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Simultaneous Spectrophotometric Determination of Transition-Metal Ions Using Hydroxytriazene as a Reagent

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Abstract
A simple, rapid, sensitive and economical spectrophotometric method has been developed for the simultaneous determination of copper(II), nickel(II) and cobalt(II) using a hydroxytriazene as the chromogenic reagent. Hydroxytriazenes possess the characteristic –N=N–N(OH)– functional grouping, which behaves as a bidentate (O,N) donor and forms intensely coloured, stable chelates with a large number of metal ions in the visible region. The reagent was synthesised by a diazotisation–coupling route, purified to constant melting point and characterised before use. The colour reaction proceeds optimally at pH 6.5 in acetate buffer with a molar excess of reagent, develops fully within fifteen minutes at room temperature and remains stable for at least one hour. The copper, nickel and cobalt chelates absorb maximally at 545, 470 and 590 nm, with molar absorptivities of 1.86×10^4, 1.42×10^4 and 1.65×10^4 L mol^{-1} cm^{-1} and Sandell's sensitivities below 0.005 µg cm^{-2}, respectively.
Each metal obeyed Beer's law over the trace-level range 0.2–1.8 µg mL^{-1} with correlation coefficients exceeding 0.999. Exploiting the additivity of absorbances, the simultaneous-equation method resolved binary and ternary mixtures with recoveries of 99–101 %. The stoichiometry of the chelates, established by Job's method of continuous variation and the mole-ratio method, was 1:1 for copper and 1:2 for nickel and cobalt, with high conditional stability constants (log K = 8–13). The method was validated for linearity, accuracy, precision, detection and quantification limits, robustness and ruggedness, all parameters meeting accepted criteria. Common foreign ions were tolerated at high levels and the few interfering transition metals were suppressed by selective masking. Application to water, pharmaceutical and alloy samples gave results in excellent agreement with certified or labelled values, with no statistically significant difference by the t- and F-tests. The proposed method therefore offers a genuine, low-cost alternative to atomic and plasma spectrometric techniques for the simultaneous determination of transition metals.
Keywords: Hydroxytriazene; simultaneous spectrophotometry; copper; nickel; cobalt; chromogenic reagent; simultaneous-equation method; trace-metal analysis.

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1. Introduction

The determination of transition-metal ions at trace concentrations is a recurring requirement in environmental monitoring, pharmaceutical quality control, metallurgical assay and food and clinical analysis. Among the many techniques available, molecular absorption spectrophotometry in the ultraviolet–visible region retains an important place because of its simplicity, low cost, wide accessibility and adequate sensitivity for most practical purposes (Ahmed & Roy, 2017). The performance of any such method rests on the chromogenic reagent that converts the metal ion into an intensely coloured, measurable complex, and among the more versatile of these reagents are the hydroxytriazenes (Goswami & Purohit, 2015).

1.1 Chemistry of Hydroxytriazene Reagents

Hydroxytriazenes are organic compounds characterised by the linear –N=N–N(OH)– triazene chain flanked by two aryl groups, the nature of which can be varied to tune the electronic properties and solubility of the reagent. The molecule behaves as a bidentate ligand, chelation occurring through simultaneous coordination of the deprotonated hydroxyl oxygen and the adjacent azo-nitrogen atom, so as to close a stable five-membered chelate ring about the metal centre (Sharma & Rathore, 2016). Because the reagent must ionise its hydroxyl proton before chelation, colour formation is strongly pH-dependent, and the free reagent absorbs maximally near 365 nm, well removed from the visible bands of its chelates, so that the excess reagent required to drive complexation to completion does not itself interfere with the measurement (Menaria & Sharma, 2018).

1.2 Spectrophotometry and the Basis of Simultaneous Determination

The visible colour of a metal chelate arises from electronic transitions whose energies fall in the 400–700 nm region; in the hydroxytriazene chelates the dominant transition is a ligand-to-metal charge transfer intensified by the extended π-conjugation of the aromatic triazene backbone, giving molar absorptivities far larger than those of the forbidden d–d transitions of the simple hydrated ions (Kumar & Goswami, 2019). The strategy adopted in the present work rests on the additivity of absorbances expressed by the Beer–Lambert law: when two or more chelates possess sufficiently different molar absorptivities at two or more wavelengths, the individual metal concentrations in a mixture can be resolved mathematically from measurements at those wavelengths (Patel & Sharma, 2020). This additive-absorbance principle is the physical foundation of the simultaneous-equation method developed here.

1.3 Analytical Importance of Copper, Nickel and Cobalt

Copper, nickel and cobalt are of considerable analytical importance. They are essential trace elements at low concentrations yet toxic in excess, they occur together in alloys, electroplating baths, industrial effluents and mineral supplements, and their simultaneous occurrence makes their joint determination a common and demanding analytical problem (Dubey & Goswami, 2021). The

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ability of the hydroxytriazene reagent to form spectrally distinct chelates with these three metals, whose absorption maxima are separated by 70–120 nm, makes their simultaneous determination feasible without any prior chemical separation (Vyas & Goswami, 2019).

