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A Prepared Azo Reagent for Cloud Point Extraction, Spectrophotometric Determination, and Removal of Ni(II) from Environmental Samples

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ABSTRACT

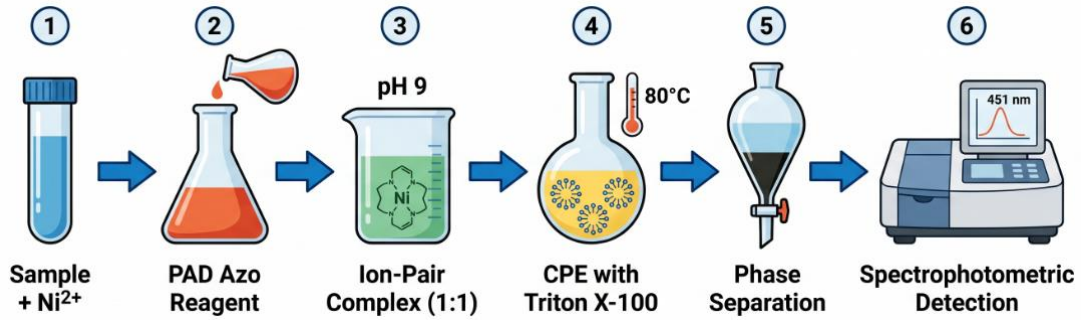
The nickel (II) ion was determined, separated, and removed using the azo reagent 2-2-[phenylazo]doxycycline (PAD), which had previously been created by a coupling reaction. The metal was extracted as an ion-pair association complex by the cloud point extraction (CPE) technique into the surfactant-rich (organic) phase, and was subsequently estimated after the optimum conditions and the factors governing the extraction efficiency had been established. The ion-pair complex was monitored spectrophotometrically at λ_{max} = 451 nm. Optimization of the complexation and extraction conditions gave the following optimum values: pH = 9, a Triton X-100 volume of 0.7 mL, a heating time of 10 min and an equilibration temperature of 80 °C. The thermodynamic parameters of the CPE process in the Triton X-100 phase were also evaluated and found to be ΔH = 0.15126 kJ, $\Delta G = -57.6472$ kJ and $\Delta S = 163.7350$ J mol^{-1} K^{-1}, indicating a spontaneous, endothermic and entropy-driven process. The extracted compound was prepared in stoichiometric ratio of 1:1, (metal : ligand). The method was effectively utilised for the analysis and removal of nickel in fish, vegetables, plant leaves and water with a 10 mL sample volume under the optimised condition and good results were obtained.

KEYWORDS: *cloud point extraction; nickel(II); azo reagent; spectrophotometric determination; thermodynamics; water purification.*

HIGHLIGHTS

- Ni(II) can be determined and removed by cloud point extraction using the azo reagent PAD.
- Ni(II) is extracted as a 1:1 ion-pair complex measured at λmax = 451 nm.
- Optimum CPE conditions: pH 9, 0.7 mL Triton X-100, 10 min at 80 °C.
- Thermodynamics drives extraction, which is endothermic and spontaneous.
- results that are in line with FAAS when applied to fish, vegetables, and water

Graphical Abstract



1- INTRODUCTION

One of the most significant and useful uses of surfactants in analytical chemistry is cloud point extraction (CPE), a separation, enrichment, and preconcentration technique[1]. Many non-ionic surfactants' aqueous solutions turn murky above a specific temperature called the cloud point. [2]. Above this temperature the solution separates into two phases: a bulk aqueous phase with surfactant monomers at a concentration near the critical micelle concentration and a surfactant-rich phase with a very small volume.

Micellar systems have a number of advantages over traditional separation techniques, such as low cost, operational safety, and a high capacity to concentrate a wide range of analytes with high recoveries and large preconcentration factors [3].

When consumed in excess of the recommended levels, nickel can be hazardous to human health; allergic reactions are the most frequent side effect. Food is the principal route of exposure to this element. Foods may naturally contain nickel, as a result of pollution, or as a result of food processing and storage, such as when the metal migrates from cans into the food they contain. [4][5Therefore, it is necessary to determine it using sensitive and eco-friendly methods. For this purpose, CPE provides a green-chemistry method using reagents made from drug compounds like PAD. [6]. Procedures for enrichment, separation and preconcentration have been documented for the determination of nickel in different matrices by flame atomic absorption spectrometry (FAAS) [7], along with related studies on manganese preconcentration [8].

In the hydrometallurgical processing of laterite nickel ores, nickel and cobalt have also been separated using solvent extraction. These systems can combine mixed-sulphide or mixed-hydroxide precipitation with a single extractant (direct solvent extraction) or a combination of two or more extractants (synergistic solvent extraction) [9]. Several azo reagents have been developed recently for this sort of separation. The reagent 2-[2-(cefpodoxime proxetil)azo]-2-paracetamol (CefAP) was characterised using UV-Vis, FTIR and 1H NMR spectroscopy and C.H.N analysis and was used to identify Ni^{+2} and Co^{+2} by CPE as ion-pair complexes with spectrum maxima at λ_{max} = 412 nm for Ni^{+2} and 340 nm for Co^{+2} [10].

