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AN INNOVATIVE ECO-FRIENDLY TECHNIQUE TO MEASURE CHROMIUM WITH BENZOHYDRAZIDE

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Abstract

Numerous methodologies exist for trace level analysis of Chromium (Cr) metal ions. Many of these approaches are characterized by their time-consuming nature, complexity, toxicity, and sophistication. Recently, some spectrophotometric methods have been developed that, while less sensitive and selective, offer quicker analyses for determining Cr(III) ions. We innovated suitable, swift, sensitive and selective technique for detecting Cr(III) at trace levels, utilizing Benzohydrazide (BH) as the derivatizing reagent within a 3.0% Triton X-100 surfactant system. This procedure demonstrated improved analytical characteristics. Cr-[BH]3 complex exhibited absorbance maxima at λmax 436.2 nm at pH 5. The method adhered to Lambert-Beer's law within concentration range of 0.1-4.0 µg/mL, with a 1:3 ligand to metal ratio achieved for the Cr-[BH]3 compound. Sandell's sensitivity was calculated to be 4.9 ng/cm², and the molar absorptivity was determined as $\varepsilon = 4.03 \times 10^4$ L mol^{-1} cm^{-1}. A detection limit of 4.9 µg/L was successfully established. This method proved highly effective for quantifying Cr(III) ions across diverse samples, including natural, biological, alloy, industrial, and ecological matrices.

Keywords: *Chromium, Benzohydrazide, Chelating agent, Surfactant.*

Introduction

Metals play a pivotal role in various aspects of modern society, from infrastructure and manufacturing to advanced technological applications [1]. Among them, chromium stands out as a crucial element due to its unique properties and widespread industrial uses [2]. Chromium plays a crucial role across various aspects of life, industry, and the environment due to its unique properties and diverse applications. Chromium is an essential trace element for human nutrition, playing a role in glucose metabolism and insulin regulation [3]. It is necessary for maintaining proper carbohydrate and lipid metabolism in the body [4]. Chromium compounds, such as chromium picolinate, are studied for their potential health benefits in managing conditions like diabetes. They may help improve insulin sensitivity and regulate blood sugar levels in some individuals [5].

Hexavalent chromium is highly toxic and carcinogenic [6]. Chromium deficiency disrupts normal glucose metabolism, potentially leading to insulin resistance and difficulty controlling blood sugar levels [7]. This deficiency increases the risk of developing type 2 diabetes and can also impact cardiovascular health by raising cholesterol levels and contributing to heart disease. Additionally, inadequate chromium intake may affect cognitive function and mood regulation [8]. Chronic ingestion or exposure to high levels of chromium, often through contaminated drinking water sources, has been associated with kidney damage [9]. Chromium can accumulate in the kidneys over time, impairing their function and potentially leading to kidney failure. Additionally, prolonged exposure to chromium can affect liver function, causing damage or toxicity as chromium accumulates in the liver and disrupts its metabolic processes [10].

Chromium contamination in food can occur from environmental sources or improper processing techniques [11]. Chromium can accumulate in the food chain, potentially reaching harmful levels in aquatic organisms and posing risks to ecosystems and human health [12]. Workers in industries involving chromium, such as chrome plating, welding, leather tanning, and chemical manufacturing may face potential health risks from exposure to chromium compounds [13]. Some individuals may develop allergic dermatitis upon contact with chromium-containing materials, such as in certain industrial settings or through skin exposure [14]. Chromium compounds, especially Cr(VI), can contaminate soil and water through industrial processes like electroplating, leather tanning, and chemical manufacturing [15]. Efforts to mitigate chromium pollution require stringent regulations and effective wastewater treatment methods. Chromium is a key component in stainless steel alloys, providing corrosion resistance, strength, and durability essential for construction, automotive, and aerospace industries [16]. Chromium alloys are used in medical implants and prosthetics for their biocompatibility and resistance to corrosion, ensuring long-term performance and safety in the human body. Chromium coatings protect metals from corrosion and wear, extending the lifespan of machinery and infrastructure [17]. Chromium is used in electronic components, such as circuit boards and semiconductors, due to its electrical conductivity and resistance to oxidation. Chromium-containing alloys contribute to the strength and corrosion resistance of construction materials, ensuring durability in bridges, buildings, and pipelines [18]. Chromium alloys are critical in jet engines, turbines, and automotive components due to their ability to withstand high temperatures and harsh environments [19]. Chromium's hardness,

corrosion resistance, and heat resistance make it indispensable in various industries, including aerospace, automotive, and construction. It contributes to the durability and performance of materials used in these sectors [20].