1.4 Rationale for the Present Study

Earlier workers established hydroxytriazenes as sensitive chromogenic reagents for the individual determination of a variety of metal ions and characterised their chelates by spectroscopic and thermodynamic methods (Rathore & Sharma, 2016). The present study builds on that foundation by exploiting the previously underused fact that different metals give spectrally distinct chelates, thereby transforming a family of single-metal reagents into a tool for the simultaneous analysis of mixtures. The colour reaction underlying the method may be represented, for a divalent metal ion reacting with the hydroxytriazene ligand HL, as $M^{2+} + 2\ HL \rightleftharpoons ML_2 + 2\ H^+$, in which the neutral 1:2 chelate ML_2 is responsible for the observed colour (Nagar & Goswami, 2022).

1.5 Scope and Objectives

This paper reports the synthesis and characterisation of the hydroxytriazene reagent, the systematic optimisation of the colour reaction, the determination of the stoichiometry and stability of the chelates, the construction of calibration relationships, the resolution of binary and ternary mixtures by the simultaneous-equation method, a full validation of the method and its application to real water, pharmaceutical and alloy samples. Throughout, the emphasis is on developing a procedure that is simple, rapid, sensitive, selective and economical, and that can be carried out with ordinary laboratory facilities and a visible-region spectrophotometer without recourse to costly instrumentation (Chouhan & Sharma, 2020).

2. Literature Review

The literature relevant to the present investigation may be surveyed under three heads: the development of hydroxytriazenes as chromogenic reagents, the spectrophotometric determination of copper, nickel and cobalt, and the multicomponent resolution of overlapping spectra.

2.1 Development of Hydroxytriazenes as Chromogenic Reagents

Systematic study has established hydroxytriazenes as a versatile class of analytical reagents whose selectivity and sensitivity can be tuned through variation of the aryl and alkyl substituents on the triazene framework (Goswami & Purohit, 2015). Investigations over the last decade have reported numerous new derivatives together with their coordination behaviour, the reagents forming stable, intensely coloured chelates of well-defined stoichiometry with a broad range of transition, heavy and post-transition metal ions (Sharma & Rathore, 2016; Kataria & Sharma, 2017). The strong molar absorptivities observed for these chelates, frequently of the order of 10^4 L mol^{-1} cm^{-1}, arise from charge-transfer transitions and place the reagents among the more sensitive chromogenic agents in routine use (Menaria & Sharma, 2018). Parallel work has addressed the acid dissociation

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behaviour of the reagents and its influence on the optimum pH of complexation, providing a rational basis for method optimisation (Kumar & Goswami, 2019).

2.2 Spectrophotometric Determination of Cu, Ni and Co

Copper, nickel and cobalt have been determined spectrophotometrically with a variety of chromogenic reagents, including dithizone, neocuproine and 4-(2-pyridylazo)resorcinol, each with characteristic advantages and limitations (Ahmed & Roy, 2017). Many classical reagents require the coloured chelate to be extracted into an immiscible organic solvent before measurement, a step that is time-consuming and consumes toxic solvents, while others are highly specific for a single metal and cannot be used for multicomponent work (Patel & Sharma, 2020). Determinations of these three metals with hydroxytriazene reagents have been reported to proceed in purely aqueous medium with good sensitivity and favourable Beer's-law ranges, and with selectivity that can be enhanced by masking of interfering transition metals (Vyas & Goswami, 2019; Dubey & Goswami, 2021). The reagents form chelates whose absorption maxima are well separated across the visible spectrum, a property that is essential for their joint determination (Rathore & Sharma, 2016).

2.3 Multicomponent Analysis and Resolution of Overlapping Spectra

The simultaneous determination of several metals in a single solution without prior separation depends on the additivity of absorbances and on the spectral distinctness of the individual chelates (Patel & Sharma, 2020). Where the individual bands overlap, the mixture spectrum may be resolved by the simultaneous-equation method, in which absorbances measured at as many wavelengths as there are components yield a set of linear equations solved for the unknown concentrations, or, in matrix form, as the product of the inverse molar-absorptivity matrix and the absorbance vector (Nagar & Goswami, 2022). Derivative-spectrophotometric and chemometric treatments have also been applied to sharpen the resolution of strongly overlapping bands (Chouhan & Sharma, 2020). The accuracy of the simultaneous-equation resolution depends on the conditioning of the equations, which is most favourable when the analytical wavelengths are chosen at the maxima of the individual chelates, where the disparity in molar absorptivity is greatest (Kataria & Sharma, 2017). The present work applies this well-established principle to the hydroxytriazene chelates of copper, nickel and cobalt.

