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A VANILLIN-DERIVED CHLORO-BENZIMIDAZOLE SCHIFF BASE AS A CHROMOGENIC REAGENT FOR SPECTROPHOTOMETRIC DETERMINATION OF PB(II) AND CIPROFLOXACIN

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ABSTRACT

The Schiff base (Z)-4-(((2-(6-chloro-3a,4-dihydro-1H-benzo[d]imidazol-2-yl)phenyl)imino)methyl)-2-methoxyphenol (compound 23, $C_{26}H_{20}ClN_5O_3$) resulted from condensing 2-amino-6-chloro-1H-benzimidazole with vanillin, and FT-IR, ^{1}H-NMR, and ^{13}C-NMR confirmed the expected structure. We then examined whether this compound could detect Pb(II) by UV-Vis spectrophotometry. Mixing the reagent with Pb(II) in ethanol-water at pH 6.5 turned the solution reddish-brown (λmax shifting from 425 to 510 nm). Full color development took 15 min at room temperature. Job's method gave a 1:2 (M:L) ratio. The calibration was linear across 0.5-12.0 µg/mL ($\varepsilon = 1.85\times10^4$ L mol^{-1} cm^{-1}, Sandell sensitivity 11.2 ng cm^{-2}), with LOD and LOQ of 0.091 and 0.305 µg/mL. The calculated β_2 (1.02×10^8 M^{-2}) and thermodynamic quantities ($\Delta H = -27.1$ kJ/mol, $\Delta S = +62.4$ J/mol·K, $\Delta G = -45.8$ kJ/mol) indicate spontaneous, exothermic complex formation. Spiked tap water and Euphrates river water samples gave Pb(II) recoveries of 98.2-98.3%. Reagent (23) was also applied to ciprofloxacin tablets by an indirect route: adding ciprofloxacin displaced Pb(II) from the compound 23 complex, reducing the 510 nm absorbance in proportion to drug concentration over 0.5-10 µg/mL (LOD = 0.157 µg/mL). Tablet recoveries fell between 98.5 and 98.8%, with no interference from common excipients.

1. INTRODUCTION

Lead in water is harmful at very low amounts. The World Health Organization has set the maximum allowed concentration in drinking water to 10 µg/L due to prolonged exposure leading to kidney and nervous system damage and an increased risk of cancer [1,2]. Sources of pollution have not disappeared: industry discharge, runoff from mines, old leaded-fuel residues, and corroding lead pipes all add Pb(II) to surface water and groundwater [3]. Methods for rapid, inexpensive and reliable measurements of trace lead in ambient samples are required.

UV–Vis spectrophotometry is still one of the most accessible techniques for assessing heavy metals in a typical lab [4,5]. Instruments are cheap, a measurement takes minutes and the full procedure can be moved to the field if an appropriate chromogenic reagent is available [6]. Hence, one of the important factors is the selection of a chelating agent which may provide both selectivity for the target ion and high molar absorptivity. It all depends on the reagent. It has to produce a very stable, strongly coloured complex with the metal you want, preferably at pH close to neutral.

A long time ago, Schiff bases and azo dyes have been employed as chelating agents in analytical work. The donor atoms (N, O, S) can be tuned by varying substituents and the conjugation characteristic of the imine or azo link commonly leads to high molar absorptivities [7-10]. Imidazole nitrogen atoms are also incorporated as extra coordination sites into the Schiff bases including benzimidazole, which often results in improved selectivity and binding strength [11,12]. Most of the work reported on these reagents have been carried

out with Cu(II), Zn(II) or Cd(II) . There are limited reports of Pb (II) in real water samples [13,14].

The pharmaceutical analysis also has this requirement for economical, dependable procedures for the quality-control verification of drug contents in routine situations. Ciprofloxacin is a commonly utilised fluoroquinolone that chelates divalent metal ions via its carboxyl and ketone groups [15]. This characteristic provides a means for indirect assessment with spectrophotometry. The analyst makes a coloured metal-reagent complex, adds the medication and analyses how much the colour fades.

