasks were diluted with distilled water. Reagent blank solution prepared similarly. Against reagent blank solution the absorbance of the complex was measured at a selected wavelength (λ_{max}). The absorbance values were referred to the pre-determined calibration plot to compute the amount of the metal ion.

3.2.9.1 Water samples

By using whatmann filter paper, water samples (250ml) were filtered and concentrated HNO_3 (10ml) were added to the water samples in distillation flask (500ml). By applying the recommendation method the samples was digested in the presence of KMnO_4 . After cooling the solution by adding NH_4OH , the solution has got neutralized. In a calibration flask (25ml) digest solution was transferred and using deionised water up to the mark solution was diluted.

3.2.9.2 Soil samples

Soil samples like roadside, agricultural, contaminated soil were taken. The soils were air dried and in a 100ml flask, 100mg of accurately weighing homogenate soil samples were taken. By using oxidising agent the samples were digested. Using

no 41 filter paper the content of the flask was filtered in to a volumetric flask (25ml) and by using dilute NH_4OH , solution was neutralised and diluted with distilled water.

3.2.10 Derivative spectrophotometric procedure

Derivative spectrum of the solution containing the complex species

In a volumetric flask (10ml), metal ion solution ($1 \times 10^{-3}\text{M}$), 3 ml of buffer solution of desired pH and the reagent (usually 10 fold molar excess to metal ion) solutions are taken. The contents on the flaks were diluted with distilled water. Its absorbance spectrum in a suitable wavelength region is recorded against the reagent blank, prepared under identical conditions, the derivative (first order) spectra was recorded from zero order spectrum with 9 degrees of freedom, speed, scan and fast in a wave length region 300-650nm against the reagent blank prepared identically. By peak zero method the derivative amplitude was measured. This technique is applied in the estimation of metal ions in various water samples and soil samples. Various diverse ions were also studied when they interface with the complexes. The Beer's law verification and the different amounts Vs amplitude plots are drawn for all metal ions when they interact with the synthesized reagent. The experimental investigations and the results are discussed in next chapters.

3.3 A brief description of instruments employed in the present investigation

3.3.1 UV-Visible spectrophotometer

For carrying out analytical measurements in the present work Shimdzu, Japan make model No UV (160A) UV-Visible double beam spectrophotometer was used.

3.3.2 Digital pH meter

For measuring the pH of different buffer solutions, ELICO digital pH meter model No LI-120 manufactured by M/S ELICO private ltd. India was employed in the present study with temperature compensate arrangement. The reproducibility of measurement within ± 0.01 pH.

3.3.3 Digital electronic balance

Sartorius BS/BT2245 model electronic analytical balance having maximum capacity of 220gm and sensitivity of ± 0.1 mg was employed for weighing purpose.