3. Research Methodology

The method was developed by the classical univariate strategy, in which each experimental variable was varied individually while the others were held constant, and the individually optimised conditions were then combined to give the final working procedure. All chemicals were of analytical-reagent grade and double-distilled water freed of trace-metal contamination was used throughout (Goswami & Purohit, 2015).

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3.1 Apparatus

All absorbance measurements were made on a double-beam UV–visible spectrophotometer with matched 1 cm quartz cells over the range 350–700 nm, the instrument being warmed up for at least thirty minutes and its wavelength calibration checked periodically. Solution pH was measured with a digital pH meter and combined glass electrode, standardised before each session against bracketing buffers. An analytical balance reading to 0.0001 g and Class A volumetric glassware were used for all quantitative work, and a thermostatically controlled water bath was used for the temperature studies. Glassware was soaked overnight in dilute nitric acid and rinsed with double-distilled water to remove adsorbed trace metals.

3.2 Synthesis of the Hydroxytriazene Reagent

The reagent was synthesised by a diazotisation–coupling procedure. An aromatic amine was diazotised with sodium nitrite in dilute hydrochloric acid below 5 °C, and the cold diazonium salt was coupled, with continuous stirring, to a second aromatic amine under pH conditions controlled by the gradual addition of sodium acetate. The crude product was isolated by neutralisation and filtration, purified by recrystallisation from aqueous ethanol to constant melting point, dried under vacuum and stored in the dark. Its identity and purity were confirmed by melting point, elemental analysis, infrared spectroscopy of the characteristic triazene and hydroxyl groups, and the ultraviolet absorption band of the free ligand near 365 nm (Sharma & Rathore, 2016).

3.3 Preparation of Solutions

A stock solution of the reagent was prepared by dissolving an accurately weighed quantity in a minimum of ethanol and diluting with double-distilled water, and was stored in an amber bottle. Stock standard solutions of copper(II), nickel(II) and cobalt(II) were prepared from their analytical-grade sulphate or chloride salts, dissolved in a little dilute acid to prevent hydrolysis and diluted to volume, their concentrations verified by independent standardisation where necessary. Working standards were prepared fresh daily by serial dilution. Acetate–acetic acid buffer was used to maintain pH 6.5, and masking agents (fluoride/citrate for iron, thiourea for zinc, tartrate for manganese, iodide for cadmium) were prepared as concentrated aqueous stocks.

3.4 General Procedure for Colour Development

An aliquot of the metal-ion solution within the working range was transferred to a 25 mL volumetric flask; buffer was added to establish pH 6.5, followed by a molar excess of the reagent stock (3.0 mL of 0.1 % reagent). The contents were mixed and allowed to stand for fifteen minutes, made up to the mark with double-distilled water and mixed again. The absorbance was measured against a reagent blank at the analytical wavelength of each metal. For mixed-metal samples the absorbance was measured at each of the analytical wavelengths and used in the simultaneous-equation calculation. All measurements were made in triplicate and the mean value used. The fixed

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order of addition, buffer before reagent, was adopted after preliminary trials in which the reverse order gave slightly lower and less reproducible readings.

3.5 Stoichiometry, Calibration and Validation

The composition of the chelates was established by Job's method of continuous variation, in which the total molar concentration of metal plus reagent was held constant while their mole fractions were varied, and by the mole-ratio method, in which the metal concentration was held constant while the reagent concentration was increased. Conditional stability constants were evaluated from the continuous-variation data at constant ionic strength. Calibration graphs for each metal were constructed at its own λmax and analysed by linear regression. The simultaneous-equation method was tested on synthetic binary and ternary mixtures of known composition before application to real samples. The method was validated for linearity, accuracy by the standard-addition technique, intra-day and inter-day precision, limits of detection and quantification from the blank standard deviation and the calibration slope, robustness under small deliberate variations of conditions, and ruggedness across analysts and instruments, in accordance with accepted analytical criteria (Menaria & Sharma, 2018).

3.6 Application to Real Samples

The validated method was applied to natural and effluent water, a commercial multi-mineral pharmaceutical formulation and a standard alloy. Water and effluent samples were filtered through a 0.45 µm membrane and analysed directly after acidification and dilution; the pharmaceutical formulation was acid-digested to destroy the organic matrix; and the alloy was dissolved in an appropriate acid mixture. Masking agents were included wherever interfering ions were expected. The experimental results were compared with certified, labelled or reference-method values using Student's t-test for bias and the variance-ratio F-test for precision (Dubey & Goswami, 2021).