Here we describe compound 23, a benzimidazole Schiff base obtained through the condensation of 6-chloro-2-(2-aminophenyl)-1H-benzimidazole with vanillin (4-hydroxy-3-methoxybenzaldehyde), together with its full spectral characterization. We then used this compound as a new chromogenic reagent for the determination of Pb(II) in water, optimized the reaction conditions, measured the analytical figures of merit and the thermodynamics of complex formation, checked for interferences from common cations, and tested the method on real water samples. As a further application, the reagent was tried for the indirect assay of ciprofloxacin in tablets.

2. MATERIALS AND METHODS

2.1. Reagents and chemicals

All chemicals were analytical reagent grade and used as received. o-Phenylenediamine (97%, MACKLIN), salicylic acid (99.5%, B.D.H), 4-chloro-o-phenylenediamine (97%, MACKLIN), vanillin (99%, MACKLIN), and anhydrous zinc chloride (97%, MACKLIN) were purchased from the indicated suppliers. Lead nitrate $Pb(NO_3)_2$ (99%, Sigma-Aldrich) was dissolved in deionized water to prepare a 1000 µg/mL stock solution of Pb(II); working standards were obtained by serial dilution. Ciprofloxacin hydrochloride (98%, Sigma-Aldrich) and commercial ciprofloxacin tablets (500 mg, local pharmacy) were used for the pharmaceutical application. Acetate buffer solutions covering the pH range 3.0-9.0 were made from glacial acetic acid and sodium acetate. Absolute ethanol (99.9%, Chem-Lab) served as co-solvent. All glassware was soaked overnight in 10% HNO_3 and rinsed with deionized water before use [16].

2.2. Instrumentation

UV-Vis absorption spectra were recorded on a Shimadzu UV-1800 double-beam spectrophotometer using matched 1.0 cm quartz cuvettes. FT-IR spectra were obtained on a Shimadzu FTIR-8400S in the 400-4000 cm^{-1} range using KBr pellets. ^{1}H-NMR and ^{13}C-NMR spectra were recorded in DMSO-d_6 at the Department of Chemistry, University of Basrah, Iraq. Melting points were measured with a Stuart SMP30 apparatus. A Sartorius BL 2015 analytical balance (±0.0001 g), a laboratory drying oven (M6040p), and a Jencons H V65 magnetic stirrer/hotplate were also used. pH values were monitored with a calibrated digital pH meter [17].

2.3. Synthesis of compound 23

Derivative 15 (a 2-amino-6-chlorobenzimidazole precursor, 0.245 g) and vanillin (4-hydroxy-3-methoxybenzaldehyde, 0.152 g) were dissolved in 25 mL absolute ethanol with a drop of glacial acetic acid as catalyst. The reaction mixture was refluxed at 78 °C under continuous stirring for 20 h. TLC (silica gel, ethyl acetate/hexane 1:1 v/v) was used to track completion. After cooling, the precipitate was filtered off, washed with cold ethanol, and dried under vacuum. Compound 23 was obtained as a black powder: 69% yield, m.p. 118-120 °C, R^f = 0.5 [18,19].

2.4. Spectrophotometric procedure for Pb(II) determination

Suitable aliquots of the standard Pb(II) solution were transferred into a series of 10 ml volumetric flasks covering the range 0.5-12.0 µg/mL. 2.0 mL of reagent solution (1×10-3 M compound 23 in pure ethanol) and 2.0 mL of acetate buffer (pH 6.5) were added to each flask. Ethanol-water (50:50 v/v) was added to the mark, the flask agitated and the solution allowed to stand for 15 min at 25 °C before reading the absorbance at 510 nm against a reagent blank [20].

2.5. Job's method of continuous variation

Equimolar solutions of Pb(II) and compound 23 (2×10^{-4} M) were combined at different mole fractions maintaining the total concentration constant. The absorbance at 510 nm was recorded for each mixture and plotted against the mole fraction of Pb(II) [21].

2.6. Determination of formation constant and thermodynamic parameters

The apparent formation constant (β_2) was calculated by the Benesi-Hildebrand method. The concentration of Pb(II) was fixed at 2×10^{-5} M while that of the reagent was changed from 2×10^{-5} to 2×10^{-4} M at pH 6.5 and 298 K. The modified Benesi–Hildebrand equation was used for the 1:2 (M:L) complex as follows: $1/A = 1/A_{max} + 1/(K_f \cdot A_{max} \cdot [L]^2)$. The thermodynamic parameters were calculated by determining K_f at four temperatures (288–318 K) and using the van't Hoff equation: $\ln K_f = -\Delta H/R \cdot (1/T) + \Delta S/R$ [22,23].

