

CHROMIUM DETERMINATION IN JATROPHA PLANT SAMPLES BY ASABT USING UV-VIS SPECTROPHOTOMETRIC METHOD

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Abstract

A very simple, selective and sensitive method is developed for the spectrophotometric determination of Cr (III). The reagent 5-Acetylsaliylamide-2-iminothiophenol (ASABT) provides a yellow colored solution with Chromium (III) in an aqueous medium. The maximum absorption of the reagent λ_{max} shows at 350 nm. The maximum absorption of the pH range was observed at 3.0-5.0. The Beer's law validity was 0.05-3.46 $\mu\text{g/ml}$. The Molar absorption factor i.e., $5.78 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ and Sandell's sensitivity 0.00173 $\mu\text{g/ml}$. The Specific absorption factor was $0.00916 \text{ ml g}^{-1}\text{cm}^{-1}$. The composition of metal and ligand (M: L) obtained in Job's and Molar quantitative relation technique, are in 1:1 (M: L) ratio and 1.002×10^{-3} was calculated as the stability constant of the complex. For full-colour improvement, a ten molar chemical reagent in excess was necessary. Chromium (III) of concentration i.e., 2.12 $\mu\text{g/ml}$ was used in the calculation of standard deviation for a set of ten various determinations was calculated as 0.0013 $\mu\text{g/ml}$. The calculated Relative standard deviation of the system was calculated as 0.5849% and Mean absorbance was found to be 0.220 ± 0.01 . The results obtained are in excellent agreements with the nominal values.

Key Words: Chromium determination, ASABT, Spectrophotometry and Plant Samples.

Introduction

In recent years, herbal medicine has been marked to cure diseases for more than 80% of the world's population. The traditional herbal medicine plays a major role in their primary health care¹. Jatropha roots are used for the treatment of leprosy and stem latex possess anticoagulant activity.^{2, 3} The leaves of Jatropha gossypifolia are used for the treatment of fevers, carbuncles, itches, sores on the tongues of babies, stomachache, renal disease and blood purifier.⁴ The leaf is used for healing wounds.⁵ Due to importance of plant parts, there is need to find the trace elements and accumulation of those elements for lively applications. Among all those Cr was recognizable and important. Chromium is a lustrous, brittle and hard metal. Its color is silver-grey and can be highly polished. In nature, Chromium exists in two unique stable oxidation states trivalent (Cr-III) and hexavalent (Cr-VI). Chromium is the 21st most abundant mineral in the earth. In the US soils, the average concentration of Chromium is around 40 mg/kg.⁶ Chromium (III) utilization is increased by some industries like steel, leather, textile, and electroplating. This research focuses on the recent developments in the field of plant interactions and the utilization of Chromium in biology.

Chromium is an essential nutritional trace element that improves the body's responses to the hormone insulin, which is essential for blood sugar control⁷. The role of Chromium in the organism is parallel to insulin role in the organism. In the presence of organic chromium in the decreased level of insulin is enough to acquire a comparable organic activity.^{8, 9} Chromium did not have any effect on antibody agent in the reaction it doesn't join with immunization against infections caused by bovine rhinotracheitis (IBR).¹⁰ Chromium no longer have an impact on antibody reaction to mixed vaccination towards infections bovine rhinotracheitis (IBR). Chromium

deficiency additionally leads to an increase in hematological parameters i.e. hemoglobin, hematocrit, erythrocytes, leucocytes and suggest erythrocyte quantity. These plants were existing abundantly in all regions. For this work, these plants were collected at botanical garden, Y.V. University, Kadapa, A.P, and India ¹¹.

For the determination of chromium in natural plants, various methods, such as 1) spectrophotometric method, 2) catalytic method, 3) Chemiluminescence method, 4) atomic absorption, and 5) neutron activation methods have been recommended ¹². Many spectrophotometric methods for the determination of chromium have been reported with some chromogenic reagents such as BZATDT in plant samples and ASABT for Se determination.

The better results of ASABT method suggest that the reagent was more selective for Cr (III) determination. As per the above signified, it was confirmed that the reagent ASABT was more sensitive than others for determination of Cr. This paper describes determination of Cr and analytical properties using new reagent viz., the 5-Acetyl-salicylamide-2-Iminothiophenol (ASABT). Since the reagent is more sensitive and it is also used for the determination of Se in various water, soil and medicinal plant samples¹³. The spectrophotometric method was less expensive instrumentation than atomic absorption and atomic emission methods. The spectrophotometric method gives greater sensitivity when compared to other methods. The proposed method, when compared with other spectrophotometric methods, is more sensitive and selective. It also offers advantages like reliability and reproducibility in addition to its simplicity instant color development and less interference. The results obtained through UV- Visible spectrophotometer have been compared with those obtained through the ICP-OES ¹⁴.