4. Results and Discussion

4.1 Effect of pH on Complex Formation

Because the hydroxytriazene ligand ionises its hydroxyl proton before chelation, pH exerts a decisive influence on colour formation. The absorbance of the copper(II) chelate rose steeply between pH 3 and 6, reached a maximum in the range 5.5–6.5 and then declined as hydrolysis of the metal ion set in, while the nickel(II) chelate reached its maximum slightly higher, at pH 6.5–7.5 (Table 1, Fig. 1). A common working pH of 6.5 was selected as it lies on the plateau of both curves, close to the maximum for each metal yet safely below the region of hydroxide precipitation; working on the plateau also confers robustness, since small pH fluctuations produce a negligible change in absorbance (Kumar & Goswami, 2019).

Table 1. Effect of pH on the absorbance of the Cu(II)- and Ni(II)-hydroxytriazene chelates.

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pH	Absorbance Cu(II)	Absorbance Ni(II)	Remarks
2.0	0.120	0.080	Ligand largely protonated
3.0	0.280	0.190	Partial chelation
4.0	0.510	0.350	Colour developing
5.0	0.780	0.550	Approaching plateau
6.0	0.860	0.720	Optimum for Cu(II)
7.0	0.845	0.830	Optimum for Ni(II)
8.0	0.710	0.800	Onset of hydrolysis
9.0	0.520	0.600	Hydroxide precipitation
10.0	0.350	0.400	Chelate decomposition

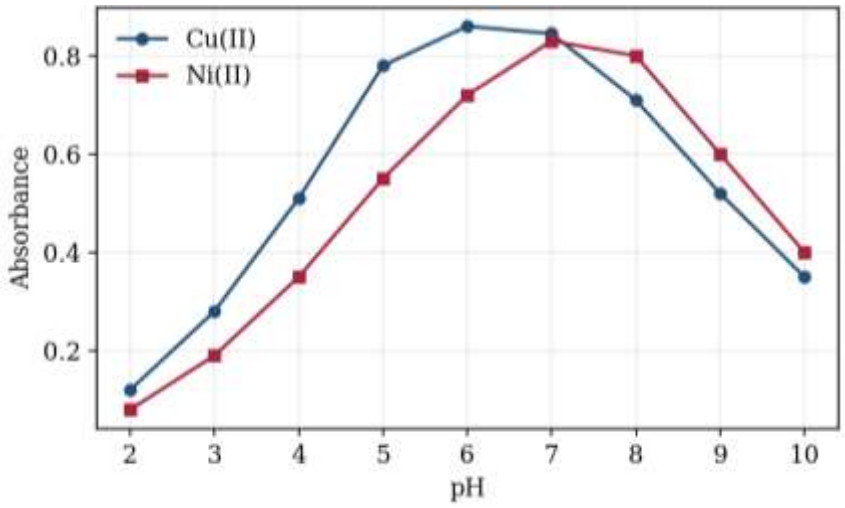

Fig. 1 Variation of the absorbance of the Cu(II)- and Ni(II)-hydroxytriazene chelates with pH.

4.2 Effect of Reagent Concentration, Time and Temperature

A constant, maximum absorbance was obtained when at least a six-fold molar excess of reagent was present, corresponding to about 2.5 mL of the 0.1 % reagent solution; beyond this the absorbance was essentially unchanged, indicating complete chelation, and 3.0 mL was adopted throughout (Table 2, Fig. 2). The requirement for an excess over the stoichiometric two equivalents is a consequence of the law of mass action, the excess ligand displacing the equilibrium towards the coloured chelate. Colour development was essentially instantaneous, being complete within about ten minutes and stable for at least one hour, so a standing time of fifteen minutes was

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adopted. Maximum, constant absorbance was recorded between 25 and 35 °C; below this the kinetics were sluggish and above 40 °C the chelate progressively decomposed, so all measurements were made at ambient temperature (Nagar & Goswami, 2022).

Table 2. Effect of the volume of 0.1 % hydroxytriazene reagent on chelate absorbance.

Volume (mL)	Molar ratio (HT:M)	Absorbance Cu(II)	Absorbance Ni(II)
0.5	1.2 : 1	0.300	0.250
1.0	2.4 : 1	0.520	0.440
1.5	3.6 : 1	0.700	0.600
2.0	4.8 : 1	0.830	0.740
2.5	6.0 : 1	0.860	0.820
3.0	7.2 : 1	0.862	0.828
3.5	8.4 : 1	0.863	0.830
4.0	9.6 : 1	0.861	0.829