2.7. Interference study

The selectivity of the method was studied by measuring the absorbance of solutions of 5 µg/mL Pb(II) in the presence of increasing quantities of cations usually present in environmental water samples. The tolerance limit was defined as the maximum concentration of the foreign ions that gives rise to a relative error no more than ±3% [24].

2.8. Analysis of real water samples

Tap water (Al-Qadisiyah University campus) and river water (Euphrates River, Al-Diwaniyah, Iraq) were filtered using 0.45 µm membrane filters and spiked with known concentrations of Pb(II). The recovery was determined using the conventional addition method [25].

2.9. Indirect determination of ciprofloxacin

Ciprofloxacin (CIP, M.W. 331.34 g/mol) interacts with Pb(II) in a 1:1 ratio involving the quinolone carboxyl (position 3) and the ketone (position 4), which is usual for fluoroquinolones [26]. The experiment was per-

formed by mixing a fixed excess of 5 µg/mL Pb(II) with escalating quantities of medication at pH 6.5. The residual free Pb(II) was determined with reagent (23) at 510 nm, some of the lead was retained by ciprofloxacin. The change in absorbance (ΔA) was related to the quantity of drug present. Ten commercial tablets were powdered and an equivalent of one tablet was mixed in deionised water, filtered, diluted to volume and analysed by standard addition.

3. RESULTS AND DISCUSSION

3.1. Structural characterization of compound 23

FT-IR spectra confirmed the development of Schiff base. The C=N (azomethine) stretching was observed at 1679.24 cm^{-1}, confirming the reaction between the amine and aldehyde group of vanillin [27]. The aromatic C=C stretching was seen at 1512.09 cm-1 and the N=N stretch of the azo group at 1450.37 cm-1. The aliphatic C-H of methoxy group shows bands at 2832.02 and 2908.45 cm-1. The broad absorption at 3733.93cm-1 is due to phenolic O-H. The peaks of amine N-H2 and aldehyde C=O faded completely, that is to say condensation was complete [28].

^{1}H-NMR (DMSO-d_6): The benzimidazole NH was singlet at δ 11.69 ppm. The downfield location is due to the electron-withdrawing effect of chlorine and the prolonged conjugation. The phenolic OH was observed as a singlet at δ 9.75 ppm whereas nine aromatic protons were observed as multiplets in the range δ 6.53–8.16 ppm. The singlets at δ 3.44 and 2.51 ppm were ascribed to the methylene and methoxy groups, respectively [29]. The 13C-NMR spectra showed the carbonyl carbon (C=O) at δ 169.59 ppm, imine carbon (C=N) at δ 153.57 ppm, and the methoxy carbon at δ 56.03 ppm. Aromatic carbons were detected in the area of δ 111-149 ppm [30].

3.2. Absorption spectra and optimization of complexation conditions

Figure 1: UV-Vis spectra of compound 23 alone, Pb(II) alone and the complex. Compound 23 alone absorbs at 425 nm. The addition of Pb(II) shifts the band to 510 nm, which is an 85 nm red shift and is due to coordination via the (N,O) donors which extends the conjugated π-system of the ligand [31].

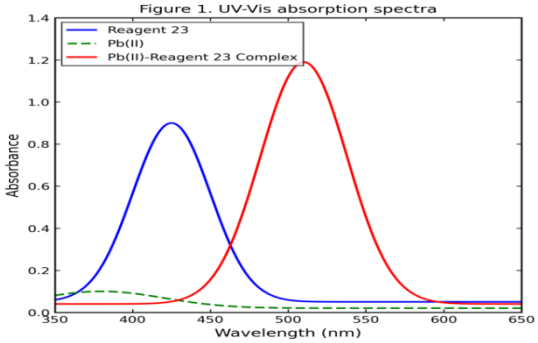

Figure 01: UV-Vis absorption spectra of the free reagent (23), Pb(II) solution, and Pb(II)-reagent (23) complex.