Several analytical techniques are available for analysis of chrmomium, including atomic absorption spectroscopy [21], Ultra violate visible spectrophotometry [22] and ICP-AES [23]. These methods often require expensive equipment and are known for their time-consuming and labor-intensive nature. Consequently, there is a growing need for innovative methods capable of accurately detecting chromium ions at very low concentrations. Spectrophotometric techniques are widely preferred due to their simplicity, precision, rapidity, and affordability of instrumentation (Korai, MB et al., 2019). Several spectrophotometric approaches for metal analysis have emerged as alternatives to traditional solvent extraction methods, utilizing micellar systems. Recently, spectrophotometric procedures with reduced sensitivity and selectivity for Cr(III) ion determination have been established. In current research work, a swift, selective and practical technique was developed for the trace analysis of Cr(III) using benzohydrazide (BH) in 3.0% Triton X-100. This technique has proven effective in accurately determining Cr(III) ions across a range of real-world samples, including foodstuffs, pharmaceuticals, biological specimens, and environmental samples.

Material and method

The equipment used includes a UV-Vis spectrophotometer (Cecil CE 9500), an atomic absorption spectrometer (Perkin Elmer Analyst-800), an IR spectrometer (Hitachi), and a pH/conductivity meter (HACH Sension156).

Preparation of Reagents

A 1000 µg/L stock solution of chromium (II) ions was meticulously prepared using high-purity salts sourced from Merck, Darmstadt, Germany and dissolved in double-distilled water. Solutions containing various metal ions were also meticulously prepared from salts to evaluate potential interferences [24].

The BH solution of 4×10^{-4}M was prepared by dissolving 0.0544g of BH in the minimum amount of ethyl alcohol in a 1000 mL flask, followed by addition of 3.0% Triton X-100 to reach the final volume. The 3.0% Triton X-100 solution was prepared by dissolving 3g of Triton X-100 in a 100 mL calibrated flask and adjusting the volume with distilled water. Buffers spanning pH 1 to 10 were prepared according to the methods described by Perrin (2012) [25]. This involved combining specific volumes of KCl (0.2 M) and HCl (0.2 M) for pH 1-4, volumes of CH_3COOH (0.2 M) and CH_3COONa (0.2 M) for pH 5-6, volumes of KH_2PO_4 (0.1 M) and NaOH (0.1 M) for pH 7-8, and volumes of $Na_2B_4O_7$ (0.025 M) and HCl (0.1 M) for pH 9-10. All chemicals used were of analytical grade and sourced from Merck/Fluka, ensuring high purity.

General Method for Determination of Chromium (III) Ions

Chromium (III) ion solutions ranging from 0.06 to 10 µg/mL were prepared. To each 10 mL volumetric flask, 2 mL of Benzohydrazide (BH) reagent solution, 2 mL buffer pH 5, and 2 mL 3.0% Triton X-100 were mixed. Solution was thoroughly mixed by shaking, and deionized water was mixed to reach final

volume. Cr-$[BH]_3$ absorbance was measured at λ_{max} 436.2 nm using BH as the blank.

Chromium (III) ions analysis from alloy

In a process, 5.0 mL of NIST 1643 underwent digestion with 10 mL of nitric acid concentrated, 10 mL of sulphuric acid (20%), and 2 mL of hydrogen per oxide. Subsequently, the resulting solution was evaporated and reduced. Following this, the sample underwent dilution, neutralization, and filtration. In order to create a complex, the sample solutions were put into a measuring flagon and combined with 2 mL of pH 5 buffer, 3.0% Triton X-100, and Benzohydrazide (BH). The outcomes are shown in Table 4.