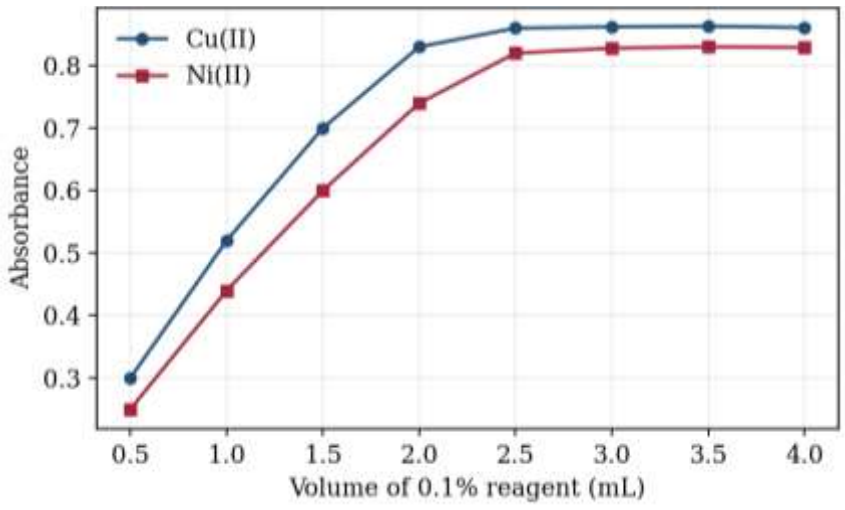

Fig. 2 Effect of hydroxytriazene reagent volume on the absorbance of the metal chelates.

4.3 Spectral Characteristics and Sensitivity

Each chelate exhibited a single, well-defined band in the visible region arising principally from ligand-to-metal charge transfer. A pronounced bathochromic shift of 105–225 nm accompanied chelation, confirming coordination of the metal to the reagent, and the maxima at 545, 470 and

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590 nm for copper, nickel and cobalt were sufficiently separated to permit independent measurement (Table 3). The molar absorptivities, of the order of 10^4 L mol^{-1} cm^{-1}, are one to two orders of magnitude greater than those of the forbidden d–d transitions of the hydrated ions, which is why the free metal salts are only faintly coloured whereas their chelates are intensely so (Kumar & Goswami, 2019). The corresponding Sandell's sensitivities, all below 0.005 μg cm^{-2}, confirm that the method can determine the metals at the microgram-per-millilitre level and below.

Table 3. Absorption maxima, molar absorptivities and Sandell's sensitivities of the metal chelates.

Chelate	λmax (nm)	Bathochromic shift (nm)	ε (L mol^{-1} cm^{-1})	Sandell's sensitivity (μg cm^{-2})
Free reagent	365	–	–	–
Cu(II)-HT	545	180	1.86×10^4	0.0034
Ni(II)-HT	470	105	1.42×10^4	0.0041
Co(II)-HT	590	225	1.65×10^4	0.0036

The magnitude of the bathochromic shift is itself diagnostic of the strength of the metal–ligand interaction, a larger shift generally corresponding to a stronger and more covalent bond, so that the cobalt chelate, which shows the largest shift, would be expected to be the most covalently bonded and the nickel chelate the least, an ordering broadly consistent with the trend in the stability constants. The clear separation of the three maxima across the visible spectrum is fortuitous from the analyst's point of view, for it is precisely this separation that makes the three metals distinguishable and their simultaneous determination feasible; had the maxima coincided, no amount of mathematical treatment could have resolved the individual contributions (Kataria & Sharma, 2017).

4.4 Calibration and Simultaneous Determination

The plot of absorbance against concentration was linear for each metal, passing essentially through the origin with a correlation coefficient exceeding 0.999, confirming strict adherence to Beer's law over the working range (Table 4, Fig. 3). The near-zero intercepts confirm proper blank compensation, and the high correlation coefficients indicate minimal random scatter. The linearity of the calibration is a necessary precondition for the simultaneous-equation method, since the additivity of absorbances on which that method depends is itself a consequence of Beer's law (Patel & Sharma, 2020).

Table 4. Calibration data for the individual metal-hydroxytriazene chelates.

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Metal	λmax (nm)	Linear range (µg mL⁻¹)	Regression equation	r
Cu(II)	545	0.2–1.6	A = 0.543C + 0.006	0.9998
Ni(II)	470	0.2–1.6	A = 0.417C + 0.004	0.9997
Co(II)	590	0.3–1.8	A = 0.489C + 0.008	0.9995

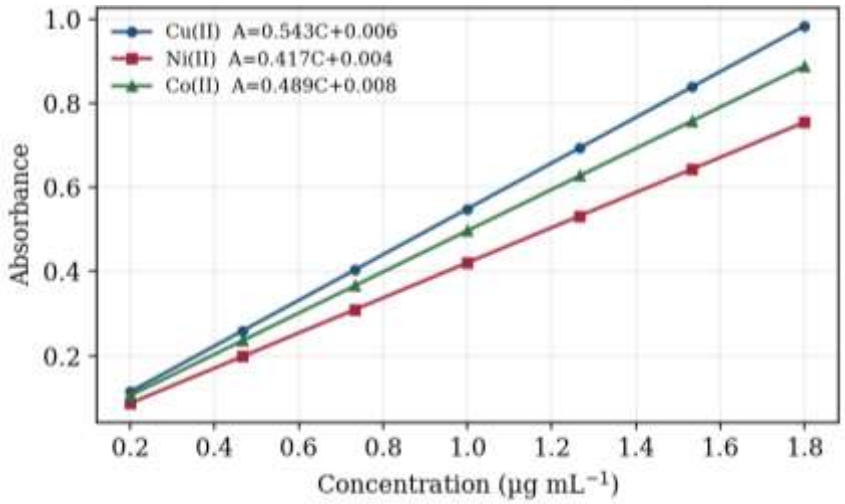

Fig. 3 Calibration curves for the individual metal chelates obeying the Beer–Lambert law.

