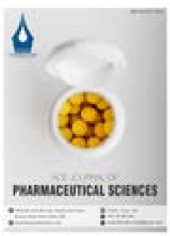
ACE PUBLICATIONS
www.theacepublications.com
ACE JOURNAL OF PHARMACEUTICAL SCIENCES

Research Article



Simple Cloud Point Extraction-spectrophotometric for the Determination for Cadmium (II) Using -3-Anilino-1-Phenylimino-Thiourea

[1]Ali Ihssan Hamza Najm, [2]Lilyan Q. Alwan and [3]Sura Mohammed Jasim
[1]Department of Chemistry, College of Science, University of Babylon, Babylon, Iraq
[2]Department of Applied Geology, College of Science, University of Babylon, Babylon, Iraq
[3]Department of Biology, College of Science, University of Babylon, Babylon, Iraq

Key Words
Cadmium ion, Triton X-114 ,Cloud point extraction, 3-anilino-1-phenylimino-thiourea

Corresponding Author
Ali Ihssan Hamza Najm,
Department of Chemistry, College of Science, University of Babylon, Babylon, Iraq
sci676.ali.ehsan@uobabylon.edu.iq

Received: 15th July 2025
Accepted: 20th August 2025
Published: 30th September 2025

Citation: Ali Ihssan Hamza Najm, Lilyan Q. Alwan and Sura Mohammed Jasim, 2025. Simple Cloud Point Extraction-spectrophotometric for the Determination for Cadmium (II) Using -3-Anilino-1-Phenylimino-Thiourea. J. Res. Stud. Biosci., 5: 1-7, doi: 10.36478/acepharma.2025.1.7

Abstract
Cadmium (II) was pre-concentrated through a cloud-point extraction (CPE) method after forming a complex with 3-anilino-1-phenylimino-thiourea (R.). Triton X-114, a non-ionic surfactant, was employed to facilitate the extraction process, and the resulting mixture was analyzed by ultraviolet-visible (UV-Vis) spectrometry. Several experimental parameters affecting the efficiency of the CPE process were optimized, including pH, the concentration of reagent (R), the volume of Triton X-114 (0.4 mL), equilibration temperature and time (55°C), and centrifugation conditions (4,000 rpm for 20 minutes). The optimal pH was determined to be 4.7, and the extraction was performed in a 5 mL solution containing 2.5% (v/v) of Triton X-114 and 1×10^{-3} mol·L^{-1} of (R). Under these optimized conditions, the method achieved a limit of detection (LOD) for cadmium (II) of 0.0091 µg·mL^{-1} and a limit of quantification (LOQ) of 0.02 µg·mL^{-1}. The linearity of the calibration curve was maintained over the concentration range of 0.012 to 3.0 µg·mL^{-1}. The method also demonstrated good precision, with a relative standard deviation (RSD) of 6.3% for seven replicates (n = 7) at a cadmium (II) concentration of 6.3 µg·mL^{-1}.

INTRODUCTION

A key area of interest in analytical chemistry is the separation and preconcentration of metal ions, with cloud point extraction (CPE) emerging as a widely used technique[1,2]. In this context, cadmium (II) was pre-concentrated by forming a complex with 3-anilino-1-phenylimino-thiourea, followed by CPE. The resulting extract was then analyzed using ultraviolet-visible (UV-Vis) spectrophotometry. The cadmium (II) complex is selectively extracted into the surfactant-rich phase of Triton X-114[3].

Conventional liquid-liquid extraction methods typically involve large volumes of high-purity organic solvents, making them time-consuming, labor-intensive, and environmentally hazardous due to the challenges associated with solvent disposal. In contrast, cloud point extraction offers a greener alternative by minimizing solvent usage, reducing operator exposure, shortening extraction time, and lowering disposal costs[4-11].