Analysis of chromium (III) from waste-water specimen

The sample for this study i.e. 2L of waste water was mainly collected from Sohu Kanasira, a village in District Khaipur, Sindh, Pakistan. For the digestion process, the material was filtered and combined with 2 mL of hydrogen peroxide and 4 mL of concentrated nitric acid. After that, it was pre-concentrated at roughly 110 ^oC, and a volume of 25 mL was created. After that, the sample was placed in a volumetric flask and 3.0% Triton X-100, 2 mL of pH 5 buffer and 2 mL of benzohydrazide (BH) were added. Cr-$[BH]_3$ absorbance was noted, as in table 3.

Analysis of chromium (III) from spout water specimen

A 1000 mL sample of tap water was taken from the hamlet of Naroo Dhoro in the district of Khairpur, Pakistan. It was then cooked in an oven for preconcentration, filtered, and combined with 2 mL of concentrated nitric acid. Ultimately, the sample was put into a calibrated bottle and combined with 2 mL of pH 5 buffer, 2 mL of benzohydrazide (BH), and 2 mL of Triton X-100 (3.0%). Cr-$[BH]_3$ absorbance were noted, as in Table 5.

Analysis of Chromium (III) ions from food specimens

2g of each Fish and cow's flesh, using microwave digestion were broken down into 10 mL of HNO_3 and 2 mL of H_2O_2. After diluting the solution with 50 millilitres of deionized water, it was filtered. Benzohydrazide (BH) 2 mL, pH 5 buffer 2 mL, 3.0% Triton X-100 2 mL, and tartrate solution 2 mL were finally put onto a calibrated flagon (Jamaluddin et al., 2014). The measured complex absorbances are shown in table 4**.**

Analysis of Chromium (III) from pharmacological specimen

Ultimately, 25 grams of a tablet (multi-vitamin) was pulverised and subjected to digestion using 2 mL of hydrogen peroxide and 10 mL of conc. nitric acid. The mixture was then evaporated until it was completely dry. Deionized water was added after leaching the

residues with sulphuric acid (0.5M). Lastly, the solutions were placed into a calibrated flagon, containing two millilitres each of mixed reagent BH, buffer of pH 5, tartarate solution, and Triton X-100 (3.0%). The measured complex absorbances are shown in table 3.

Results and discussion

The interaction between Cr(III) ions and Benzohydrazide (BH) resulted in charge transfer transitions. BH contributed electron pairs from both the oxygen of the carbonyl group (C=O) and the nitrogen of the amino group ($-NH_2$) (Fig. 1).

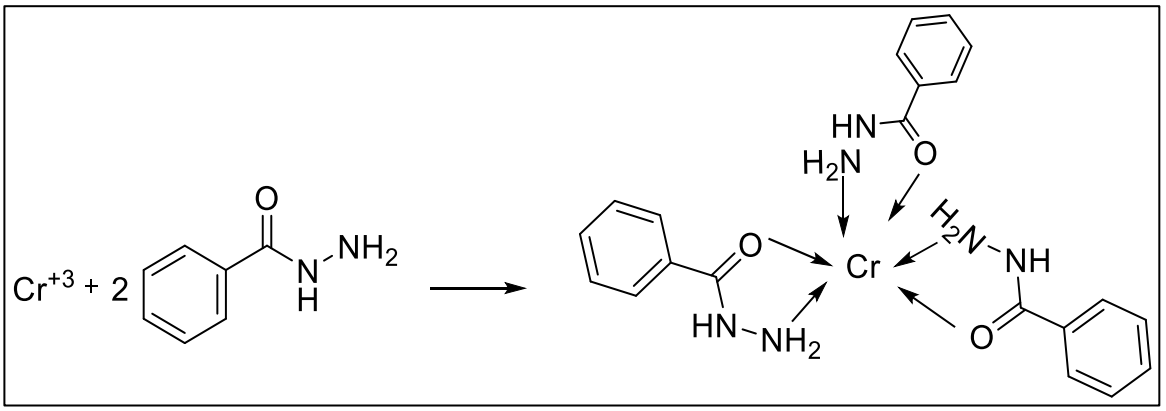

Figure 1 Proposed structure for [Benzohydrazide]-Chromium(III) complex.

In Figure 2, The reagent BH displayed absorption bands at λ_{max} 285.2 nm, attributed to the (n→π*) transitions within the molecule, specifically involving the charge transfer (L→LCT) from the amino group ($-NH_2$) nitrogen and the carbonyl group (-C=O) oxygen. These measurements were conducted in a surfactant solution containing Triton X-100 (3.0%).

