RASĀYAN *J. Chem.*
Vol. 19 | No. 1 | 556-563 | January-March | 2026
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

SYNTHESIS AND CHARACTERIZATION OF CINNAMALDEHYDE 4-HYDROXY METHYL PHENYL HYDRAZONE DERIVED FROM AN ISOLATED HYDRAZINE OF *Agaricus bisporus:* APPLICATION IN THE SPECTROPHOTOMETRIC DETERMINATION OF COBALT (II) IN BIOLOGICAL AND ENVIRONMENTAL SAMPLES

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ABSTRACT

In this work, 4-hydroxy methyl phenyl hydrazine is isolated from *Agaricus bisporus* that was collected from the Lambasingi region. Cinnamaldehyde-4-hydroxy methyl phenyl hydrazone (CHMPH) is then synthesised, characterised, and used for the spectrophotometric determination of cobalt (II). The hydrazone was created via a condensation reaction between the mushroom extract and cinnamaldehyde, and it was identified by elemental analysis, IR, NMR, and mass spectroscopy. The CHMPH–Co(II) complex demonstrated a 1:1 stoichiometry, a linear Beer's law range of 0.029–0.29 µg/mL, a molar absorptivity of 8.4×10^4 L·mol^{-1}cm^{-1}, and Sandell's sensitivity of 0.0007 µg/cm² under ideal conditions (pH 9.0, λmax 410 nm, and Triton X-100 5 %). High accuracy and precision (RSD < 2.5%) were shown by the good regression coefficient of 0.99985 and the detection and determination limits of 0.0047 µg/mL and 0.0147 µg/mL, respectively. The suggested approach demonstrated findings similar to AAS approaches with errors within ±2% when used to determine Co (II) in a variety of matrices, including tea leaves, vitamin B_{12} tablets, alloys, and biological samples, including blood and urine. These results show that CHMPH is a straightforward, sensitive, and selective reagent for determining cobalt (II), with potential uses in analytical chemistry, biological, and environmental settings.

Keywords: Cobalt (II), Mushrooms, Tea Leaves, Vitamin B_{12}, Micellar Medium, Biological Samples.
RASĀYAN *J. Chem.*, Vol. 19, No.1, 2026

INTRODUCTION

Hydrazones, known for their versatility in analytical chemistry, continue to draw attention for their ability to form stable and selective complexes with transition metals. Researchers have evaluated the possible analytical uses of hydrazones and their derivatives. One significant class of widely recognised analytical reagents are hydrazones.[2–7] Keeping in view the analytical potential of hydrazones, the formation and characterisation of cinnamaldehyde 4-hydroxy methyl phenyl hydrazone (CHMPH), its biological and analytical applications, as well as the extraction of hydrazine from brown mushrooms (*Agaricus bisporus*), were studied. Based on the above derivative and direct spectrophotometric techniques to determine the amount of cobalt (II) in vitamin B_{12}, alloy samples, vehicle exhaust, tea leaves and biological samples were studied utilising CHMPH. Cobalt is vital for vitamin B_{12} and has a significant role in bacteria's nitrogen fixation. In medicine, Cobalt-60 is employed as an effective tracer in radioactive studies and anticancer therapy. It is a crucial component of vitamin B_{12} (cyanocobalamin), and cobalt is also vital to human nutrition. Deficiency of cobalt results in stunted growth, and in excess concentrations, it inhibits enzyme activity and biosynthesis.[8-9] Several analytical techniques have been reported in the literature for the determination of hydrazone derivatives; among them, spectrophotometric techniques are the most popular due to their ease of use and lower cost.[10] The development of a method that does not rely on solvent extraction is important. Micellar solutions increase the stability of metal complexes and serve as effective media for the detection of metal chelates.[11-14]