CPE has proven effective for extracting and pre-concentrating metal ions by forming poorly water-soluble complexes[12,13]. For instance, 3-anilino-1-phenylimino-thiourea has been effectively employed as a complexing agent for the selective separation and preconcentration of silver under acidic conditions[4]. This ligand is known for forming stable complexes with a variety of metal ions under suitable conditions, making it valuable in both liquid–liquid extraction[14] and solid-phase extraction methods[15].

In the present study, a new cloud point extraction method was developed for the preconcentration and determination of cobalt, utilizing 3-anilino-1-phenylimino-thiourea as the chelating agent prior to UV-Vis spectrophotometric analysis.

MATERIALS AND METHODS

Experimental: An appropriate amount of (R) was first dissolved in 5 mL of 0.1 M NaOH to prepare the reagent solution, which was subsequently adjusted to a concentration of 1×10^{-3} mol·L^{-1} in a 50 mL volumetric flask. Similarly, a measured quantity of lead nitrate was dissolved in distilled water to obtain a standard lead solution with a concentration of 6 µg·mL^{-1}. In addition, 2.5 mL of Triton X-114 was diluted with distilled water in a 50 mL volumetric flask to prepare a 5% (v/v) solution

Instrumentation: The absorption of the complex formed was measured in order to ascertain the cadmium (II) content. A pH meter, Inolab, WTW, 720 (Germany), was used to measure the solutions' pH. A Shimadzu UV-vis1650 spectrophotometer from Tokyo, Japan, was employed. The water bath model (OPTIMA, Japan) was utilised to achieve appropriate temperatures for aqueous samples. The aqueous phase and SRP were separated using a medifuge centrifuge model.

General CPE Procedure: Add 1 mL (1 x 10-3) mol and 3 µg.mL-1 of Cd (II) standard solution to a 5.0 mL volumetric flask.X-114 as a micellar and L-1 of (R) as a complex agent with 0.4 mL (5% (v/v)) of Triton To finish the phase separation process, the solution is moved to a test tube and heated in a water bath at 55°C for 20 minutes. After that, it is centrifuged for 15 minutes at 4000 rpm. To treat the viscosity and finish the necessary volume in the cuvette for analysis, 3.0 mL of ethanol absolute is added to the rich phase of the surfactant after the aqueous phase has been easily poured[16].

The analytical signal is recorded at λ max (525) nm to measure the absorbance of the sample and ascertain its cadmium (II) content.

RESULTS AND DISCUSSIONS

Absorption spectra: The absorption spectra of the [Cd(II) – (R.)] complex by CPE, cadmium(II), and reagent (R) were obtained using a Shimadzu uv-vis1650 spectrophotometer. The results are displayed in Figure 1; the complex exhibits the maximum absorption (λ max) at (525 nm) and can be regarded as proof of its formation.

In the CPE procedure, pH is crucial and must be adjusted before proceeding to the next step in the extraction process. It also plays a significant role in the embedding process for analyses that generate [metal-ligand] complexes, so the extraction's outcome depends on figuring out the pH at which the complex forms[17]. Therefore, a series of experiments were conducted to assess the impact of pH on the extraction process by creating similar solutions with 3 µg.mL-1 of cadmium (II) standard solution and 1 mL 1x10-3 mol.L-1 of (R) in addition to 0.4 mL (5% (v/v)) of TritonX-114 within pH ranges (3.3-6.1) that were adjusted with various buffer solutions. The experimental results of this test are displayed in Figures (2 a and b) following the recording of absorbance for each sample at 525 nm.

The extraction procedure was conducted using the following parameters: 3 µg/mL of Cd(II), 1 mL of a 1×10^{-3} mol/L solution of reagent R, and 0.4 mL of 5% (v/v) Triton X-114. The mixture was maintained at 55°C for 20 minutes to achieve maximum extraction efficiency.