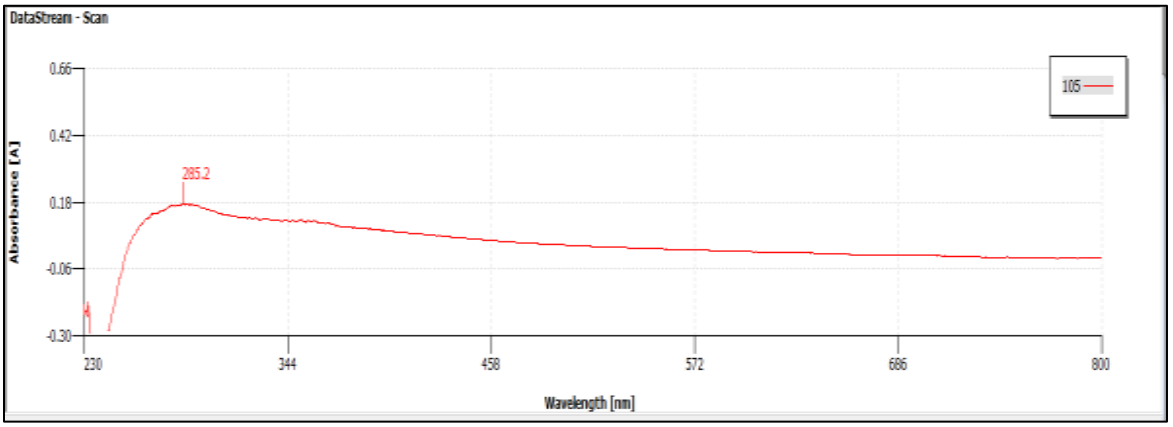

Figure 2. Benzohydrazide reagent UV/Vis spectrum at (λmax 285.2 nm)

The absorption spectrum of Cr-$[BH]_3$ revealed peaks at λ_{max} 436.2 nm, attributed to the transition (n→π*) from BH reagent to Cr (L→MCT), specifically involving the amino ($-NH_2$) and carbonyl (C=O) groups, as illustrated in Figure 3. For 120 minutes, the Cr (III)-

complex remained stable and had a consistent maximum absorbance.

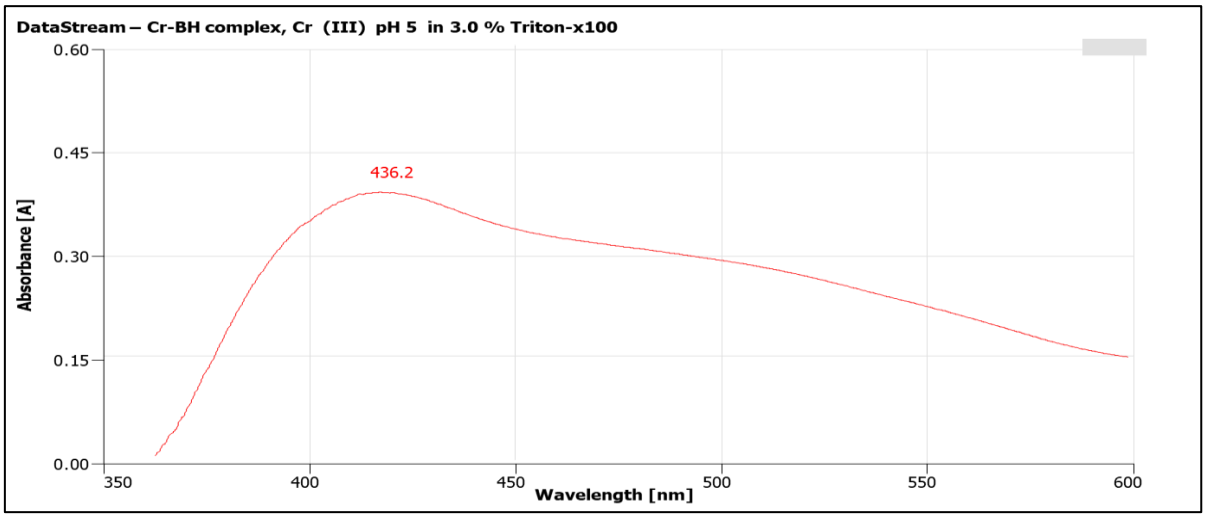

Figure 3 Absorption spectrum of Cr-BH Complex

For Cr (III) and benzohydrazide , the stoichiometric molar ratio was determined using Job's approach [26]. Plots of absorbance vs Cr (III) for the chromium (III) complex were shown, with the equimolar mole fraction of 1:3 for Cr-$[BH]_3$ complex (Fig. 4).