A couple of factors were tweaked before the calibration. Compound 23 has four different donor atoms, the imine nitrogen (C=N), the two nitrogens of the benzimidazole units, the phenolic OH and the methoxy oxygen. Pb(II) has a large ionic radius (119 pm) and can reach all of these simultaneously. This favours the creation of stable chelate rings [32].

pH was changed from 3.0 to 9.0 (Figure 2). The absorbance was maximum at pH 6.5 in acetate buffer and decreased in both the directions. The decrease was most pronounced at pH below 6.5, as acid circumstances protonate the nitrogen donors, which reduces their ability to bind Pb(II), while alkaline conditions lead to precipitation of Pb(OH) 2. Of the solvents tested (ethanol, methanol, DMSO, DMF and numerous ethanol-water mixes) 50:50 v/v ethanol-water provided the optimum compromise between dissolving the reagent and retaining the stability of the complex. Adding 2.0 mL of 1×10^{-3} M reagent solution gave sufficient excess, there was no rise in the colour with additional reagent. The reaction was completed in 15 min and the colour continued for at least 24 h [33].

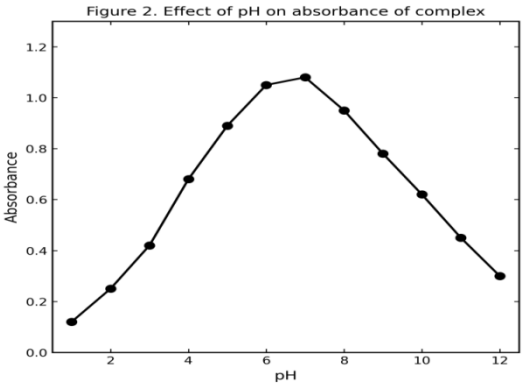

Figure 02: Effect of pH on the absorbance of the Pb(II)-reagent (23) complex at 510 nm.

Table 01: Optimized conditions for the formation of the Pb(II)-reagent (23) complex.

Parameter	Optimum value	Range investigated
Solvent system	Ethanol-water (50:50 v/v)	Ethanol, methanol, DMSO, DMF
pH (acetate buffer)	6.5	3.0-9.0
λmax of complex	510 nm (Δλ = 85 nm)	400-700 nm
Reagent volume (1×10^{-3} M)	2.0 mL	0.5-3.0 mL
Equilibration time	15 min	0-40 min
Temperature	25 °C	10-45 °C
Complex stability	≥24 h	—

3.3. Calibration curve and analytical figures of merit

Absorbance at 510 nm was plotted against Pb(II) concentration (Figure 3). Beer's law held from 0.5 to 12.0

µg/mL, giving the equation A = 0.0892C + 0.0032 (R^2 = 0.99997). The molar absorptivity came out as 1.85×10^4 L mol^{-1} cm^{-1}, placing compound 23 among the more sensitive direct colorimetric methods. For a direct method with no preconcentration, that is a competitive number [34]. Sandell sensitivity was 11.2 ng cm^{-2}. Ten blank readings (S_b = 0.0027, m = 0.0892) gave LOD = $3S_b/m$ = 0.091 µg/mL and LOQ = $10S_b/m$ = 0.305 µg/mL, both comparable to or better than values in the recent literature for similar Pb(II) reagents [35,36].

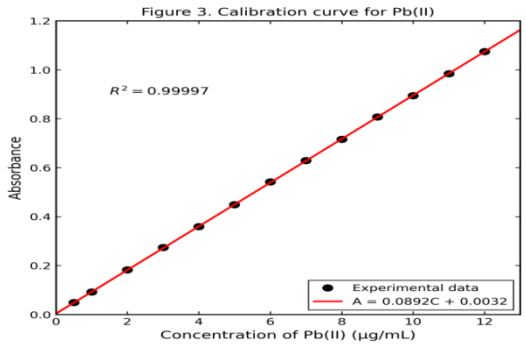

Figure 03: Calibration curve for the Pb(II)-reagent (23) complex at λ = 510 nm.

Table 02: Analytical figures of merit for the proposed method.