Materials and Methods

Apparatus

A Shimadzu (Model- 1800) UV-VIS spectrophotometer and ELICO model LI-610 pH meter with combination electrodes were used for measurements of absorbance and pH respectively. ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry Model-7000) methods were used for the quantitative analysis of heavy metals in all samples.

Reagents and solutions

All chemicals used were of the analytical reagent grade of the highest purity available procured from Merck. Double distilled de-ionized water and glass vessels were cleaned soaking in acidified solutions of $K_2Cr_2O_7$ followed by washing with $Con.HNO_3$, and was rinsed several times with high purity deionized water. Stock solutions and plant samples were kept in polypropylene bottle.

Preparation of reagent (ASABT)

The methanolic solution (60 ml) containing 5-Acetyl salicylamide (1.5g, (or) 0.0098 mol) was mixed to 2-iminothiophenol (1.36g, (or) 0.0098 mol) in 60 ml of methyl alcohol. The subsequent combination was taken in 250 ml round bottom flask. To this, 10 ml of 1 molar sodium acetate solution and two or three drops of $Conc. H_2SO_4$ were added. The reaction mixture was allowed to reflux for 10 hours. On cooling the reaction mixture, the ash colored product was collected. It was gathered with the aid of filtration and washed several times with warm water and 50 percent methanol and with n-hexane at the end. This compound could be re-crystallized from methanol and dried in vacuum. The percentage of yield became 91gms and the melting point of ASABT was $86^{\circ}C$.

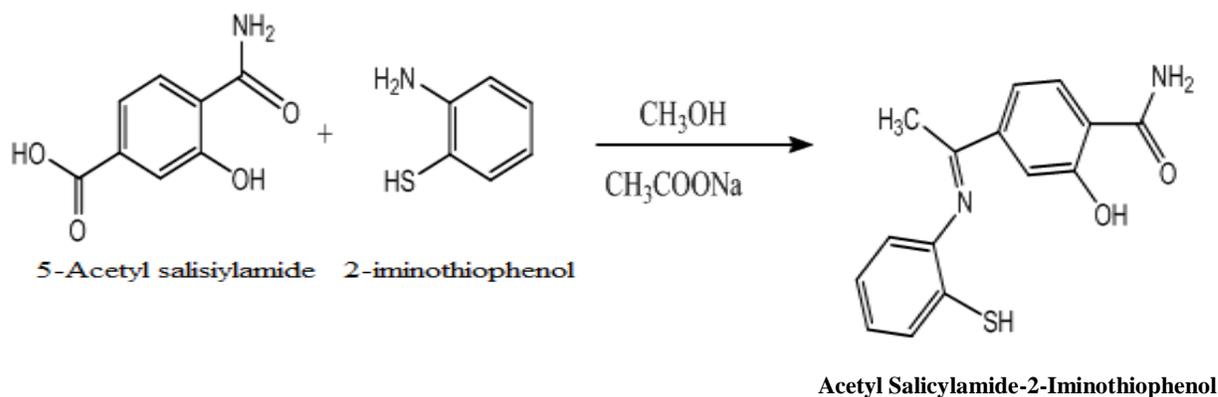


Fig. 1. Preparation and Structure of ASABT reagent

Characterization of reagent (ASABT)

The reagent has been synthesized and characterized by IR, ¹HNMR and mass spectral data. Infrared spectrum of ASABT shows bands at 3377, 3167, 2334, 1714, 1613, 1583, 1662, 1583, 1470, 1442, 1153 and 1021 respectively corresponding to ν (O-H) symmetric stretch, ν (C-H) stretch (pyridine) (sp^2 -CH), ν (OCH₃) stretch, ν (C = O) Aromatic stretch, ν (C = N) Schiff base, ν (C (-OCH₃)) Aromatic stretch, ν (C = N) azomethine stretch, ν (C - C) Aromatic stretch, ν (H - C=N) (azomethine stretch), ν (C-H) (Aromatic ring), ν (C - O) stretch, ν (C - N) stretch. ¹HNMR spectrum of ASABT (DMSO-d₆) showed signals at 6.75 – 7.43 (7H), 9.3 (¹H) due to Hydroxyl proton, 3.7 (3H) due to -CH₃ group, 5.4 (¹H) due to -SH group, 6.4 (2H) due to -NH₂. Mass spectrum of ASABT shows a signal at 286 (M+1) corresponding to its molecular ion peak. The molecular formula of the reagent is C¹⁵ H¹⁴ N² O² S (Molecular weight-287).