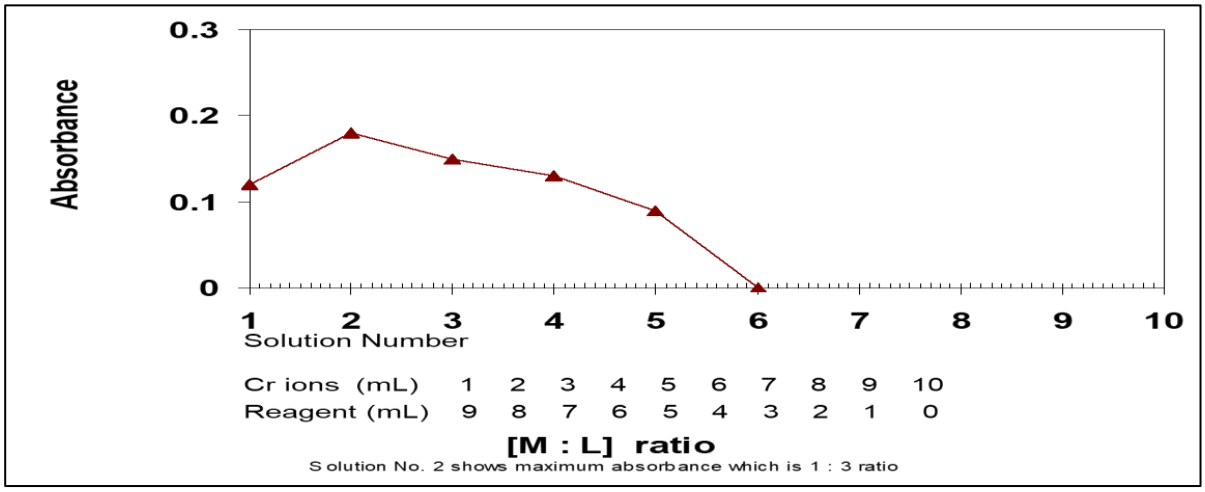

Figure 4Influence of chromium quantities on the absorbances of Cr-[BH]3

By increasing the concentration of Benzohydrazide from 0.5 to 10.0×10^{-4}M even though keeping constant concentrations of Cr(III) at 1.0mM, the effect of the concentration of Benzohydrazide on the Cr-$[BH]_3$ compound absorbance was observed. It was noted that the optimal Benzohydrazide molar ratio for achieving maximum absorbance remained consistent at 4.0×10^{-4} M when the Cr(III) concentration was maintained at 1.0 mM. Figure 5 illustrates the optimized concentration of BH under constant Cr (1.0

mM) conditions.