However, a decrease in absorption values as the solution moves towards alkalinity could be the result of the complex's instability at basic media, which causes the complex to dissociate. As a result, the ideal pH for complex formation that does not require buffer solution was determined to be 4.7

Effect of (R) Concentration: As the transport medium for metal ions to the rich phase of the surfactant, reagent

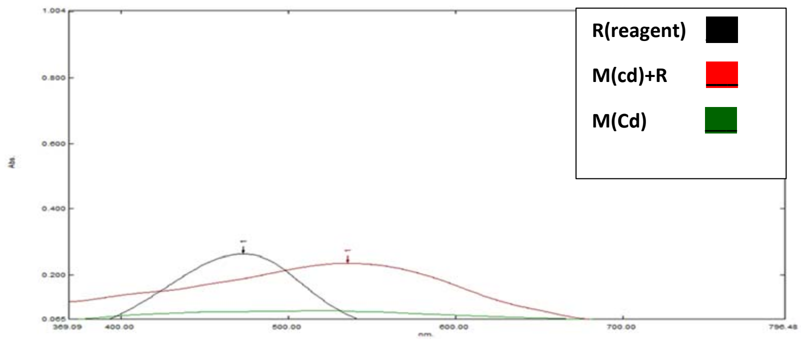


Fig. 1: Absorption spectrums of: cadmium (II) ions, complexing agent (R), and [Cd (II)-R] complex

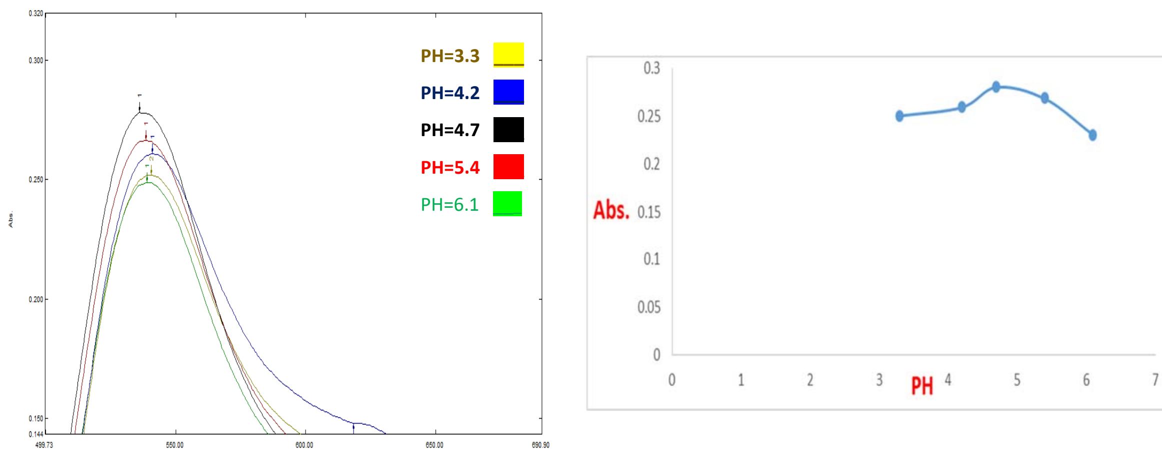


Fig. 2: (a) UV-Vis absorption spectra of the [Cd(II)-R] complex at varying pH levels. (b) Effect of solution pH on the extraction efficiency of Cd(II)