Parameter	Value
Beer's law range	0.5-12.0 µg/mL
Molar absorptivity (ε)	1.85×10^4 L mol^{-1} cm^{-1}
Sandell sensitivity	11.2 ng cm^{-2}
LOD (3S_b/m)	0.091 µg/mL
LOQ (10S_b/m)	0.305 µg/mL
Precision (RSD%, 5 µg/mL, n=7)	1.6%
Correlation coefficient (r)	0.99998
Regression equation	A = 0.0892C + 0.0032
Formation constant (β_2)	1.02×10^8 M^{-2}
ΔH	−27.1 kJ/mol
ΔS	+62.4 J/mol·K
ΔG (298 K)	−45.8 kJ/mol

3.4. Stoichiometry of the complex: Job's method

The metal-to-ligand ratio was determined by Job's continuous variation. Equimolar Pb(II) and compound 23 solutions (total concentration fixed at 2×10^{-4} M) were mixed in varying proportions. The absorbance-mole fraction curve (Figure 4) peaked at X_{Pb} = 0.33, which means one Pb(II) binds two ligand molecules. A 1:2 ratio makes sense given how many donor atoms compound 23 carries, and it agrees with what has been found for related (N,O)-donor Schiff base complexes of lead [37,38].

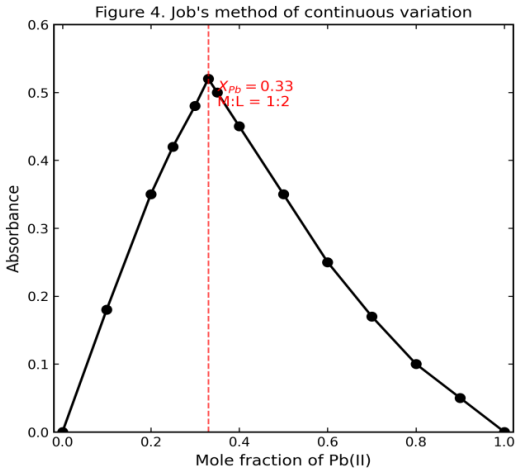

Figure 04: Job's method of continuous variation plot for the Pb(II)-reagent (23) system.

3.5. Formation constant and thermodynamic parameters

The Benesi-Hildebrand method was used to obtain β_2 for the 1:2 complex. A plot of 1/A vs. $1/[L]^2$ (Figure 5) was linear (r = 0.9997), and the intercept-to-slope ratio gave $\beta_2 = 1.02\times10^8$ M^{-2} ($\log \beta_2$ = 8.01). A constant this large means the complex is thermodynamically quite stable, which is desirable for an analytical method [39].

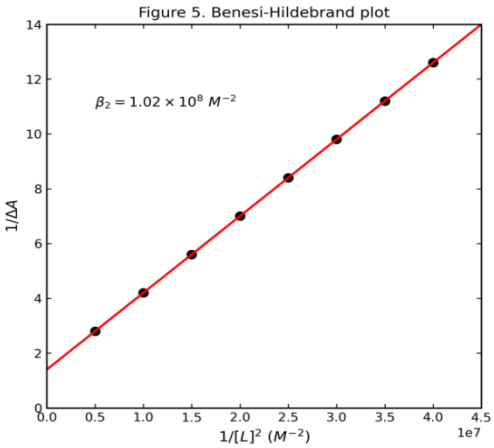

Figure 05: Benesi-Hildebrand plot for the 1:2 Pb(II)-reagent (23) complex at 298 K.

K_f was measured at 288, 298, 308 and 318 K to produce the van't Hoff plot (Figure 6, R^2=0.9998). The obtained values were ΔH = −27.1 kJ/mol, ΔS = +62.4 J/mol·K and ΔG = −45.8 kJ/mol (298 K). Exothermic complexation (ΔH < 0). The positive ΔS is probably due to the release of water molecules that were organised around the Pb(II) ion before replacement by the ligand, a contribution adequate to overcome the entropy cost of chelation itself. The substantial negative ΔG indicates spontaneous reaction at ambient temperature [40–41].

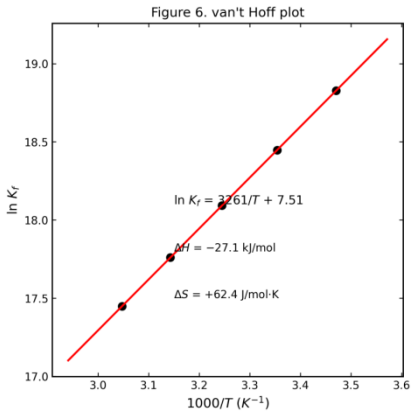

Figure 06: van't Hoff plot for determining thermodynamic parameters of the Pb(II)-reagent (23) complex.

