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Spectrophotometric Determination of Lead (II) Chelation by $CaNa_2EDTA$ Using PAR (4-(2-Pyridylazo) resorcinol)

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ABSTRACT

The spectrophotometric determination of Lead(II) chelation was carried out using PAR (4-(2-pyridylazo)resorcinol) as a chromogenic reagent. The UV–Visible absorption spectrum of the Pb^{2+}–PAR complex showed a maximum absorbance at 520 nm, which was selected as the analytical wavelength. The effect of incubation time indicated that complex formation was time-dependent, reaching equilibrium at approximately 25–30 minutes. The chelating activity of $CaNa_2EDTA$ was evaluated by monitoring the decrease in absorbance of the Pb^{2+}–PAR complex, with increasing chelator concentration resulting in a corresponding increase in percentage chelation. The observed inverse relationship between absorbance and chelation confirms the effective binding of Pb^{2+} ions by $CaNa_2EDTA$. Overall, the method proved to be simple, sensitive, and reliable for the determination of Pb^{2+} and its chelation and is applicable to the assessment of a wide range of chelating agents, including synthetic and plant-derived compounds.

Keywords: Lead(II), Spectrophotometry, PAR (4-(2-pyridylazo)resorcinol), Chelation, $CaNa_2EDTA$, Metal–ligand complex

INTRODUCTION

Lead is a widespread environmental pollutant with no known biological function in living systems [1], [2]. Its pervasive and non-biodegradable nature facilitates its presence in air, water, soil, and the food chain, leading to bioaccumulation following human exposure through ingestion, inhalation, and occupational contact [3], [4]. This accumulation often results in toxic effects, even at low concentrations [5], [6]. Therefore, the reliable determination and control of Pb^{2+} are essential in environmental and toxicological studies [7], [8].

Although advanced techniques such as atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS) provide high sensitivity, their high cost and operational complexity limit their routine application [9], [10], [11]. Spectrophotometric techniques offer a simpler and more cost-effective alternative. 4-(2-Pyridylazo)resorcinol (PAR) is a well-established chromogenic reagent that forms stable, intensely coloured complexes with metal ions, including Pb^{2+}, enabling quantitative determination using UV–Visible spectrophotometry [12]. The formation of the Pb^{2+}–PAR complex can be influenced by competing ligands, making this system suitable for evaluating metal chelation processes [13], [14].

Chelation therapy remains a primary strategy for the treatment of lead poisoning, with calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) being one of the most used chelating agents [15]. $CaNa_2EDTA$ effectively binds Pb^{2+} ions to form stable, water-soluble complexes that can be excreted from the body [16]. The strong affinity of EDTA for lead ions can be exploited analytically to study metal–ligand interactions through competitive complexation reactions. In this context, spectrophotometric methods based on ligand competition provide a simple approach for evaluating metal chelation. By allowing $CaNa_2EDTA$ to compete with PAR for Pb^{2+} ions, a decrease in the intensity of the Pb^{2+}–PAR complex can be monitored as a function of chelator concentration. Therefore, the present study aims to evaluate the chelation efficiency of $CaNa_2EDTA$ toward Pb^{2+} ions using a PAR-based spectrophotometric assay. The method was further optimized with respect to reaction conditions, including chelator volume, incubation time, and colour development, to establish a reliable and reproducible analytical procedure.

MATERIALS AND METHODS

Chemicals

Lead acetate trihydrate ($Pb(CH_3COO)_2 \cdot 3H_2O$), 4-(2-pyridylazo)resorcinol (PAR), and calcium disodium ethylenediaminetetraacetate ($CaNa_2EDTA$) were procured from Loba Chemie Pvt. Ltd., India. All other chemicals used were of analytical grade.

Preparation of Solutions

Preparation of Pb^{2+} Stock Solution (100 µM): A 100 µM Pb^{2+} stock solution was prepared by dissolving 1.898 mg of lead(II) acetate trihydrate ($Pb(CH_3COO)_2 \cdot 3H_2O$; molecular weight: 379.3 g/mol) in distilled water and making up to 50 mL. Concentrations are expressed as Pb^{2+} equivalents.

Preparation of PAR Solution (1 mM): A 1 mM solution of 4-(2-pyridylazo)resorcinol (PAR) was prepared by dissolving 10.65 mg of the reagent (molecular weight: 213 g/mol) in 50 mL of 10% (v/v) ethanol in distilled water (5 mL ethanol and 45 mL distilled water). The solution was stirred until complete dissolution was achieved.