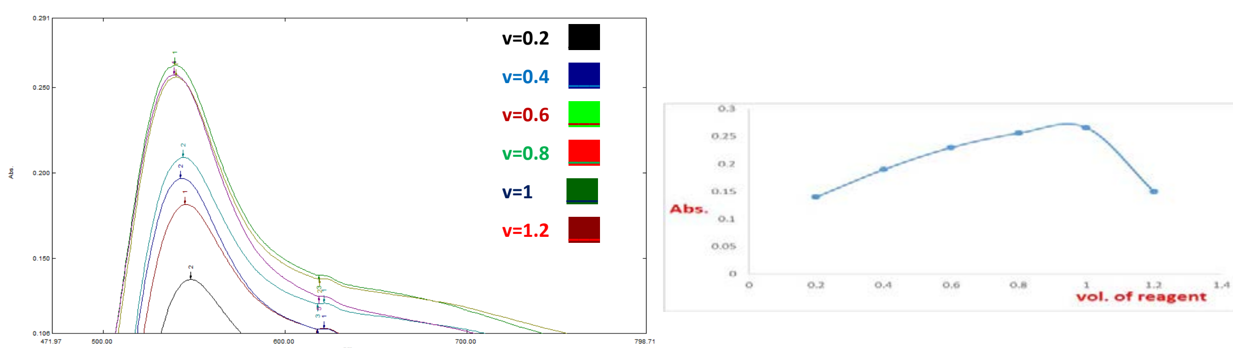


Fig. 3: (a) Absorption spectra of [Cd (II) -R] complex under effect of concentration of reagent (R), (b) Effect of Reagent Concentration on the Extraction Efficiency of Cd(II): The experiments were conducted at pH 4.7, using 3 µg·mL-1 of Cd(II) and 0.4 mL of 5% (v/v) Triton X–114. The mixture was equilibrated at 55 °C for 20 minutes to evaluate the influence of reagent concentration on the extra

concentration is one of the variables that influences the CPE process[18]. As a result, in order to extract and transfer the greatest quantity of lead ions to SRP, the right amount of reagent must be known. In this work, similar solutions with varying reagent volumes (0.2-1.2) mL 1x 10-3 mol were prepared in order to test the impact of reagent concentration on the extraction process in CPE.L-1 with 0.4 mL of 5% (v/v) TritonX-114 at

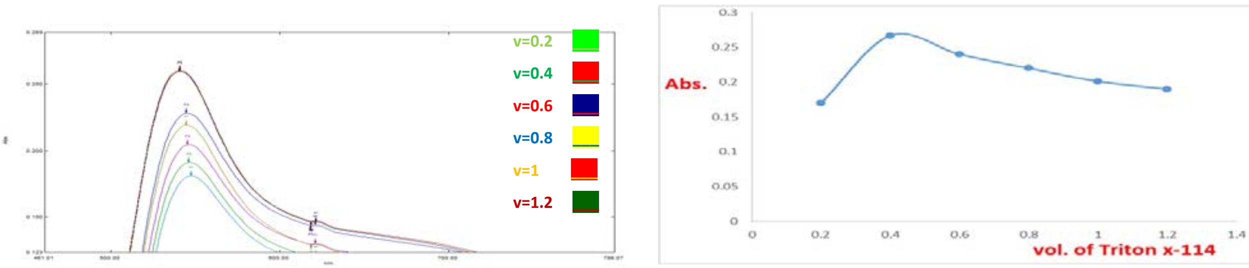


Fig. 4: (a) Absorption Spectra of the [Cd(II)–R] Complex: The absorption behavior of the [Cd(II)–R] complex was investigated with varying concentrations (b) Influence of Triton X-114 Concentration on the Extraction Efficiency of Cd(II):Experiments were performed at pH 4.7 with a Cd(II) concentration of 3 µg·mL-1 and 1 mL of 1 × 10-3 mol·L-1 of reagent (R). The mixture was equilibrated at 55 °C for 20 minutes to assess the impact of Triton X-114 concentration on the extraction efficiency.