This work also aligns with the United Nations Sustainable Development Goals, particularly SDG 3 (Good Health and Well-Being) and SDG 6 (Clean Water and Sanitation). By developing a simple and sensitive spectrophotometric method for detecting trace levels of Cobalt (II) in biological and environmental samples, the study contributes to improving health-related monitoring and ensuring the safety of water and food resources. Despite several reports on synthetic hydrazone reagents, limited studies have focused on utilising naturally derived hydrazine compounds for analytical applications. In this study, hydrazine isolated from *Agaricus bisporus* was used to synthesise a new hydrazone derivative, offering an environmental friendly and economical alternative to purely synthetic reagents. This approach not only demonstrates the potential of bio-derived materials in analytical chemistry but also addresses the need for simple, solvent-free spectrophotometric methods for trace metal determination, particularly for Cobalt (II) in biological and environmental matrices. *Agaricus bisporus,* used for the preparation of hydrazones, is one of the most widely grown and edible types of mushrooms in the world. Browm mushrooms are available in two varieties: Portobello mushrooms, which are fully grown and have a big, meaty cap, and cremini (baby bella) mushrooms, which are younger and harder. These mushrooms are well-known for their nutritional content, earthy flavour, and culinary versatility. *Agaricus bisporus* belongs to the kingdom Fungi and phylum Basidiomycota. It belongs to the family *Agaricaceae.* The nutritional and health benefits of brown mushrooms include B vitamins (B_2, B_3, and B_5), protein, fibre, and antioxidants, including ergothioneine, which may help prevent cellular damage. Their low-calorie and fat content make them a nutritious complement to a variety of diets.[15] Due to their umami-rich flavour, brown mushrooms are frequently used in cooking and go well with pasta, soups, stir-fries, and grilled foods. They are also being researched for possible therapeutic uses, such as their anti-inflammatory and antioxidant capabilities. Nevertheless, they also include 4-hydroxymethylphenylhydrazine, a substance that has been investigated for its possible pharmacological characteristics.[16] Brown mushrooms are a nutrient-dense food that is moderately high in zinc, iron, and copper and high in potassium. Potassium improves nerve signalling, muscle function and regulates blood pressure. A potent antioxidant, selenium promotes thyroid and immune system health. Red blood cell formation, brain function, and energy production are all supported by copper. Other elements that show potential biological applications include magnesium, which promotes heart health, and zinc, which helps in wound healing, among other functions.[17] *Agaricus bisporus* contains three naturally occurring hydrazine compounds, namely N-methyl-N-formyl hydrazine and 4-(Hydroxymethyl)phenyl hydrazine, Agaritine (β-N-[γ-L-(+)-glutamyl]-4-hydroxymethylphenylhydrazine).

EXPERIMENTAL

Materials and Methods

Extraction of Hydrazones

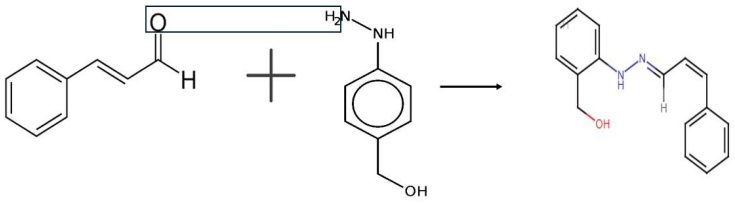
Agaricus bisporus was collected from the Lambasingi area in Visakhapatnam, India. The mushrooms were dried for 72 hours, after they were dried and powdered using a blender. Dried powder was taken in a measured quantity and dissolved in ethanol for two days in an orbital shaker with an incubator operated at a speed of 3000 rpm and a temperature of 40°C. The solution was filtered using a Buchner funnel and finally rotary evaporated to get the crude extract of the hydrazine sample.

Preparation of CHMPH

In a round-bottom flask, 13.2 mL of cinnamaldehyde and brown mushroom extract were mixed and refluxed for 4 hours. After cooling the solution, by simple filtration, the product was separated. A crude sample of $C_{16}H_{15}ON_2$ was obtained with a yield of 80 %. Using hot ethanol, the resultant product was recrystallised twice. Pure and pale yellowish crystals of Cinnamaldehyde-4-hydroxy methyl phenyl hydrazone (CHMPH), having a melting point of 185°C, were obtained. The scheme of reaction is shown in Fig.-1.

Analytical Characterization

Using Fourier-transform infrared (FTIR), nuclear magnetic resonance (NMR), and mass spectrometry, structural analysis was performed to validate the molecular structure and functional groups of the newly synthesised reagent (CHMPH). Comprehensive analytical data about CHMPH is shown in Table-1.



Cinnamaldehyde 4 hydroxymethyl phenyl hydrazine CHMPH

Fig.-1: Reaction Showing the Synthesis of Hydrazone (CHMPH)

Table-1: IR and NMR Data of CHMPH

Types of stretching	IR Spectral bands (cm^{-1})	Functional groups	The 1H NMR (300 MHz) spectrum in DMSO	Assignments	The ^{13}C NMR (300 MHz) spectrum in DMSO
NH	3400	NH	δ 11.57(S,1H)	-CH=CH-	δ 134.98
OH	3300	OH (phenolic)	δ 3.45 (S, 1H)	C3-Phenyl ring	δ 130.41
C=O	1708	N=C-H	δ 7.5 (S, 1H)	C4-Phenyl ring	δ 129.35
C=N	1685	Phenyl ring	δ 7.60-7.25 (D,2H)	C=n	δ 71.71
		HC=CH	δ 3.5 (D,2H)	C-OH	δ 70.06

Preparation of the Reagent

In the present study, 0.252g of CHMPH was mixed with 100 mL of dimethyl formamide to prepare a 0.01 M Solution, and it was found to be stable for 24 hours in the presence of TritonX-100. Required working solutions were prepared from stock solutions.