Preparation of $CaNa_2EDTA$ Solution (100 µM): A 100 µM $CaNa_2EDTA$ solution was prepared by dissolving 1.87 mg of calcium disodium EDTA (molecular weight ≈ 374 g/mol) in distilled water and making up to 50 mL.

Experimental Protocol

Spectrophotometric Determination of Pb^{2+}–PAR Complex

The absorption spectrum of the Pb^{2+}–PAR complex was determined spectrophotometrically based on its complexation with PAR, following a modified method of [17]. A 1.0 mL aliquot of 100 µM Pb^{2+} solution was mixed with 100 µL of PAR reagent and allowed to stand for 10 min for complete color development, forming an orange–red complex. The absorption spectrum was recorded over the wavelength range of 480–540 nm using a UV–Vis spectrophotometer against a reagent blank. The wavelength of maximum absorbance (λ_max) was determined and used for subsequent measurements. All measurements were performed in triplicate.

Effect of Pre-incubation Time with $CaNa_2EDTA$ on Pb^{2+}–PAR Complex Formation

The effect of pre-incubation time on Pb^{2+}–PAR complex formation was evaluated by measuring absorbance at 520 nm. Reaction mixtures were prepared by combining 950 µL of 100 µM Pb^{2+} with 50 µL of 100 µM $CaNa_2EDTA$ to obtain final concentrations of 95 µM Pb^{2+} and 5 µM $CaNa_2EDTA$ in a total volume of 1.00 mL. The mixtures were pre-incubated at room temperature for 5–30 min. Following pre-incubation, 100 µL of PAR reagent was added, and the solutions were allowed to stand for 10 min for color development. Absorbance was then measured at 520 nm using a UV–Vis spectrophotometer (final volume: 1.10 mL). All measurements were performed in triplicate. Experimental conditions were established based on preliminary optimization.

Effect of $CaNa_2EDTA$ Concentration on Pb^{2+} Chelation

The effect of $CaNa_2EDTA$ concentration on Pb^{2+} chelation was investigated spectrophotometrically by monitoring the formation of the Pb^{2+}–PAR complex at 520 nm. Reaction mixtures were prepared by combining 950 µL of 100 µM Pb^{2+} solution with appropriate volumes of $CaNa_2EDTA$ solution to obtain final concentrations in the range of 0.00–5.00 µM in a total volume of 1.00 mL.

The mixtures were incubated at room temperature for 25 min to allow chelation equilibrium. Subsequently, 100 µL of PAR reagent was added, and the solutions were allowed to stand for 10 min for complete color development. Absorbance was measured at 520 nm using a UV–Vis spectrophotometer against a reagent blank (final volume: 1.10 mL). All measurements were performed in triplicate.

The percentage of Pb^{2+} chelation was calculated using the expression: % Chelation = $(A_0 - A) \div A_0 \times 100$, where A_0 represents the absorbance of the control (absence of $CaNa_2EDTA$) and A represents the absorbance in the presence of $CaNa_2EDTA$.

Statistical Analysis

Results are presented as mean ± standard deviation (SD). Data were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

The UV–Visible absorption spectrum of the Pb^{2+}–PAR complex recorded over the wavelength range of 480–540 nm is presented in Figure 1. The absorbance increased progressively from 0.052 ± 0.001 at 480 nm to a maximum of 0.105 ± 0.002 at 520 nm. Beyond this wavelength, the absorbance decreased to 0.076 ± 0.002 at 540 nm. The increase in absorbance between 480 and 520 nm was gradual, followed by a corresponding decline from 520 to 540 nm.

The effect of incubation time on the absorbance of the Pb^{2+}–PAR complex measured at 520 nm is shown in Figure 2. The absorbance increased progressively with time, from 0.013 ± 0.002 at 5 min to 0.017 ± 0.001 at 10 min and 0.028 ± 0.002 at 15 min. This increase continued to 0.033 ± 0.001 at 20 min and 0.045 ± 0.001 at 25 min, reaching

0.046 ± 0.001 at 30 min. The most pronounced rise in absorbance was observed between 10 and 25 min, after which only a slight increase was recorded between 25 and 30 min.