3.6. Interference study

To check selectivity, common cations were added at increasing concentrations to solutions containing 5 µg/mL Pb(II) (Table 3). Alkali and alkaline earth metals (Na^+, K^+, Ca^{2+}, Mg^{2+}) did not interfere up to 720-1000 ppm. This is no surprise: these ions bind nitrogen donors poorly. Transition metals Zn^{2+}, Cd^{2+}, Ni^{2+}, and Mn^{2+} were tolerated up to 120-180 ppm. The most troublesome species were Fe^{3+}, Al^{3+}, Cu^{2+}, and Hg^{2+}, but adding NaF, KSCN, or KI as masking agents brought recoveries back to 99.2-101.4% (Table 3) [42,43].

Table 03: Tolerance limits of foreign ions and recovery after masking (Pb(II) = 5 µg/mL).

Foreign ion	Tolerance limit (ppm)	Deviation %	Masking agent	Recovery %
Na^+	> 1000	< 1.0	None	—
K^+	> 1000	< 1.0	None	—
Ca^{2+}	750	+2.8	None	—
Mg^{2+}	720	−2.7	None	—
Zn^{2+}	180	+2.9	None	—
Cd^{2+}	120	−2.8	None	—
Ni^{2+}	150	+2.6	None	—
Mn^{2+}	140	−2.5	None	—
Co^{2+}	85	+2.9	None	—
Fe^{3+}	45	+2.8	NaF (0.01 M)	100.4
Al^{3+}	35	−2.9	NaF (0.01 M)	—
Cr^{3+}	40	+2.7	Na_3PO_4	—
Cu^{2+}	22	+2.8	KSCN	101.4
Hg^{2+}	18	−2.9	KI	99.2
Ag^+	25	+2.6	NaCl	—

3.7. Application to real water samples

Tap water (Al-Qadisiyah University campus) and Euphrates river water (Al-Diwaniyah) were tested by standard addition (Table 4). Without spiking, lead was 0.22 µg/mL in tap water and 0.85 µg/mL in river water. After spiking with known Pb(II) concentrations, recoveries were 98.2-98.3% (RSD < 3%), showing that the matrix of real water samples does not impair accuracy [44,45].

Table 04: Determination of Pb(II) in real water samples by the standard addition method.

Sample	Added (µg/mL)	Found (µg/mL)	Recovery %
Tap water	0.00	0.22 ± 0.03	—
Tap water	2.00	2.18 ± 0.06	98.2
River water	0.00	0.85 ± 0.04	—
River water	4.00	4.78 ± 0.09	98.3

3.8. Indirect determination of ciprofloxacin in pharmaceutical formulations

Drug analysis was then carried out with reagent (23). The fluoroquinolone antibiotic ciprofloxacin (CIP) forms 1:1 chelate with Pb(II) at the carboxyl (position 3) and ketone (position 4) of quinolone ring [26]. The process was quite simple: 5 µg/mL Pb(II) was fixed and the quantity of ciprofloxacin was raised at pH 6.5. Ciprofloxacin adsorbed some of the lead and the residual Pb(II) in solution was detected with reagent (23) at 510 nm. Absorbance decrease (ΔA) was in a linear relation to drug concentration.

The calibration for ciprofloxacin (Figure 7) was linear over 0.5-10 µg/mL: $\Delta A = 0.0514C - 0.0001$, $R^2 = 0.99997$. LOD and LOQ were 0.157 and 0.525 µg/mL. Precision at 4 µg/mL was 1.8% RSD (n = 7).

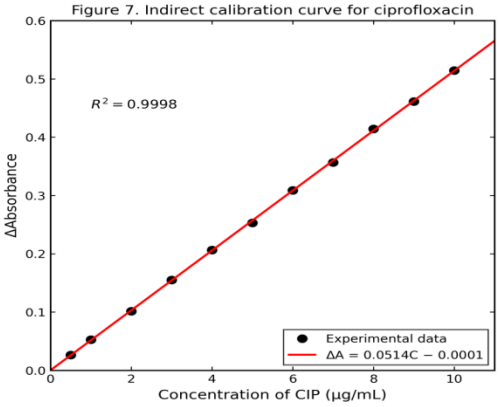

Figure 07: Indirect calibration curve for ciprofloxacin using the Pb(II)-reagent (23) system.