Equipment and Reagents Required

Using a cell with a path length of 10 nm, measurements were performed with a UV-visible spectrophotometer (Model 1800, Shimadzu, Japan). Digital pH-mv-Temp was used to measure pH levels. The pH meter's mode was the WTW-720 inoLab (Germany). The JEIO TECH (Korea) BS-11 digital water bath shaker model was utilised. Cobalt sulphate solution. $CoSO_4.7H_2O$ (0.2811g) was dissolved in hot double-distilled water to prepare a $1x10^{-2}$ M solution in a 100-mL volumetric flask.

Statistical Analysis

Origin software is a powerful tool for Beer's Law analysis, allowing for accurate determination of molar absorptivity, concentration, and deviations from linearity through statistical analysis and visualisation. Statistical significance was tested using ANOVA and Origin software.

RESULTS AND DISCUSSION

Like any other Schiff's base reagent, the new reagent, Cinnamaldehyde 4-hydroxy methyl phenyl hydrazone (CHMPH), was simple to prepare. The metal ions and ligands coordinate and form a complex in a basic medium. The absorption spectra of Cobalt (II) and CHMPH under the optimum conditions are shown in Fig.-2. At different concentrations, the zero-order and derivative spectra are represented in Fig.-3 and 4 (a) and 4(b).

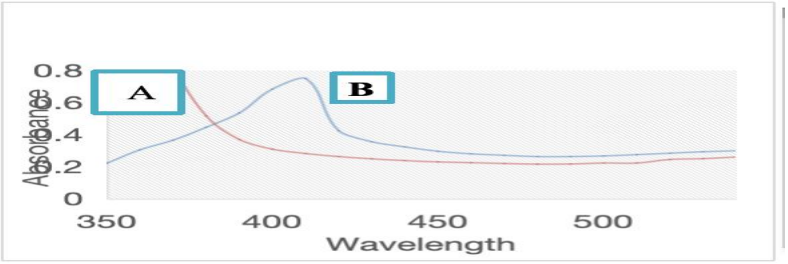


Fig.-2: Effect of Reagent (CHMPH) Concentration
(A= Reagent, B= Complex) CHMPH: 2 $X10^{-3}$ M, Co: $2X10^{-4}$ M pH: 9.0 Triton-X-100 (5%), (λ max: 410 nm)

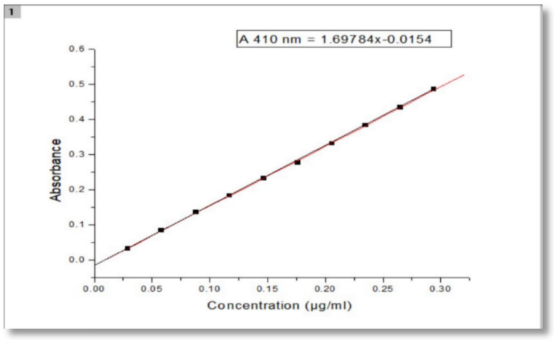
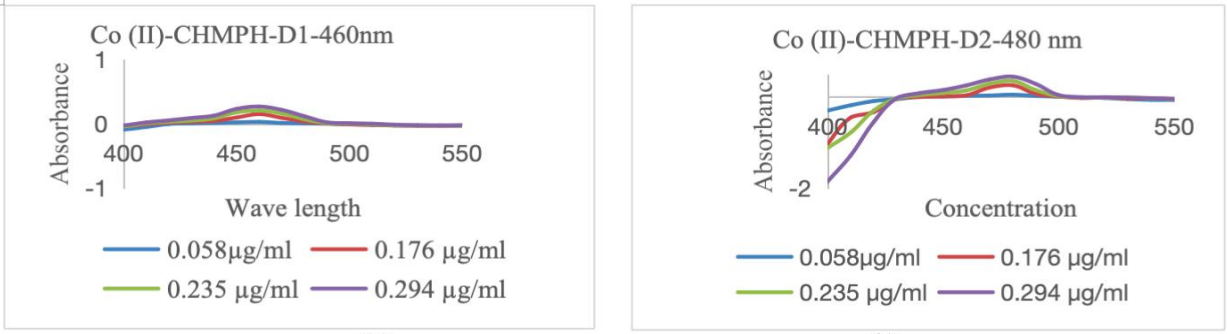