Table 1 shows the effect of $CaNa_2EDTA$ concentration on the absorbance of the Pb^{2+}–PAR complex at 520 nm and the corresponding percentage chelation. The absorbance decreased progressively with increasing $CaNa_2EDTA$ concentration, accompanied by a corresponding increase in percentage chelation. At 0.000 µM, the absorbance was 0.103 ± 0.001 with 0.00% chelation. Increasing the concentration to 0.313 µM reduced the absorbance to 0.092 ± 0.002, corresponding to 10.68% chelation. Further increases to 0.625 µM and 1.250 µM resulted in absorbance values of 0.080 ± 0.001 and 0.068 ± 0.002, with chelation percentages of 22.33% and 33.98%, respectively. At higher concentrations of 2.500 µM and 5.000 µM, the absorbance decreased to 0.055 ± 0.001 and 0.046 ± 0.001, corresponding to 46.60% and 55.34% chelation.

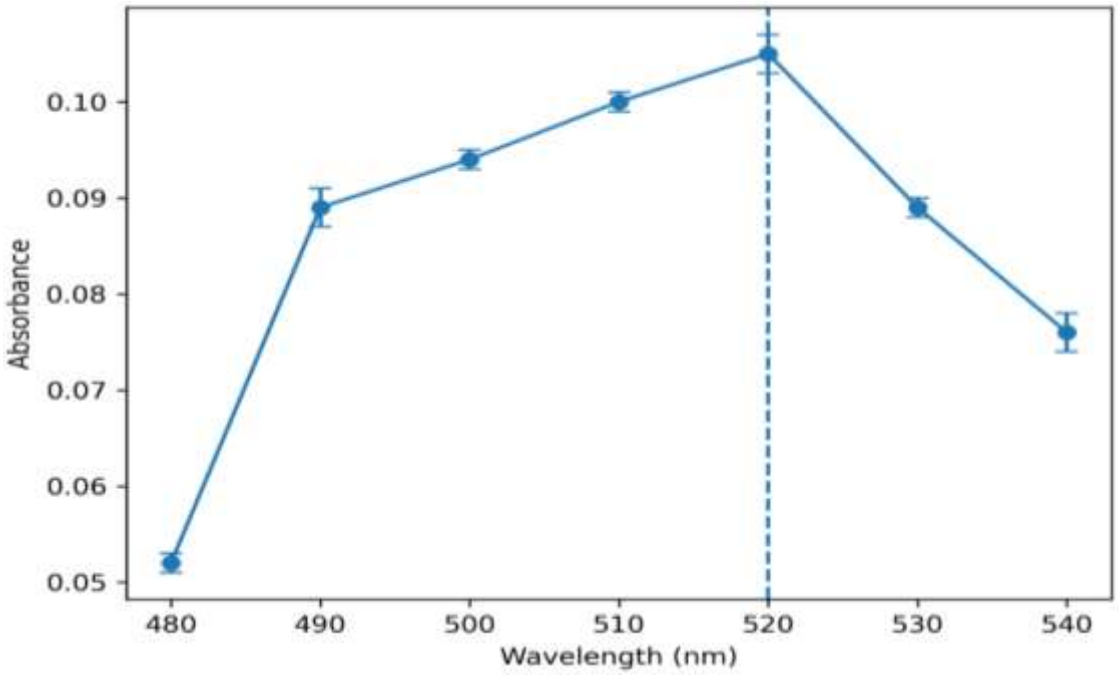

Figure 1: UV–Visible absorption spectrum of the Pb^{2+}–PAR complex recorded over the wavelength range of 480–540 nm. Data are presented as mean ± standard deviation (n = 3). The maximum absorbance (λmax) was observed at 520 nm (indicated by the dashed line) and selected as the optimum wavelength for subsequent spectrophotometric measurements.

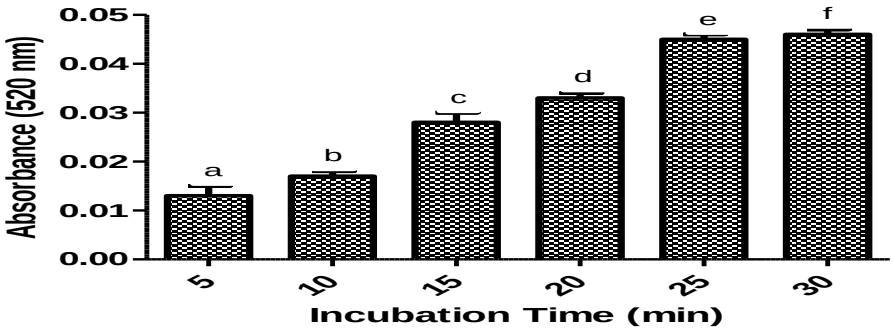

Figure 2: Effect of incubation time on the absorbance of the Pb^{2+}–PAR system measured at 520 nm following pre-incubation with $CaNa_2EDTA$. All assays were performed as described in the Materials and Methods section. Data are presented as mean ± standard deviation (n = 3).