pKa values of reagent

The pKa values were determined by recording the UV-Visible spectra of 1X10⁻³ M solutions of the reagent at various pH values and by taking the arithmetic mean of the values obtained from the measurements at different wavelengths determined spectrophotometrically using Phillips and Merrit method. The values of deprotonation of ASABT were 7.0 (pK₁) and 10.0 (pK₂).

ASABT solution

By using methanol, the stock solutions are prepared with the reagent's concentration (1 X 10⁻³ M). This has been prepared by dissolving a suitable amount of reagent individually. The stock solution was made by taking 0.0068 g of the reagent (ASABT) in a 25 ml volumetric flask. The correct weighed amount of the reagent was dissolved in methanol solvent and diluted on top of the mark with the equivalent solvent.

Chromium (III) solution

A (1 X 10⁻³ M) inventory solution of chromate was prepared by dissolving 0.3312 g of Potassium chromate in Milli-Q water. To this solution, added a few drops of concentrated Nitric acid and made up to the mark in a 100 ml volumetric flask. Aliquots of this solution had been standardized with EDTA titration using xylenol orange as an indicator. Dilute solutions were prepared from this stock solution.

Buffer solution

With the help of the literature and by following strategies preferred for the preparation of buffer solution. The following solutions were utilized for the preparation of buffers solution given below. 1M Sodium acetate + 0.1M hydrochloric acid (0.5 – 3.0), 0.2M Sodium acetate + 0.2M acetic acid (3.5 – 6.0), 1M Sodium acetate + 0.2M acetic acid (6.5 – 7.5), 2M Ammonia + 2M ammonium chloride (8.0 – 12.0) buffer solutions are prepared in distilled water. Suitable portions of these solutions are mixed to get the desired pH.

Preparation of samples (Sample preparation)

The collected various parts of the medicinal plants were washed several times with water, finally with deionized water. The species were dried in shade and ground to fine powder with the help of mechanical grinder and stored in an air tight glass container. These samples were used for further analysis.

Medicinal plant samples

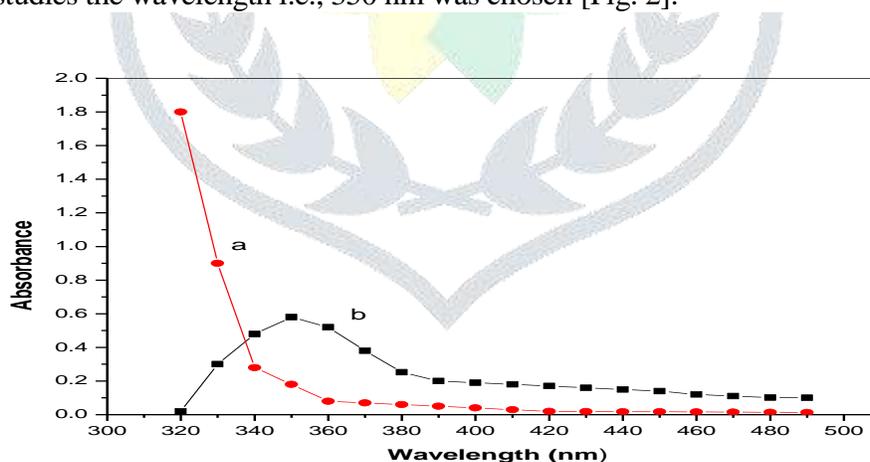
Sample preparation of the plant samples was carried out by microwave digestion. Approximately 1g (dry mass) of plant sample materials were weighed directly into the PTFE vessels, to which 10 ml of concentrated Nitric acid was added and the vessels were capped immediately. The digestion programme consisted of a ramp of time for 10 min. till the temperature reach to 150⁰C. The power was 800 W. After the completion of program vessels was cooled, vented and opened and then 2 ml of 30% Hydrogen peroxide as added and filtered the solutions into 25 ml volumetric flasks and made up with double distilled water. The blanks were prepared by following similar digestion procedure without plant material.¹⁵

Results and Discussion

A light greenish coloured solution was formed instantaneously when 5-Acetyl salicylamide-2-iminothiophenol (ASABT) was added to Cr (III) solution. To this reaction mixture, 10 ml buffer solution of pH 4.0 was added. Consequently, the coloured compound formed was used for the determination of Chromium (III) in the aqueous medium. By using the spectrophotometric technique, the coloured reaction was examined thoroughly for the determination of Chromium (III) in an aqueous medium.