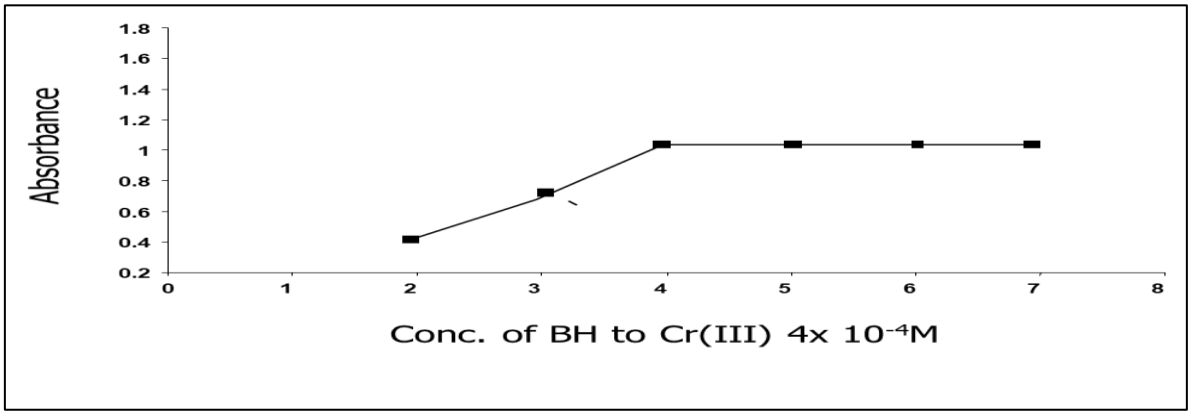

Figure 5 Influence of BH concentrations on the absorbance of Cr-BH Complex

The experiment involved mixing 2 mL of Cr(III), 2 mL of Benzohydrazide (4.0×10^{-4} M), 2 mL of buffer (pH 5), and 2 mL of Triton X-100 (3.0%) in a 10 mL calibrated flask for creation of complex. Triton X-100 (3.0%), optimized for the study and used above its critical micellar concentration, facilitated the complexation process. pH 5 was identified as optimal for achieving consistent maximum absorption. A calibration curve was then generated from 0.1 to 4.0 µg/mL concentrations, measured at λ_{max} 436.2 nm, demonstrating a high correlation coefficient ($R^2 = 0.9994$) as shown in Figure 6.

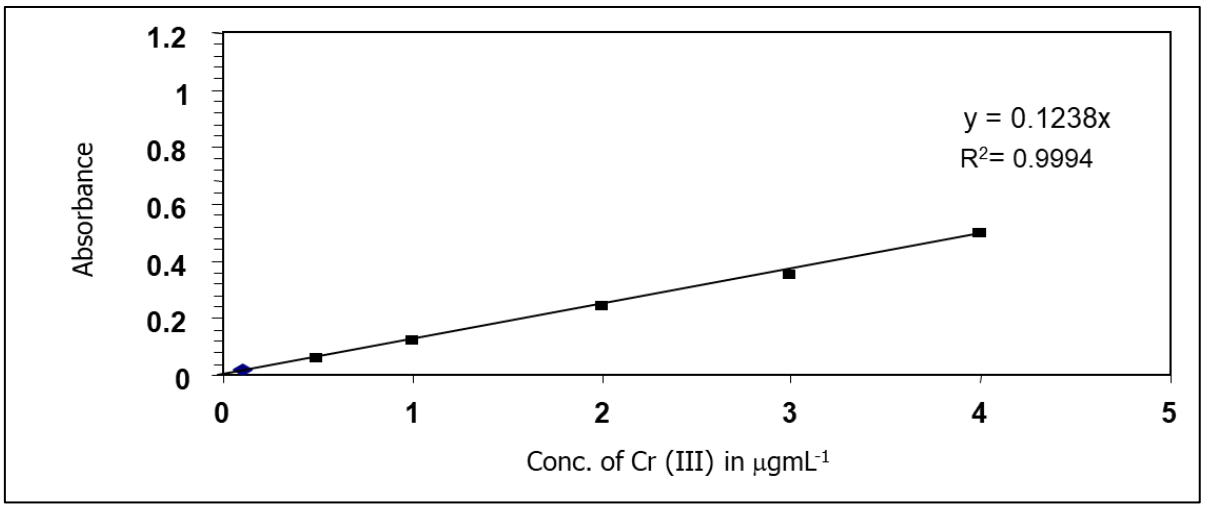

Figure 6 Calibration curve of Cr(III) -BH

The values for limit of detection, Sandell's sensitivity and coefficient of molar absorptivity were determined as 4.9 $ngmL^{-1}$, 4.9 $ngcm^{-2}$ and 4.03×10^4 $mol^{-1}cm^{-1}$ at λ_{max} 436.2 nm, respectively, as shown in Table 1. The improvements in selectivity and sensitivity of the proposed method over previously described extraction approaches are evident in Table 6.