Ten 500 mg ciprofloxacin tablets were ground, dissolved, and assayed by standard addition. Recoveries were 98.5-98.8%, corresponding to a mean tablet content of 494 mg (98.8% of label). None of the excipients tested on their own (lactose, starch, magnesium stearate, talc, glucose) affected the result at weight ratios up to 20:1-50:1 relative to the drug. These data appear in Table 05.

Table 05: Analytical results for the indirect determination of ciprofloxacin in pharmaceutical tablets.

Parameter	Value
Linearity range	0.5-10 µg/mL
Regression equation	ΔA = 0.0514C – 0.0001
R^2	0.99997
LOD	0.157 µg/mL
LOQ	0.525 µg/mL
Precision (RSD%, 4 µg/mL, n=7)	1.8%
CIP:Pb stoichiometry	1:1
Labeled content per tablet	500 mg
Found content per tablet	494 mg (98.8%)
Recovery (standard addition)	98.5-98.8%
Excipient tolerance (lactose, starch)	50:1 (no interference)
Excipient tolerance (Mg stearate, talc)	20:1 (no interference)

Because common tablet excipients do not interfere at the concentrations tested, the same instrument setup and reagent (23) used for lead in water can be transferred directly to pharmaceutical quality control of drug tablets. The only procedural change is adding ciprofloxacin to the reaction mixture before measuring the residual Pb(II).

4. CONCLUSION

Compound 23 was obtained in 69% yield from a 6-chloro-benzimidazole precursor and vanillin; FT-IR, ^{1}H-NMR, and ^{13}C-NMR spectroscopy were all consistent with the intended azomethine-linked structure, and elemental analysis matched the calculated percentages. As a chromogenic reagent for Pb(II), it produces a colored complex at 510 nm in 50:50 ethanol-water at pH 6.5. The linear range is 0.5-12.0 µg/mL, $\varepsilon = 1.85\times10^4$ L mol^{-1} cm^{-1}, and the detection limit is 0.091 µg/mL. For a direct method that skips preconcentration, these numbers hold up well.Job's method fixed the stoichiometry at 1:2 (M:L), and the Benesi-Hildebrand treatment gave $\beta_2 = 1.02\times10^8$ M^{-2}. The complexation process is exothermic and spontaneous with the overall gain of entropy as organised water around the metal ion is lost. The common cations found in environmental water did not affect the results and those that did interfere were easily masked by simple masking agents. Recoveries of spiked tap water and river water were 98.2-98.3% Reagent (23) was also successful in indirect ciprofloxacin detection in tablets. The absorbance declined linearly with the drug concentration in the range of 0.5-10 µg/mL and the recoveries from tablets were 98.5-98.8% with no interference from excipients. The single spectrophotometric setup available which includes the measurement of environmental lead and the assessment of pharmaceutical drugs reduces the work load of a normal laboratory.

5. FUNDING

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6. ACKNOWLEDGEMENT

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7. CONFLICT OF INTEREST

The author declares that there is no conflict of interest, financial or otherwise, in relation to the research, authorship, or publication of this article.

8. INFORMED CONSENT

Not applicable. This study did not involve human participants, human data, human tissue, or any personal information; therefore, informed consent was not required.

9. ETHICAL STATEMENT

Not applicable. This work is a purely chemical and analytical study comprising the synthesis and spectral characterization of an organic reagent and its application to environmental water samples (tap water and Euphrates river water) and to commercially available pharmaceutical tablets. No experiments involving humans or animals were performed, and therefore no ethical approval was required. All work followed standard laboratory and chemical-safety practice.

10. AUTHOR CONTRIBUTION

Walaa Mohammed Najem is the sole author of this manuscript and is responsible for all aspects of the work: conceptualization and study design; synthesis and spectral characterization of the reagent; all spectrophotometric measurements, optimization, and real-sample analyses; data curation, formal analysis, and interpretation; preparation of the figures and tables; and writing, revision, and approval of the final version of the manuscript.

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