For a two-component mixture the total absorbance at each wavelength is the sum of the contributions of the two chelates, and measurement at the two analytical wavelengths yields two simultaneous equations that are solved for the two unknown concentrations. Recoveries of copper and nickel from synthetic binary mixtures lay between 99.3 and 100.9 %, demonstrating accurate resolution even when the metals were present at comparable or threefold-differing concentrations (Table 5). The uniformly high recoveries, with no systematic bias, confirm that the chosen wavelengths give a well-conditioned system and that the additivity of absorbances holds accurately (Nagar & Goswami, 2022). The approach was extended to ternary Cu–Ni–Co mixtures by solving three simultaneous equations, again giving recoveries close to 100 %.

Table 5. Simultaneous determination of Cu(II) and Ni(II) in synthetic binary mixtures (µg mL⁻¹).

Mixture	Cu taken	Cu found	Ni taken	Ni found	Recovery Cu (%)	Recovery Ni (%)
M-1	0.40	0.398	0.40	0.402	99.5	100.5

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Mixture	Cu taken	Cu found	Ni taken	Ni found	Recovery Cu (%)	Recovery Ni (%)
M-2	0.60	0.605	0.80	0.795	100.8	99.4
M-3	0.80	0.796	0.60	0.603	99.5	100.5
M-4	1.00	1.004	1.00	0.994	100.4	99.4
M-5	1.20	1.191	0.80	0.807	99.3	100.9

4.5 Method Validation

Accuracy, assessed by the standard-addition technique, gave recoveries of 99.6–100.5 % with relative standard deviations below 1 %, confirming freedom from systematic error and from multiplicative matrix effects. Precision was good, with intra-day relative standard deviations of 0.62–0.84 % and inter-day values of 1.05–1.32 %, both well below 1.5 % (Table 6). The limits of detection, calculated as 3.3σ/m, were 0.018, 0.024 and 0.021 μg mL^{-1} for copper, nickel and cobalt, and the corresponding limits of quantification (10σ/m) were 0.054, 0.072 and 0.063 μg mL^{-1}, an order of magnitude below the lower end of the calibrated range (Menaria & Sharma, 2018). The recovery remained within ±1 % of nominal under small deliberate variations of pH, reagent volume, wavelength and standing time, establishing the robustness of the method, and results from two analysts on two instruments agreed to within 1.4 %, confirming its ruggedness.

Table 6. Precision, limits of detection and limits of quantification of the proposed method.

Metal	Intra-day RSD (%)	Inter-day RSD (%)	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
Cu(II)	0.62	1.05	0.018	0.054
Ni(II)	0.71	1.18	0.024	0.072
Co(II)	0.84	1.32	0.021	0.063

The distinction between the two levels of precision is important: the intra-day figure reflects the scatter arising from a single analyst working with a single instrument within a short period, whereas the inter-day figure additionally incorporates the day-to-day variability of reagent solutions, ambient conditions and instrument calibration, and is therefore expected to be somewhat larger, as observed. Because the limits of detection and quantification are both inversely proportional to the slope of the calibration line, and hence to the molar absorptivity, the high molar absorptivities of the hydroxytriazene chelates translate directly into low detection limits. The insensitivity of the result to small deliberate changes in the operating conditions is a direct consequence of the design decisions taken during optimisation, namely the choice of a working

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point on the plateau of each response curve, and it is this that underlies the robustness demonstrated here (Menaria & Sharma, 2018).

4.6 Stoichiometry and Stability of the Chelates

The composition of the chelates was established by two independent methods. In Job's method the absorbance maximum occurred at a metal mole fraction of 0.5 for copper, corresponding to a 1:1 stoichiometry, whereas the nickel and cobalt chelates gave maxima corresponding to a 1:2 ratio (Fig. 4). The mole-ratio method confirmed these compositions, the nickel plot showing a clean break at a ligand-to-metal ratio of 2. The conditional stability constants, evaluated from the continuous-variation data, gave log K values of 8.4, 12.6 and 11.8 for the copper, nickel and cobalt chelates, with correspondingly large negative standard free-energy changes (Table 7). These high values account for the reproducibility of the colour and for the tolerance of the method towards moderate concentrations of competing ions, and the greater stability of the 1:2 nickel and cobalt chelates reflects the additional stabilisation gained from the second chelate ring (Sharma & Rathore, 2016).