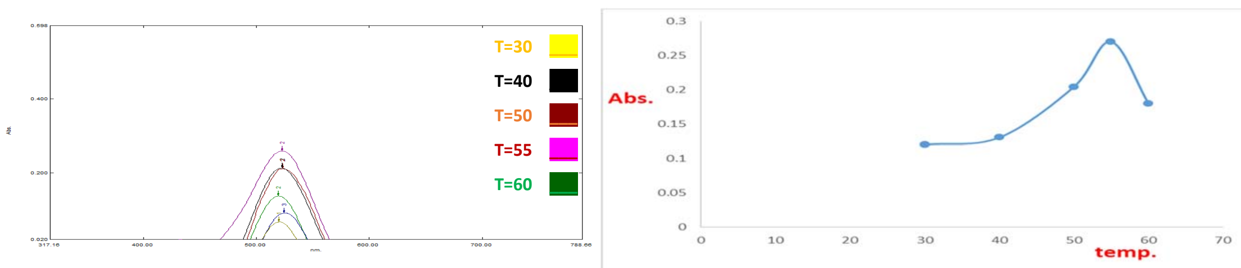


Fig. 5: (a) Absorption Spectra of the [Cd(II)-R] Complex: The spectra were obtained to investigate how the equilibration temperature affects the [Cd(II)-R] complex. (b) Influence of Equilibration Temperature on Cd(II) Extraction Efficiency: The study was performed at pH 4.7, employing 3 µg/mL of Cd(II), 1 mL of a 1×10^{-3} mol/L solution of R, and 0.4 mL of Triton X-114 at a concentration of 5% (v/v). The temperature was varied while keeping the heating duration fixed at 20 minutes to assess its impact on extraction performance.

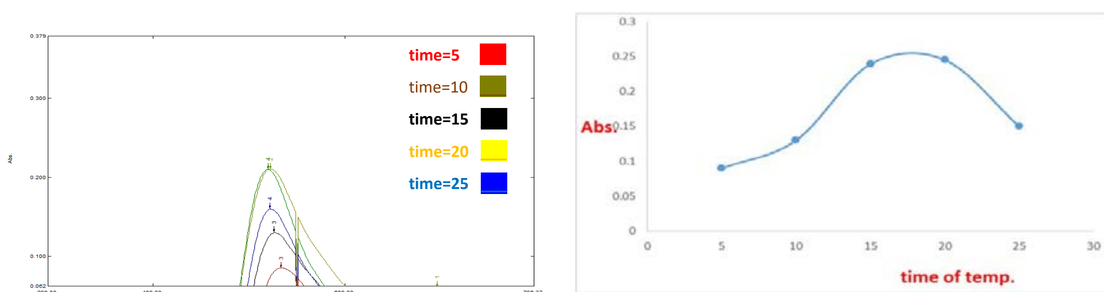


Fig. 6: (a) Absorption spectra of the [Cd(II)–R] complex as a function of incubation time.(b) Impact of Equilibration Time on Cd(II) Extraction Efficiency:

pH 4.7 and 3 µg.mL-1 of cadmium (II) standard solution. Following the CPE procedure, the complex's absorption spectra were acquired at various levels, as shown in Figure (3) (a), which illustrates how this effect behaved. Since surfactant is an extraction medium that allows the pre-concentration agent to be optimised to reach maximum extraction efficiency by lowering the volume ratio of the surfactant-rich phase to the aqueous phase, surfactant concentration is one of the factors influencing the action of CPE[19]. Therefore, using different volumes within "between 0.2 and 1.2 mL of 5% (v/v) solution" Triton X-114, the effect of surfactant concentration on [Cd(II)-R] extraction was investigated. The outcomes of using the general CPE procedure are shown in Figures (4 a and b).

According to Figure (3 a and b), one millilitre of the reagent can be used because it uses less reagent and permits maximum extraction; high concentrations of (R) are not necessary and have resulted in a significant decrease in absorbance values.