Absorption spectra of ASABT and the complex solution

The absorption spectra containing Cr (III) and ASABT complex solution were taken against reagent blank. A graph is drawn between absorbance on Y- axis and wavelength (nm) on X-axis and was shown in Fig. 2. At pH 4.0, the reagent of ASABT was taken against water as blank and the wavelength varies from 250 - 600 nm. At 350 nm, the Cr (III) complex solution shows more absorption and the chemical reagent blank has less absorbance. Hence, for additional studies the wavelength i.e., 350 nm was chosen [Fig. 2].



**Fig. 2. Absorption spectra of a). ASABT Vs Water blank
b). Cr (III)-ASABT complex Vs ASABT solution**

Effect of pH on the absorbance of experimental solution

The study of colour intensity of Cr (III) with ASABT complex with respect to the impact of pH was studied and pH was established. The graph indicates that the complex shows maximum and constant absorbance in the pH range of 3-5.¹⁶ Hence, the pH 4.0 was chosen for subsequent studies [Fig. 3]. Figure 3 shows that the pH increases and absorbance value remains constant.

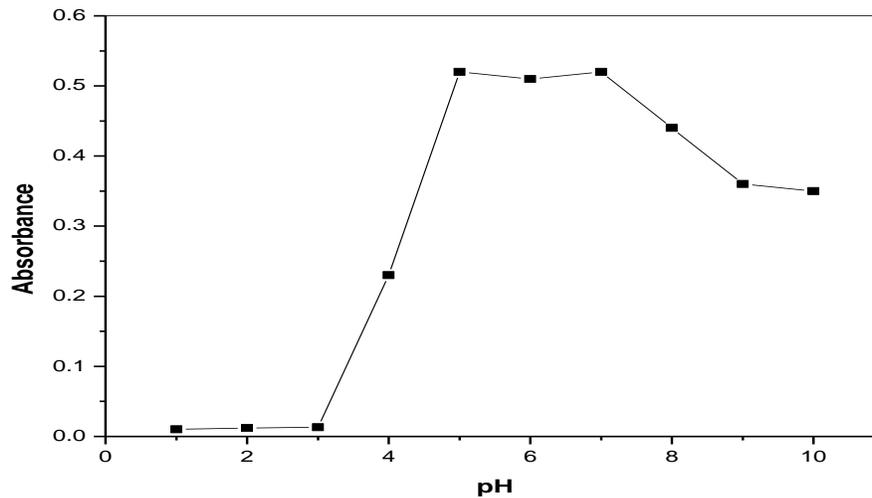


Fig. 3. Effect of pH on absorbance of Cr (III) complex [Wavelength = 350 nm.]

Absorption spectra of reagent and metal complexes

The effect of ASABT concentration and absorbance was studied at $[\text{Cr (III)}] = 1 \times 10^{-3} \text{ M}$, $\text{pH} = 4.0$ & $\lambda_{\text{max}} = 350 \text{ nm}$ and the results were given in Table 1. As per procedure, the quantitative relation of the reagent to the metal ions that is essential for complete colour improvement was established¹⁷. The information in Table 4 indicates that a ten-fold molar excess chemical reagent was comfortable for complete colour improvement. Thus additional studies were employed for the development of 10-fold molar chemical reagent to Chromium (III) element. The obtained results were good agreement with other parameters.

Table 1. Effect of ASABT concentration and the absorbance of the Cr (III) complex. $[\text{Cr (III)}] = 1 \times 10^{-3} \text{ M}$, $\text{pH} = 4.0$ & $\lambda_{\text{max}} = 350 \text{ nm}$]

Cr (III): ASABT	Absorbance
1:05	0.589
1:10	0.653
1:15	0.676
1:20	0.68
1:25	0.675
1:30	0.662

Optimum time required for the absorbance of Chromium (III) complexes

Based on the procedure, the absorbance of metallic element i.e., Chromium (III) with ASABT complex was estimated to determine the time stability of the complex at various time intervals. The absorbance of Chromium (III) complex was estimated at 350 nm. The concentration of [ASABT] and [Cr (III)] was $1 \times 10^{-3} \text{ M}$ and $1 \times 10^{-3} \text{ M}$ respectively.¹⁸ The colour development was immediate and remains constant for over twenty-four hours.