Fig.-3: Calibration Curve-Beer's Law
CHMPH: 2 X10^{-3} M Co: 2X10^{-5} M



(a) (b)

Fig.-4: Derivative Spectra at different Concentrations
CHMPH: 2 X10^{-3} M Co: 2X10^{-5} M pH: 9.0 Triton-X-100 (5%)

Precision, Accuracy, Data and Optical Characteristics

Table-2 provides the Cobalt (II)-CHMPH regression equation, correlation coefficients, Molar absorptivity, Sandell's sensitivity, and Beer's law limitations. In contrast to the zero-order technique, it was therefore seen that the peak location moved towards higher wavelengths and that Beer's law range was also enhanced in derivative spectra.

Table-2: Co (II)-CHMPH Analytical and Physicochemical Characterisation

Name of the parameter	Values
pH range	8.0 to 10.0
Optimum pH range	8.0 to 9.0
Wavelength (λ max)	410 nm
Mole reagent is required for colour development	10 folds
Molar absorptivity (L/mol/cm)	8.4x10^4
Sandal's sensitivity (µg/cm^2)	0.0007
Beer's law validity range(µg/mL)	0.029-0.29
Optimum concentration range(µg/mL)	0.058-0.265
Detection limit(µg/mL)	0.0047
Determination limit(µg/mL)	0.0147
Standard deviation(µg/mL)	0.001581
Composition of complex (M:L)	1:1
Stability constant	2.67x10^{12}
Relative standard deviation (%)	0.868
Regression coefficient	0.99985

Zero-Order Method

In the present investigations, the developed spectrophotometric techniques were proposed to detect Cobalt (II) in tea leaves, car exhaust, vitamin B_{12}, alloy samples, biological, vegetable, fruit, and food samples. The results were depicted in Tables-3 to 7.

Applications

Table-3: Determination of Cobalt (II) in Tea Leaves and Vehicle Exhaust

Sample	Actual value	Amount of Cobalt (II) (mg/g) (Present method)					
		D1	Error (%)	D2	Error (%)	Zero order	Error (%)
Vehicle exhaust	3.3	3.29	0.30	3.32	0.60	3.27	-0.97
Tea leaves	0.12	0.121	0.83	0.123	2.43	0.118	-0.17

Table-4: Determination of Cobalt (II) in Vitamin B_{12} Tablet

Vitamin B_{12}	AAS	Amount of Cobalt (II) (mg/mL) found with relative error (Current method)					
		Zero order	Error (%)	D1	Error (%)	D2	Error (%)
Basitonforte	7.42	7.38	-0.57	7.40	-0.27	7.41	-0.13

Table-5: Determination of Cobalt (II) in Alloy samples

Sample	Accepted value	Amount of Cobalt (II) (mg/g) (Current method)					
		Zero order	Error (%)	D1	Error (%)	D2	Error (%)
Eligiloy-M1712	40.00	39.36	-1.62	39.43	-1.44	39.48	-1.31
High-speed steel	9.25	9.15	-1.09	9.21	-0.43	9.23	-0.21

Table-6: Determination of Cobalt (II) in Biological Samples

Sample name	Sample source	Cobalt (II) (μg/L)			
		AAS		Present method	
		Found	RSD (%)	Found	RSD (%)
Normal adult (male)	Blood	0.2	1.2	0.15	1
	Urine	0.1	1.5	0.08	1.2
Normal adult (female)	Blood	0.15	1.3	0.1	1.1
	Urine	0.1	1.5	0.07	1
Skin disease patients (M)	Blood	0.5	1.3	0.45	1.1
	Urine	0.2	1.5	0.15	1.2
Skin disease patients (F)	Blood	0.3	1.3	0.2	1
	Urine	0.2	1.5	0.17	1.2
Anaemia patient (M)	Blood	10	1	9	1
	Urine	30	1.5	25	1.2
Anaemia patient (F)	Blood	8	1.3	6	1.1
	Urine	25	1.5	20	1.1
Heart disease patient (M)	Blood	5	1.2	4	1
	Urine	3	1.2	2	1.1
Heart disease patient (F)	Blood	4	1.5	3	1.2
	Urine	2	1	1.5	1.2
Kidney Dialysis patient (M)	Blood	7	1.5	6	1.3
	Urine	11	1.3	10	1.1
Kidney Dialysis patient (F)	Blood	8	1.5	7	1.2
	Urine	13	1.2	12	1