Table 1. Characteristics of Cr-$[BH]_3$	
Parameters	**Chromium**
Maximum absorption (λ_{max})	**436.2 nm**
Calibration range	**0.1-4.0 µg/mL**
Surfactant Triton X-100 (3.0%)	**2 mL**
Molar absorptivity	**4.03×10^4 mol^{-1} cm^{-1}**
pH	**5**
Sandell's sensitivity	**4.9 ng/cm^2**
Derivatizing reagent Benzohydrazide	**4.0×10^{-4} $mol^{-1}L^{-1}$**
Metal-Ligand ration	**1:3**
Detection Limit	**4.9 µg/L**
(R^2)	**0.9994**

The study examined the impact of various cations and anions on metal-chelate formation. $C_4H_4Na_2O_6$, $KClO_3$, and KSCN exhibited minimal interference and demonstrated that interference was present beyond 800 μgL^{-1}. And others such as lead (III), manganese (II), vanadium (III), and cadmium (II) were found to cause confirmed interference in the absorbance of complexes formed in their small concentrations.

As masking reagents, edetic acid, ascorbic acid and dimethylglyoxime were utilized to minimize the interference caused by foreign ions [27]. The NH_3 buffer masking agent effectively mitigated the varied effects of analytes on Cr(III) complex formation, as detailed in table 2.

Table 2 – **External ions/salts influence on Cr-$[BH]_3$ compound**	
Ion/ salt	**Cr (III)** (µg/L)
Cd(II)	2
Cr(III)	-
Pb(II)	3
Mn(III)	5
V(III)	5
Al(III)	10
Zn(II)	50
KCN	50
$Na_2C_2O_4$	200
Na_2 citrate	500
Mg(II)	600

Validation of method

The validation of the proposed method involved comparing its results with those obtained from Atomic Absorption Spectroscopy (AAS), using certified reference materials and conducting a % recovery test at a 95% CL. The accuracy and reliability of the technique were performed using certified reference materials and tap water, with results summarized in Tables 3 to 5.

Table 3 – Analysis of chromium(III) from waste water and pharmaceutical specimens

Samples	Metal ions	Established technique μgL^{-1}	RSD%	A.A.S technique μgL^{-1}	RSD%	Recoveries
Chromium from waste water specimens						
Waste water	Cr(III)	4.0	0.3	4.2	0.1	97.21%
Chromium from pharmaceutical specimen						
Tablet Chromium(III)-GFT (27 mg per tablet)	Cr (III)	26.95	0.4	26.96	0.3	99.81%

Table 4 – Analysis of chromium from food, alloy specimens and certified materials

Samples	Certified value	Found value
Chromium from food specimens		
Cows meat	_	0.63
Fish	_	1.89
Chromium from alloy specimens		
Chromel A Ni-chrome 80-20	2.0 µg/g	1.8 µg/g
Chromium from certified materials		
NIST- 1643	18.6 µg/L	18.2 µg/L
NIST- 1643	19.0 µg/L	18.6 µg/L

Table 5 – **The percentage recovery of predetermined metal quantities incorporated into water specimens**

Analytes	Mixed quantity (μgL^{-1})	Obtained (μgL^{-1})	R (%)
Cr(III)	5.50	5.34	97.09

Table 6 – Comparison of chromium investigation

Metals	Procedures	References
Cr(III)	Calibration range 0.2-2.4 μgL^{-1}, λ_{max} 575 nm , ε 1.46×10^4 $mol^{-1}cm^{-1}$	[28]
Cr(III)	in Triton X-100, λ_{max} 295 nm , ε 5.4×10^3 $mol^{-1}cm^{-1}$	[29]
Cr(III)	in Triton X-100, D.L. 0.117 µg/L, λ_{max} 586 nm , ε 0.244×10^4 $mol^{-1}cm^{-1}$	[30]
Cr(III)	D.L. 4.9 µg/L, ε 4.03×10^4 $mol^{-1}cm^{-1}$, λ_{max} 436.2nm in 3.0% Triton X-100	Developed technique

Conclusion

The current spectrophotometric approach for determining chromium (III) ions in minute quantities is simple, swift, sensitive, repeatable, non-extractive, and adaptable. The newly proposed technique is environmentally friendly, exhibiting enhanced sensitivity and selectivity, and has supplanted antiquated solvent extraction methods known for their time-consuming, costly, and hazardous nature. The findings demonstrate significant improvements in coefficient of molar absorptivity and sensitivity compared to previously documented methodologies, as detailed in table 6. This novel technique was effectively applied to the quantification of chromium in diverse natural, alloy and ecological specimens.

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