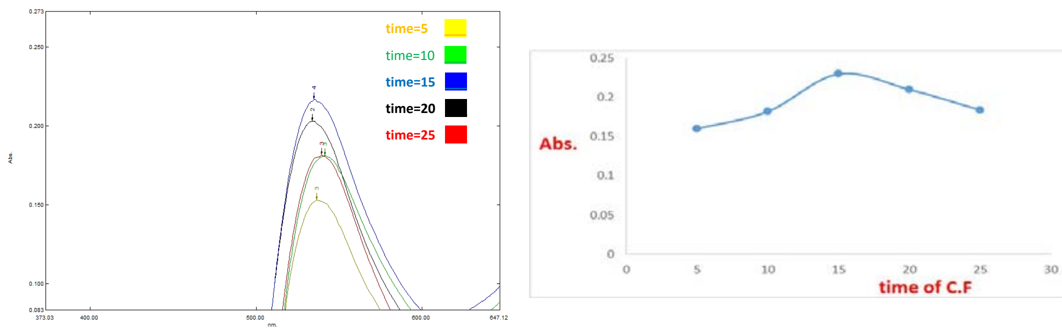


Fig. 7: (a) Absorption Spectra of the [Cd(II)-R] Complex and the Influence of Centrifugation Time; (b) Effect of Centrifugation Time on Cd(II) Extraction Efficiency:

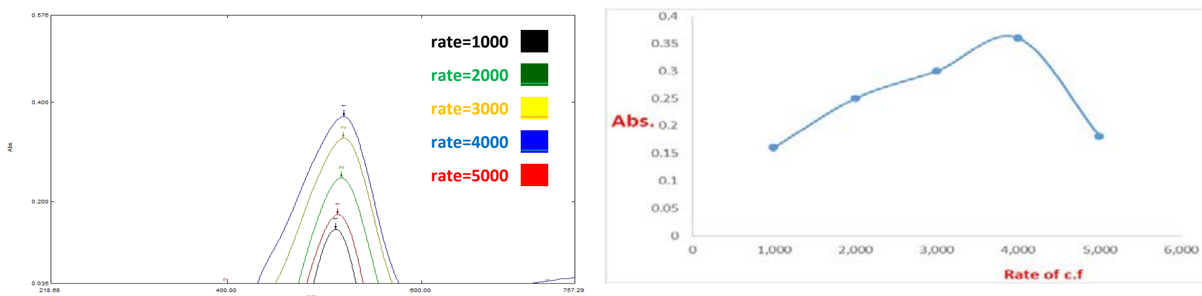


Fig. 8: (a) Absorption Spectra of the [Co(II)-R] Complex Under the Influence of Centrifugation Rate; (b) Effect of Centrifugation Rate on the Extraction Efficiency of Cd(II):

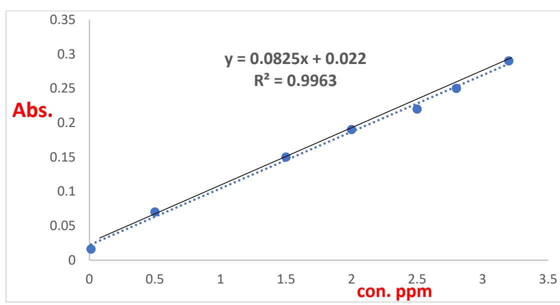


Fig. 9: Calibration curve for [Cd(II)-R] complex using CPE method

Effect of Triton -114 volume: Surfactant concentration is one of the factors influencing the action of CPE because it is an extraction medium that enables the pre-concentration agent to be optimised to reach maximum extraction efficiency by lowering the volume ratio of the surfactant-rich phase to the aqueous phase[19]. Thus, the impact of surfactant concentration on [Cd(II)-R] extraction was examined using various volumes within the range of (0.2-1.2) mL of 5% (v/v) Triton X-114. Figures (4 a and b) display the results of applying the general CPE procedure.

Figure (4 a and b) demonstrate that the extraction rate of cadmium (II) ions is raised to the highest level feasible at 0.4 mL of Triton X-114. It seems that the concentration of Triton X-114 at this point was able to capture the maximum amount of complex in SRP. Conversely, high surfactant concentrations are linked to a progressive drop in absorption values because they raise the viscosity of the rich surface phase, which lowers sensitivity[20]. Thus, the optimal concentration of Tritonx-114 was determined to be 0.4 mL, 5% (v/v), and it was utilised in all ensuing experiments.