Table-7: Determination of Cobalt (II) in Some Samples of Vegetables, Fruits and Food

Sample Name	Cobalt (II) (μg/kg)			
	AAS		Present method	
	Found	RSD (%)	Found	RSD (%)
Chicken meat	4.1	1.5	3.5	1
Chicken liver	5.3	1.3	4.8	1.1
Egg white	4.8	1.1	4	1
Egg yolk	6.2	1.5	5.8	1
Carrot	6.1	1.3	5.6	1.1
Tomato	8.2	1.5	7.6	1

Radish	7.1	1.3	6.7	1
Spinach	9.2	1.5	8.7	1.1
Black pepper	8	1	7.2	1
Cashew nut	12.8	1.5	10.8	1.1
Wheat	2.1	1.3	1.8	1
Tobacco	25.5	1.5	22.8	1.2
Rice	1.5	1.2	1.2	1

Precision and Accuracy

The accuracy and precision of the recommended techniques were assessed using five replicates of 0.117 µg/mL of cobalt (II), and the RSD values were less than 2.5%. The comparative study of spectrophotometric methods reported in the literature with CHMPH is incorporated in Table-8.

Table-8: Comparison of Spectrophotometric Methods for the Determination of Cobalt(II)

Reagent	λmax (nm)	pH	Molar absorptivity $L.mol^{-1}cm^{-1}$	Beer's law Range (µg/mL)	Reference
Iso vanillin thio semi carbazone	440	8.4	$0.135x10^4$	Up to 18.86	18
2-methylindol- 3carboxaldehyde–4-phenyl-3-thiosemicarbazone	388	8.8	$7.0x10^3$	0.39-1.38	19
Benzyl di thiosemi carbazone	589	4.0	$7.3x10^3$	1-8	20
Salicylhydroxamic acid	356	8.0	$4.4x10^3$	15 ppm	21
8-hydroxy quinoline	398	10.0	$2.74\ x10^3$	0-50 µg/mL	22
Pyridine-2-acetaldehyde salicyloyl-hydrazone	415	1.0-6.0	1.04 x103	0.5-7.0	23
2-hydroxy4-methoxy benzophenonethiosemi carbazone	410	9.0	$1.7\ x10^3$	2.9-17.6	24
Phenanhrenequinonemono semicarbazone	470	9.0-10.8	$1.7\ x10^3$	7.08ppm	25
Furfuraldehyde semi-carbazone (FAS)	356	8.1	$1.28x10^5$	0-10 µg/25mL	26
2,4-dihydroxy acetophenone oxime	390	9.5	$2.74\ x10^3$	0.12-1.8	27
2',4'-di hydroxy –5'-bromochalcone oxime	400	8.0-10.0	$2.384x10^3$	Upto 18.86	28
Cinnamaldehyde 4-hydroxybennzoylhydrazone	400	9.0	$2.171x10^4$	0.146-1.467	29
Cinnamaldehyde 4 Hydroxy Methyl Phenyl Hydrazone	410	9.0	$8.41x10^4$	0.029-0.294	Present method

CONCLUSION

When determining the amount of Cobalt (II) in pharmaceutical, environmental, food, and biological materials, the suggested technique employing CHMPH in aqueous solutions offers a highly sensitive, selective, and straightforward method. It enables routine monitoring of trace and ultra-trace levels without the need for heating or organic-phase extraction, making it a rapid and practical alternative to conventional spectrophotometric methods. Sustainable Development Goals, including SDG 3 (Good Health and Well-Being) and SDG 6 (Clean Water and Sanitation), are also supported by the method's eco-friendly and effective design, which promotes safe and trustworthy cobalt monitoring in biological and environmental matrices. This approach can be recommended as a routine tool for analytical laboratories and future studies focused on trace metal detection.

ACKNOWLEDGMENTS

We extended our thanks to the Department of Biotechnology, Nagarjuna University, Guntur, Andhra Pradesh, India, for providing the research facilities.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-6: Clean Water and Sanitation*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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Cite this article as: Ch. Kethani Devi and P. Sudhakar, *Rasayan J. Chem.*, 19(1), 556-563(2026), https://doi.org/10.317 88/RJC.2026.1919502.

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[RJC-9502/2025]