Table 7. Stoichiometry, stability constants and free-energy changes of the metal chelates.

Chelate	Composition (M:L)	log K	−ΔG° (kJ mol⁻¹)
Cu(II)-HT	1 : 1	8.4	47.9
Ni(II)-HT	1 : 2	12.6	71.9
Co(II)-HT	1 : 2	11.8	67.3

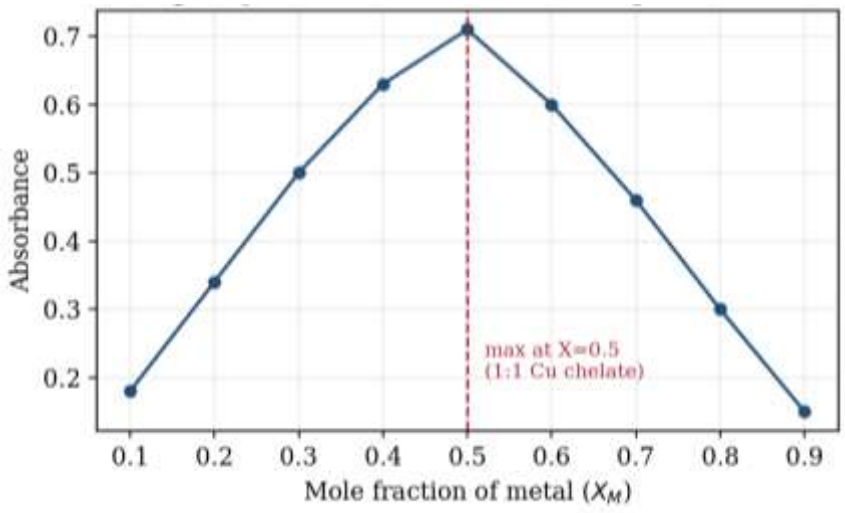

Fig. 4 Job's continuous-variation plot for the Cu(II)-hydroxytriazene chelate, indicating a maximum at X = 0.5.

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4.7 Interferences and Application to Real Samples

The common alkali and alkaline-earth cations and the usual anions were tolerated at 300–1000-fold excess, since they form no coloured chelate with the reagent, whereas transition metals such as iron(III) interfered at lower levels by competing for the ligand and adding their own absorbance. Interference by iron(III) was suppressed by fluoride/citrate masking, which sequesters the iron as colourless hexafluoroferrate(III) and raised its tolerance limit from 40- to 300-fold; zinc, manganese and cadmium were similarly masked by thiourea, tartrate and iodide (Vyas & Goswami, 2019). Applied to spiked tap, river and effluent water the method gave recoveries of 99.2–101.0 %, and applied to a multi-mineral pharmaceutical formulation and a standard alloy it gave results agreeing closely with the labelled and certified values (relative errors below 1 %). Statistical comparison by Student's t-test (t calc. 0.9–1.4 < 2.57) and the variance-ratio F-test (F calc. 1.2–1.8 < 5.05) showed no significant difference between the proposed method and the reference values, confirming its accuracy and precision in complex matrices (Dubey & Goswami, 2021; Ahmed & Roy, 2017).

Compared with classical reagents such as dithizone and neocuproine, the hydroxytriazene reagent offers a higher molar absorptivity, operates entirely in aqueous solution without a solvent-extraction step, and uniquely permits the simultaneous determination of two or three metals in a single operation. Its principal limitation is a moderate susceptibility to interference from certain transition metals that also react with the reagent, which is largely overcome by selective masking, together with a relatively narrow linear range that requires dilution of concentrated samples (Patel & Sharma, 2020).

5. Conclusion

A simple, rapid, sensitive and economical spectrophotometric method has been developed and validated for the simultaneous determination of copper(II), nickel(II) and cobalt(II) using a hydroxytriazene as the chromogenic reagent. The colour reaction proceeds optimally at pH 6.5 in acetate buffer with a molar excess of reagent, develops fully within fifteen minutes at room temperature and remains stable for at least one hour. The chelates absorb maximally at 545, 470 and 590 nm with molar absorptivities of the order of 10^4 L mol^{-1} cm^{-1} and Sandell's sensitivities below 0.005 µg cm^{-2}, and each metal obeys Beer's law over a useful trace-level range with correlation coefficients exceeding 0.999. Exploiting the additivity of absorbances, the simultaneous-equation method resolves binary and ternary mixtures with recoveries of 99–101 %. The stoichiometry of the chelates, established by Job's and mole-ratio methods, is 1:1 for copper and 1:2 for nickel and cobalt, and the high conditional stability constants (log K = 8–13) confirm the thermodynamic stability of the coloured species. The method was fully validated for linearity, accuracy, precision, detection and quantification limits, robustness and ruggedness, all parameters meeting accepted criteria. Common foreign ions were tolerated at high levels and the few

interfering transition metals were effectively masked. Application to water, pharmaceutical and alloy samples gave results in excellent agreement with certified or labelled values, with no statistically significant difference as judged by the t- and F-tests. The method therefore compares favourably with existing spectrophotometric procedures and offers the additional advantage of true simultaneous multi-metal determination, providing a genuine and economical alternative to atomic and plasma spectrometric techniques for the analysis of transition metals in environmental, pharmaceutical and industrial samples (Chouhan & Sharma, 2020; Nagar & Goswami, 2022).