Order of addition of constituents of reaction mixture

There was no adverse effect on the absorbance of the Cr (III) with ASABT complex with the addition of buffer, metal ion, and reagent.

Beer's law

For analyzation of Beer's law relevancy a linear plot between the quantity of Chromium (III) and absorbance was explained.¹⁹ The straight line complies with the condition $A_{350} = 0.24838 - 0.04359$ [Fig. 4] and the system complies Beer's law within the range of 0.05-3.46 $\mu\text{g/ml}$ of Cr (III). The Molar absorptivity property of the system was $5.78 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ and Sandell's sensitivity of the system was $0.00173 \mu\text{g/ml}$ of Cr (III) individually. The Cr (III) having the concentration of $2.12 \mu\text{g/ml}$ and the Standard deviation for ten determinations was 0.0013 .²⁰ The specific absorptivity of the system was found to be $0.00916 \text{ ml g}^{-1}\text{cm}^{-1}$. The relative standard deviation and Mean absorbance 0.5849% and 0.220 ± 0.001 respectively and has individuality.

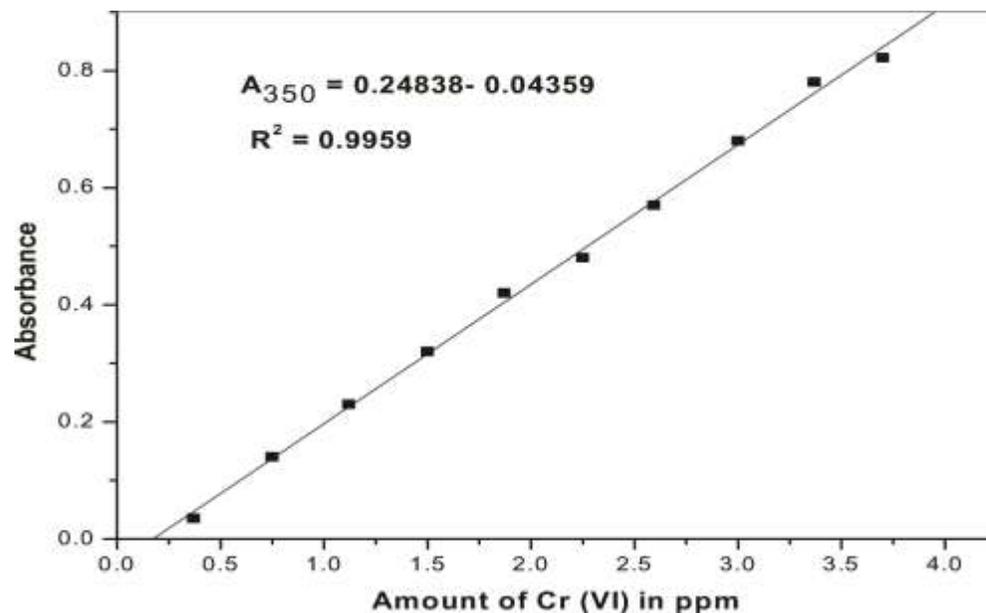


Fig. 4. Calibration plot for Cr (III) determination
[pH = 4.0, ASABT = 1×10^{-3} & Wavelength = 350 nm]

Study on effect of foreign ion determination

The effect of various ions at $\mu\text{g/ml}$ levels on the determination of Cr (III) was examined and the results were tabulated in Table 2. The presence of foreign ions was studied with a view of the relevancy of the technique for the estimation of foreign ions. Cr (III) of the concentration of $2.12 \mu\text{g/ml}$ was studied with the interference of foreign ions by std. procedure. The tolerance limit of foreign ion restricted value was calculated in fact the quantity of foreign ion particles causes $\pm 2\%$ errors in the absorbance of metal complexes.^{21, 22} However, the tolerance level for the ions V, Zn(II), Ag (I), Fe (II) and Cd (II) is found to be very low.