"Study on the Effect of Temperature and Incubation Time during Equilibration": "For the purpose of achieve the highest extraction efficiency, we conducted a temperature effect test using test samples at varying temperatures (30-60°C). The best results were obtained at 55°C, as shown in Figures (5 a and b).

According to the test results shown in Figure (5 a and b), the extracted complex's absorption values

Table 1: Analytical properties of CPE in determining Cd(II)

Conditions	Value
pH	4.7
Concentration of (R).	1 mL (1×10-3) (mol.L-1)
Concentration of surfactant	0.4 mL (5% (v/v)) of TritonX-114
Equilibrium temperature(°C)	55
Equilibration time (min.)	20
Centrifugation rate (rpm)	4000
Centrifugation time (min.)	20

progressively increased as the temperature rose to 55°C. This can be explained by the fact that a decrease in the water content causes "a reduction in the volume of the surfactant-rich phase"increasing sensitivity.

Effect of Incubation Time: Repeated tests were conducted with samples in the water bath at varying times (5-25 minutes) while maintaining other parameters at the ideal levels found in earlier studies in order to assess the impact of incubation time., The incubation time test results are displayed in Figures (6 a and b)

The role of equilibration duration in the extraction process of Cd(II) was assessed under specific conditions: pH 4.7, a Cd(II) concentration of 3 μg/mL, 1 mL of R at 1 × 10^{-3} mol/L, and 0.4 mL of Triton X–114 at 5% (v/v). All experiments were conducted at a constant temperature of 55 °C.

The analytical signal was shown to increase until it reaches the 20-minute mark, at which point it tends to stabilize as the duration increases. One could argue that 20 minutes is sufficient to accomplish a proper separation of the solution because it is better for the extraction process to go more swiftly and effectively. As a result, this point was chosen as the optimal incubation time and applied to all subsequent testing.

Effect of Centrifugation Time and Rate: In order to evaluate the impact of time on the centrifugation process, Rewrite the text while taking advantage of the similarity with the original text: the effect of centrifugation time on the extraction efficiency of cadmium (II) ions in CPE was investigated using varying periods (5-25 minutes). The time 20 min was selected as a short duration for increased extraction based on the results displayed in Figures (7 a and b).

The influence of centrifugation time on the extraction efficiency of Cd(II) was examined under the following conditions: pH 4.7, Cd(II) concentration of 3 μg·mL^{-1}, 1 mL of 1 × 10^{-3} mol·L^{-1} reagent (R), and 0.4 mL of 5% (v/v) Triton X-114. All experiments were performed at an equilibration temperature of 55 °C with a heating time of 20 minutes.

A centrifugation rate test was conducted between 1,000 and 5,000 rpm while maintaining a constant period of 15 minutes.The data collected in Figure (8 a and b) shows that the organic phase pools at the test tube's bottom and adheres to it, indicating that full phase separation is possible at 4000 rpm. 4000 rpm was selected as the optimal rate because it makes it simple to pour the aqueous phase without losing any of the analyte.

"The effect of centrifugation rate on the extraction efficiency of Cd(II) was investigated under the following conditions: pH 4.7, Cd(II) concentration of 3 μg·mL^{-1}, 1 mL of 1 × 10^{-3} mol·L^{-1} reagent (R), and 0.4 mL of 5% (v/v) Triton X-114. The experiments were carried out at an equilibration temperature of 55 °C with a heating time of 20 minutes."

The calibration curve and analytical performance of the CPE method: After applying the CPE procedure under ideal conditions derived from prior experiments and explained in Table (1), where absorbance was measured at λ max (525) nm, a linear calibration curve was established between the absorbance values versus the cadmium (II) concentration as shown in Figure (9). This was done by creating solutions containing standard concentrations of cadmium(II) ions within the ranges (0.012-3.0) μg.mL-1.

Table (1) provides an overview of the data for the calibration curve and additional analytical parameters for this work.