Similarly, the thiazole azo reagent 2-[(cefpodoxime proxetil)thiazolylazo]-1-dopamine (FTAD) was applied to the extraction of Ni^{+2} and Co^{+2} from aqueous solution by CPE into the organic phase; the reagent was identified by UV-Vis, ^{1}H NMR and FTIR spectroscopy, and the optimal conditions affecting the extraction efficiency were investigated [11]. A further reagent, 2-[(3-methoxyphenyl)azo]doxycycline, prepared from a drug compound and characterized by UV-Vis, FTIR and ^{1}H NMR spectroscopy together with C.H.N analysis, was used to determine the Mn(II) ion as an extractable ion-pair complex [12]. The reagent 2-[(cefpodoxime)azo]-2-doxycycline, synthesized by a coupling reaction and similarly characterized, was likewise used to determine the nickel(II) ion as an ion-pair complex extracted by CPE [13]. Building on these studies, the present work aims to determine and remove nickel(II) and, as an environmental application, to purify water contaminated with this ion by means of CPE using a reagent prepared from a drug compound, following a systematic optimization of the experimental conditions.

2- EXPERIMENTAL

(a) Using a quartz cell with a 10 mm path length and a Shimadzu double-beam UV-Vis spectrophotometer (model UV-1700, Japan), the absorption spectra of all the reagents and complexes examined in this work were obtained in the 190–1100 nm range.

(b) A single-beam UV–Vis spectrophotometer (TRIUP International Corp., model TRUV-74.S, Italy) was used to measure absorbance for the optimization study, and a Shimadzu FTIR-8400S device (Japan) was used to collect infrared spectra.

2-1: Reagents and Materials

All reagents were of analytical grade and employed as received, without any further purification. Throughout the work, doubly distilled water served for the dilution of standards, reagents and samples alike. The non-ionic surfactant Triton X-100, having the structure C8H17C6H4(OC2H4)n (n = 9–10, average molecular weight 625 g mol-1), was purchased from Sigma (Sigma-Ultra, > 99.6%, UK) and used as supplied. NaOH (99%), HCl (37%) and NaNO2 (99%) were all obtained from CDH (UK). A nickel(II) stock standard solution (1 mg mL-1) was prepared by dissolving 0.1598 g of NiCl2 (Merck, 99.97%) in 25 mL of distilled water in a volumetric flask. Finally, a 5×10^{-3} M solution of 2-2-[phenylazo]doxycycline (PAD) [6] was obtained by dissolving 0.1478 g of the reagent in 0.5 mL of Triton X-100, then diluting to volume with distilled water.

2-2: Recommended Procedure

An aliquot of 10 mL of the standard or sample solution containing the analyte (1×10^{-4} M, corresponding to 20 µg of Ni per 10 mL) was adjusted to pH = 9 and 0.7mL of 0.1% (w/v) Triton X-100 was added. The mixture was then heated for (10 min.) in a thermostated bath at 80°C. Almost immediately the two phases separated. The surfactant-rich phase became highly viscous and sank to the bottom of the tube. Thus, there was no need for centrifuging or cooling and the aqueous phase could be removed by simple decantation. The surfactant-rich phase was then dissolved in 5mL of ethanol and its absorbance determined at λmax = 451nm in 1cm cell against the reagent blank made similarly. The residual Ni^{+2} in aqueous phase was evaluated spectrophotometrically using dimethylglyoxime (DMG) technique [14] and the distribution ratio, D, was computed.

2-3: Preparation of Samples

About 5 g of a dried sample (fish or plant) was placed in a 250 mL conical flask, and 10 mL of concentrated HNO3 was added to it. The flask was heated on an electric hotplate until the volume had been reduced to roughly 2-3 mL. After cooling, a further 10 mL of concentrated HNO_3, along with 5 mL of concentrated H2SO4 and 4 mL of H_2O_2, were added, and the contents were once more boiled down to a volume of 2-3 mL. At this stage 10 mL of water was added, and heating was maintained until the solution turned colourless, a sign that the organic matter had been fully oxidised. The cooled digest was transferred to a 100 mL volumetric flask, filtered, and brought up to the mark with distilled water. From this solution a 5 mL aliquot was taken into a 25 mL conical

flask, treated with 1 mL of 5% (w/v) potassium iodide solution while shaking continuously, and filtered. The filtrate was then transferred to a 10 mL volumetric flask and diluted to the mark with water. Nickel(II) was afterwards determined and removed following the recommended procedure, and a blank was run in exactly the same way, only without the analyte.