Table 2. Tolerance limit of foreign ions in the determination of $2.12 \mu\text{g/ml}$ of Cr

Ion Added	Tolerance Limit $\mu\text{g/ml}$	Ion Added	Tolerance Limit $\mu\text{g/ml}$
Citrate	384	W(v)	368
Tartarate	296	Mn (II)	22
Urea	288	Pb (II)	0.82
Iodate	254	Cr (VI)	1.0
Bicarbonate	244	Zn (II)	0.13
Thio Cyanate	232	Cd (II)	0.22

Sulphate	192	Hg(II)	0.40
Oxalate	176	Ni (II)	0.23
Thio Urea	152	Fe (II)	0.22
Nitrate	124	Au (III)	0.40
Acetate	118	Pt (IV)	0.39
Phosphate	20	Tl (III)	0.25
Bromide	16	Ag (I)	0.22
Chloride	7.1	V (V)	0.20
Fluoride	4.0	Cu (II)	1.2

Applications

In order to check the validity of the method for the determination of chromium in plant samples, Chromium was determined in Jatropha leaf sample by ICP-OES and spectrophotometric method by employing the std., procedures. The Jatropha plant species were collected at Botanical garden, Yogi Vemana University, Kadapa, A.P, and India [Table 3]. The results obtained are given in Table 3 and show that the proposed method is comparable to the ICP-OES method.

Table 3. Determination of Chromium in some medicinal leaf samples

Name of the Samples	Amount of Cr Found $\mu\text{g/ml}$	
	ICP-OES	Proposed Method
a) Jatropha green leaf	1.722	1.713
b) Jatropha root	1.36	1.32

Determination of stability constants of metal complexes

Molar ratio and Job's continuous variation strategies were used to analyze the composition of the complex. Based on the data acquired from the Job's plot the acquired data was utilized for computing the stability constant of the complex.

Job's Method

For this study, Job's continuous variation method was employed and a graph was drawn between the absorbance and mole fraction of reagent as shown in Figure 5. One mole of chemical reagent reacts with one mole of metal particle and the composition of the complex of Metal ion and ligand (M: L) in the solution is in 1:1 ratio. The information obtained from the Job's curve was utilized in calculating the stability constants of the complex.²³ Since the ratio of the composition of the complex solution i.e. metal ion and ligand are in the ratio of 1:1 the subsequent equation was employed for the determination of stability constant of the complex.

$$\beta = (1-\alpha)/\alpha^2C$$

Where α = degree of dissociation and c = concentration corresponding to the point of intersection

In Job's method the values obtained are α (0.098), c (1×10^{-3}) and 1.39×10^{-6} and from which we can calculate the value for stability constant of the complexes.

The Table 4 contains physico-chemical and analytical properties of Cr (III)-ASABT complex. The molar absorptivity is $5.78 \times 10^3 \text{ L mol}^{-1} \text{ cm}^{-1}$ and the detection limits ($0.035 \mu\text{g ml}^{-1}$), calculated as three times the standard deviation of the complex in the determination of $2.12 \mu\text{g/ml}$ of Cr (III) for ten determinations is 0.0013, with Sandell sensitivity of complex ($0.00173 \mu\text{g/ml}$). The selectivity of the reagent can be due to its capability to form a more stable complex with Cr (III). The λ_{max} (nm) value was higher for Cr-ASABT method than Cr-BZATDT.

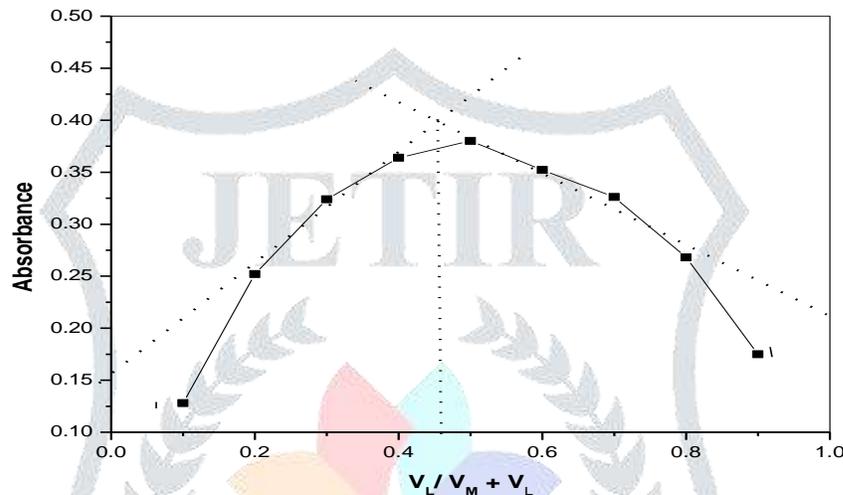


Fig. 5. Job's Curve [Cr (III) = ASABT = 1×10^{-3} , Wavelength = 350 nm & pH = 4.0.]