CONCLUSION

The cloud point extraction (CPE) method was employed for the determination of Cd(II) in aqueous solutions, using (R) as the ligand and Triton X-114 as the micellar medium. Optimization of the experimental conditions enhanced the method’s sensitivity and lowered the detection limit "Owing to its limited use of organic solvents"., this technique offers an environmentally friendly alternative to conventional extraction methods, while also being efficient, rapid, and cost-effective.

REFERENCES

1. Quina , F.H ; Hinze , w.L. Ind . Eng . chem . Res ., 1999 , 38 , 4150.
2. Bezerra ,M. A ; Bruns , R . E ; Ferreira , S.L.C., Anal. chim . Acta, 2006, 580, 251.
3. Tavallali H., Asrari E., Attaran A.M., Tebandeh M.,(2010), International Journal of Chem Tech Reserch , 2010, ISSN:0974-4290, 2: 1731-1737.

4. J.L. Manzoori, G. Karim-Nezhad, Anal. Chim. Acta 484 2003, 155.
5. J.L. Manzoori, G. Karim-Nezhad, Anal. Sci. 19, 2003 579.
6. J.L. Manzoori, A. Bavili-Tabrizi, Microchem. J. 72 2002 1.
7. E.K. Paleologos, C.D. Stalikas, M.I. Karayannis, Analyst 126 2001, 389.
8. M.F. Silva, L. Fernandez, R.A. Olsina, D. Stacchiola, Anal. Chim. Acta 342 1997, 229.
9. E.K. Paleologos, D.L. Giokas, S.M. Tzouwara-Karayanni, M.I. Karayannis, Anal. Chim. Acta 458 2002, 241.
10. M.F. Silva, L.P. Fernandez, R.A. Olsina, Analyst 123 1998, 1803.
11. R. Carabias-Martinez, E. Rodriguez-Gonzalo, J. Dominguez-Alvarez, J. Hernandez-Mendez, Anal. Chem. 71 1999, 2468.
12. M.C. Cerrato Oliveros, O. Jimenez de Blas, J.L. Pe´rez Pavo´n, B. Moreno Cordero, J. Anal. At. Spectrom. 13 1998, 547.
13. C. Garci´a Pinto, J.L. Pe´rez Pavo´n, B. Moreno Cordero, E. Romero Beato, S. Garci´a Sa´nchez, J. Anal. At. Spectrom. 11 1996, 37.
14. E. Carasek, J.W. Tonjes, M. Scharf, Talanta 56 (2002) 185.
15. J.L. Manzoori, M.H. Sorouradin, A.M. Hajishabani, J. Anal. At. Spectrom. 13 (1998) 305.
16. AKL Z. F., Hegazy M. Selective cloud point extraction of thorium (IV) using tetraazonium based ionic liquid, Journal of Environmental Chemical Engineering 2020: 8: 104185.
17. Hutapea S., Saefumillah A., Asijati E. Development of Cr (III) analytical method in dairy products by cloud point extraction using graphite furnace atomic absorption spectroscopy. AIP Conference Proceedings: AIP Publishing LLC; 2020, p. 040013.
18. Bamdad F., Raziani A. Application of surface-active ionic liquids in micelle-mediated extraction methods: pre-concentration of cadmium ions by surface-active ionic liquid-assisted cloud point extraction, Journal of the Iranian Chemical Society 2020, 17: 327- 332.
19. Khammas Z. A., Ahmad S. S. Micelle-mediating extraction combined with visible spectrophotometry for the determination of ultra trace amounts of bendiocarb insecticide in various matrices after oxidative coupling with O-Toluidine, International Research Journal of Pure and Applied Chemistry 2016, 1-16.
20. Zengin H.B., Gürkan R. Use of 2-Hydrazinobenzothiazole Modified Copolymer (s) as Potential Chelating Agent for Sensitive and Selective Determination of Low Levels of Mercury in Seafood by Ultrasound-Assisted Cloud-Point Extraction Combined with Spectrophotometry, Biological trace element research 2019, 191: 254-268.