Table 1: Effect of $CaNa_2EDTA$ concentration on the absorbance of the Pb^{2+}–PAR complex at 520 nm and the corresponding percentage chelation

$CaNa_2EDTA$ (µM)	Absorbance (520 nm)	% Chelation
0.000	0.103 ± 0.001^{a}	0.00
0.313	0.092 ± 0.002^{b}	10.68
0.625	0.080 ± 0.001^{c}	22.33
1.250	0.068 ± 0.002^{d}	33.98
2.500	0.055 ± 0.001^{e}	46.60
5.000	0.046 ± 0.001^{f}	55.34

Data are presented as mean ± standard deviation (n = 3). Different superscript letters (a–f) indicate statistically significant differences between values ($p < 0.05$).

DISCUSSION

The spectrophotometric determination of Lead(II) chelation by $CaNa_2EDTA$, employing PAR (4-(2-pyridylazo)resorcinol) as a chromogenic reagent, was examined. The UV–Visible absorption spectrum revealed a progressive increase in absorbance from 480 nm to a maximum at 520 nm, followed by a decline toward 540 nm. This confirms that 520 nm represents the wavelength of maximum absorbance (λmax) for the Pb^{2+}–PAR complex, indicating optimal complex formation and providing the most sensitive point for spectrophotometric measurement [13]. The distinct peak further suggests the formation of a stable metal–ligand complex, justifying the selection of this wavelength for subsequent analyses. This observation is consistent with findings from previous studies [11], which have also reported λmax values around 520 nm for Pb^{2+}–PAR complexes, thereby supporting the reliability and validity of the present results.

The influence of incubation time showed that absorbance increased steadily from 5 to 30 minutes, indicating that the formation of the Pb^{2+}–PAR complex is time-dependent. The most pronounced increase occurred between 10 and 25 minutes, identifying this interval as the main phase of complex formation [14]. This behavior reflects the kinetics of coordination complex formation, where Pb^{2+} ions progressively interact with PAR molecules to form a stable metal–ligand complex [10]. As the reaction proceeds, the availability of free Pb^{2+} ions decrease until equilibrium is approached. Beyond 25 minutes, only a marginal increase in absorbance was observed, indicating that the system had essentially reached equilibrium, with the rates of complex formation and dissociation becoming balanced. This plateau suggests that extending the incubation time beyond 25 minutes offers no significant analytical advantage. Therefore, an incubation time of 25 minutes is considered optimal for achieving complete complexation while ensuring efficiency and minimizing analysis time.

The effect of $CaNa_2EDTA$ concentration demonstrated a clear inverse relationship between absorbance and chelation. As the concentration of $CaNa_2EDTA$ increased, the absorbance of the Pb^{2+}–PAR complex decreased progressively, while the percentage chelation increased correspondingly, indicating a concentration-dependent (dose–response) interaction. This trend reflects the competitive binding dynamics between PAR and $CaNa_2EDTA$ for Pb^{2+} ions, with $CaNa_2EDTA$ exhibiting a higher binding affinity and stability constant for Pb^{2+}, thereby effectively displacing PAR from the complex [18]. The observed dose–response relationship suggests that increasing chelator concentration enhances the probability of Pb^{2+} sequestration until a saturation point is approached, where most available Pb^{2+} ions are bound [19], [20]. The gradual increase in chelation efficiency, reaching over 50% at the highest concentration tested, indicates that complete chelation was not yet achieved within the studied range, implying that higher concentrations may be required to reach maximal binding [21]. This behavior is characteristic of typical ligand–metal interactions and supports the reliability of the method for quantitatively evaluating chelation efficiency and comparing the potency of different chelating agents.

CONCLUSION

The results demonstrate that the PAR-based spectrophotometric method is a simple, sensitive, and reliable approach for the determination of Pb^{2+} and its chelation. The identification of λmax at 520 nm, the establishment of an optimal incubation time of 25–30 minutes, and the observed concentration-dependent decrease in absorbance confirm the robustness of the method. Importantly, the technique is applicable not only to $CaNa_2EDTA$ but also to a wide range of chelating agents, including synthetic and plant-derived compounds, making it suitable for broader studies on lead chelation.

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Conflicts of Interest

The author declares no conflict of interest.

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