Table 4. Physico-chemical and Analytical Properties of Cr (III)-ASABT Complex

S. No.	Characteristics	Results
1.	λ_{max} (nm)	350
2.	pH range (optimum)	3.0 – 5.0
3.	Mean absorbance	0.220 ± 0.001
4.	A mole of reagent required per mole of the metal ion for full color developed.	10 Fold
5.	Time stability of the complex (in Hrs)	24
6.	Beer's law validity range ($\mu\text{g/ml}$)	0.05-3.46
7.	Molar absorptivity ($\text{L mol}^{-1} \text{ cm}^{-1}$)	5.78×10^3
8.	Specific absorptivity ($\text{ml g}^{-1} \text{ cm}^{-1}$)	0.00916
9.	Sandell's sensitivity ($\mu\text{g/ml}$)	0.00173
10.	The composition of the complex as obtained in Jobs and molar ratio methods (M:L)	1: 1

11.	Stability constant of the complex	1.002X10³
12.	The standard deviation in the determination of 2.12 µg/ml of Cr (III) for ten determinations	0.0013
13.	Relative standard deviation (RSD)%	0.5849
14.	Y-intercept	-0.04359
15.	Angular coefficient (m)	0.24838
16.	Correlation coefficient (v)	0.9959
17.	Detection limit (µg ml ⁻¹)	0.035
18.	Determination limit (µg ml ⁻¹)	0.105

CONCLUSIONS

A rapid, simple and sensitive spectrophotometric method for the determination of trace amounts of chromium (VI) was studied. The reagent 5-Acetylsaliylamide-2-iminothiophenol (ASABT) provides a yellow colored solution with Chromium (III) in an aqueous medium. The maximum absorption of the reagent λ_{max} shows at 350 nm. The maximum absorption of the pH range was observed at 3.0-5.0. The Beer's law validity was 0.05-3.46 µg/ml. The Molar absorption factor i.e., $5.78 \times 10^3 \text{ L mol}^{-1}\text{cm}^{-1}$ and Sandell's sensitivity 0.00173µg/ml. The Specific absorption factor was $0.00916 \text{ ml g}^{-1}\text{cm}^{-1}$. The composition of metal and ligand (M: L) obtained in Job's and Molar quantitative relation technique, are in 1:1 (M: L) ratio and 1.002×10^{-3} was calculated as the stability constant of the complex. For full-colour improvement, a ten molar chemical reagent in excess was necessary. Chromium (III) of concentration i.e., 2.12 µg/ml was used in the calculation of standard deviation for a set of ten various determinations was calculated as 0.0013 µg/ml. The calculated Relative standard deviation of the system was calculated as 0.5849% and Mean absorbance was found to be 0.220 ± 0.01 . The proposed method has been applied successfully to the determination of chromium in jatropha plant samples.

REFERENCES

1. Paul Raj, Y. Ph.D. Thesis entitled "Determination of Trace elements Present in Environmental samples by following different Analytical Methods" submitted to Dept. of Chemistry, Dravidian University, and Kuppam on 2019.
2. Ajose, F.O. Some Nigerian plants of dermatologic importance. International journal of dermatology, 2007, 46(1): 48-55.
3. Erharuyi, O., Engel-lutz, N., Ahomafor, J., Lmieje, V., Falodun, A., Nebe, B., & Lnger, P. Anticancer activity of five forest crops used in African antiproliferative & pro-apoptotic effects. Natural product research, 2013, 28(10): 740-745.
4. Lin, J., Yan, F., Tang, L., & Chen, F. Antitumor effects of curcin from seeds of *Jatropha curcas*. Acta pharmacological sinica, 2003, 34(3): 241-246.
5. Flex-Silva, J., Giordani, R.B, Da Silva, A. A., Zucolotto, S.M, & Fernandes-Pedrosa Mde, F. *Jatropha gossypifolia* L. (Euphorbiaceae): A Review of traditional uses, Phytochemistry, pharmacology, & toxicology of these medicinal plants. Evidence-based complementary & alternative medicine, 2014, 4(1): 21-32.
6. Harzdorf, C. & Lewalter, J. Analytical methodology for the biological monitoring of chromium. Regulatory toxicology & pharmacology, 1997, 26(1): S86-S93.