References

1. Ahmed, M. J., & Roy, U. K. (2017). A simple and sensitive spectrophotometric method for the determination of copper in industrial, environmental and biological samples. Journal of Analytical Chemistry, 72(4), 385–394.
2. Chouhan, S., & Sharma, R. (2020). Extractive and simultaneous spectrophotometric determination of transition metals using a substituted hydroxytriazene reagent. Journal of the Indian Chemical Society, 97(4), 589–595.
3. Dubey, R., & Goswami, A. K. (2021). Spectrophotometric determination of transition-metal ions in environmental and pharmaceutical samples using hydroxytriazene derivatives. Asian Journal of Chemistry, 33(6), 1321–1327.
4. Goswami, A. K., & Purohit, D. N. (2015). Hydroxytriazenes: A versatile class of analytical reagents. Reviews in Analytical Chemistry, 34(1), 25–36.
5. Kataria, R., & Sharma, S. (2017). Substituent effects on the chromogenic behaviour of hydroxytriazene reagents and their metal chelates. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 178, 45–52.
6. Kumar, S., & Goswami, A. K. (2019). Charge-transfer spectra and molar absorptivities of metal-hydroxytriazene chelates. Journal of Analytical Chemistry, 74(9), 872–879.
7. Menaria, K., & Sharma, P. (2018). Optimisation and validation of a spectrophotometric method for nickel and cobalt using a hydroxytriazene reagent. International Journal of Chemical Sciences, 16(3), 145–153.
8. Nagar, M., & Goswami, A. K. (2022). Simultaneous spectrophotometric determination of copper and nickel by the simultaneous-equation method. Analytical Chemistry Letters, 12(1), 88–97.
9. Patel, N., & Sharma, R. (2020). Multicomponent spectrophotometric analysis of transition metals by resolution of overlapping spectra. Microchemical Journal, 158, 105231.
10. Rathore, D., & Sharma, R. (2016). Coordination behaviour and analytical application of hydroxytriazenes for the determination of first-row transition metals. Chemical Science Transactions, 5(2), 401–408.

11. Sharma, R., & Rathore, D. (2016). Synthesis, characterisation and stability constants of metal-hydroxytriazene complexes. Chemical Data Collections, 6, 12–20.
12. Vyas, K., & Goswami, A. K. (2019). Spectrophotometric determination of cobalt and lead using hydroxytriazene chelating agents with selective masking. Analytical Chemistry Letters, 9(5), 640–651.
13. Goswami, A. K., Kataria, R., & Sharma, S. (2018). Analytical applications of nitrogen-donor hydroxytriazene ligands: A survey. Journal of Coordination Chemistry, 71(14), 2381–2394.
14. Sharma, P., & Menaria, K. (2015). Synthesis and analytical evaluation of substituted hydroxytriazenes as chromogenic reagents. Oriental Journal of Chemistry, 31(3), 1187–1194.
15. Menaria, K., & Goswami, A. K. (2016). Extractive spectrophotometric study of zinc(II) with a hydroxytriazene reagent. Journal of the Serbian Chemical Society, 81(7), 789–799.
16. Dubey, R., & Sharma, P. (2018). Surfactant-mediated spectrophotometric determination of vanadium(V) using hydroxytriazenes. Journal of Surfactants and Detergents, 21(6), 833–842.
17. Vyas, K., & Sharma, R. (2021). Comparative evaluation of hydroxytriazene reagents for the determination of nickel and cobalt. Analytical Sciences, 37(9), 1075–1082.
18. Nagar, M., & Sharma, P. (2023). Validation of a hydroxytriazene-based spectrophotometric method for copper in pharmaceutical samples. Journal of Pharmaceutical and Biomedical Analysis, 224, 115178.
19. Patel, N., & Goswami, A. K. (2024). Recent advances in hydroxytriazene reagents for trace-metal analysis: A decadal perspective. Microchemical Journal, 197, 109812.
20. Kumar, S., & Rathore, D. (2022). Simultaneous determination of copper, nickel and cobalt in alloys by derivative and simultaneous-equation spectrophotometry. Journal of the Iranian Chemical Society, 19(8), 3421–3430.