7. Najih H., Shekho Hind Mahmoud, A. Spectrophotometric Determination of Chromium Using Pro methazine Hydrochloride - Application in Various Samples. *Jour. for Pure & Appl. Sci.*, 2016, 29 (2): 130-143.
8. Martin figuitt, Roger Newson, Lan, J., Leslie, John Fisher, EileenIngham, & Charles, P. Case. The genotoxicity of physiological concentrations of chromium [Cr (iii) & Cobalt (Co (ii)): An in vitro study. *Mutation research/Fundamental & Molecular mechanisms of mutagenesis*, 2010, 688(1-2):53-61.
9. Porrini, E., Ruggenti, P., Mogensen, C.E., Barlovic, D.P, Praga, M., Cruzado, J.M, Hojs, R., Abate, M. & De Vries, A.P. Non-protein uric pathways in loss of renal function in patients with type 2 diabetes. *Journal of Lancet Diabetes Endocrinal*, 2015, 3(5): 382-391.
10. Mark, A. & Moyad. The placebo effect & randomized trials: analysis of alternative medicine. *Journal of Urol Clin North Am.*, 2002, 29(1): 135-155.
11. Paul Raj, Y., Deepa, K., Madhusudana Reddy, A., Reddeppa M. and Shobha Rani, T. Evaluation of trace Elements in Some Medicinal Plants: *Jatropha*, *Albizia*, *Azadirachta Indica* and *Datura* Plants, *Euro. Jou. of Pharm. & Medi. Research*, 2017, 4(7): 455-465. ISSN: 2394-3211.
12. Rathore, D. P. S. and Tarafder, P. K. Spectrophotometric determination in geological samples. *Analytica Chemica Acta*, 1992, 257:129-133.
13. Paul Raj, Y., Deepa, K., Lingappa, Y., Venkata Reddy, G., Anil Kumar, A. and Shobha Rani, T. A simple Spectro photometric method for the determination of As in environmental and medicinal leaf samples using Benzil-5-Amino-1, 3, 4 – Thiadiazole-2-Thiol (BZATDT), 2016, 3(10):424-431. ISSN: 2394-3211.
14. Paul Raj, Y., Anil Kumar, A., Gangadhara, R. and Shobha Rani, T. A simple Spectro photometric method for the determination of Se in environmental and water samples using 5- Acetyl salicylamide-2- Iminothio phenol (ASABT), *IJRAR*, 2019, 6(1): 686-697. E-ISSN 2348-1269, P- ISSN 2349-5138.
15. Huie, C.W. A Review of Modern Sample Preparation Techniques for the Extraction and Analysis of Medicinal Plants. *Anal. Bioanal. Chem.*, 2002, 373: 23-30.
16. Tayone, J.C. Spectro photometric Determination of Chromium (VI) in Canned Fruit Juices *International Journal of Sciences: Basic and Applied Research*, 2015, 19(1):426-432.
17. Ahmed, M., & Reazul, M. H. A Rapid Spectro photometric Method for the Determination of Chromium in Environmental Samples using bis (Salicylaldehyde) ortho-phenylenediamine. *Research Journal of Chemical Sciences*, 2011, 1(1):46-59.
18. Cotton & Wilkinson. (1972). *Advanced inorganic chemistry*, 3rd ed., John Wiley & Sons.
19. Cary D. Christian. (1980). *Analytical Chemistry*, John Wiley & Sons, Inc.
20. Hussein, S., Rahim, A., & Farooqui, M. Studies of binary complexes of metal ions with mandelic acid by potentiometry. *Chem. J.*, 2011, 02(6): 206-209.
21. Anjani, K., Mishra, P., Mohan, M., Sunil, K., & Mishra. Equilibrium studies on bivalent transition metal chelates with biologically active Schiff's base of heterocyclic aldehydes. *Ras. J. Chem.*, 2011, 4(2): 303-319.
22. Rossetti, F.J.C, H., & Rossetti, S. Graphical methods for determining equilibrium constants.1- the system of mononuclear complexes. *Ac. chem. Scandinavica*, 1955, 9: 1166-1176.
23. Hartley, F.R., Burgess, C., & Alcock, R.M. (1980). *Solution equilibriums*, first ed. John Wiley and Sons, New York.