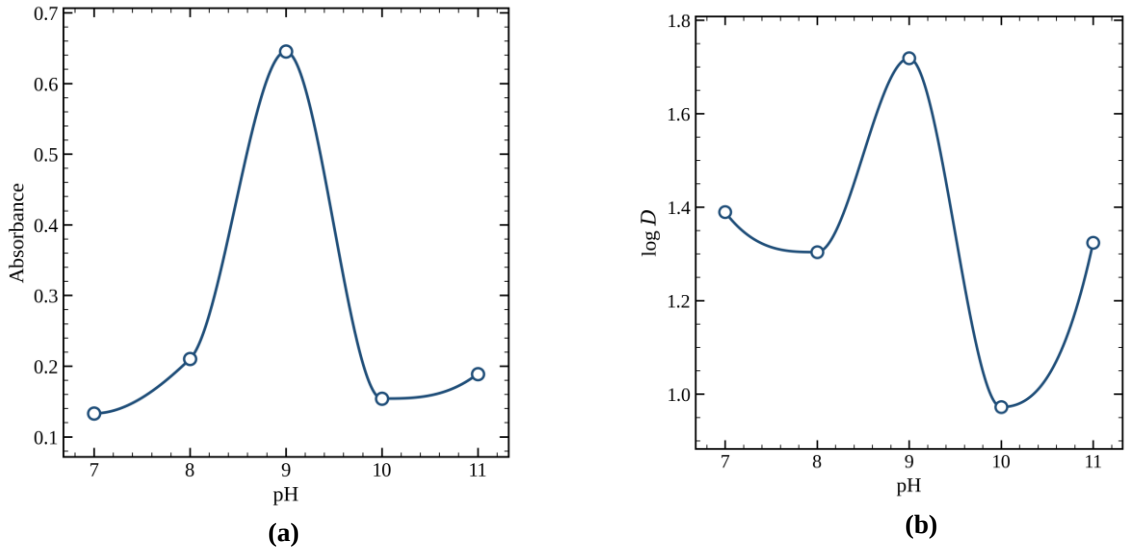
3- RESULTS AND DISCUSSION

3-1: Optimization of the Cloud Point Extraction of Ni(II)

The pH, the concentrations of Triton X-100, PAD, and Ni^{2+}, the heating time, and the equilibration temperature are some of the factors that affect how well the CPE process determines and removes Ni(II). Using the traditional one-variable-at-a-time method, each of these factors was investigated separately; the ideal values are covered below

3-1-1: Effect of pH

The optimal pH is 9, which yields the highest distribution ratio (D), as seen in Figure 1 (a and b). Protonation of the nitrogen atom in the reagent's thiazole ring occupies its lone pair below this value, which lowers D by decreasing the ligand's capacity to complex. The formation of stable, non-extractable hydroxylated Ni^{2+} species in the aqueous phase and the formation of an ion-pair complex containing the hydroxyl ion (OH^-) as the counter-anion, which is more stable in the aqueous phase and is thus retained there, are the two reasons why D decreases above the optimum value.

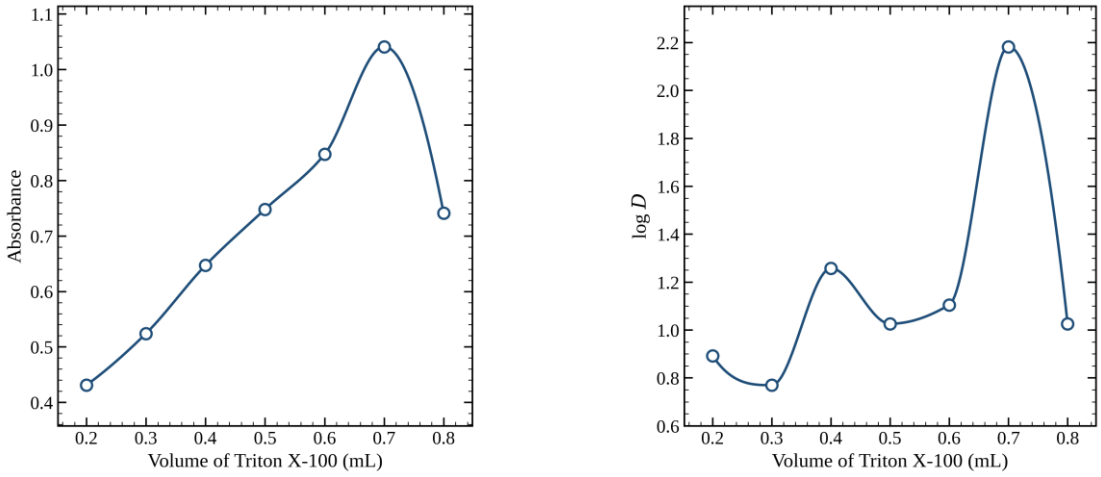


(a) **(b)**

Figure 1: effect of pH on (a) the extraction efficiency (absorbance) and (b) the distribution ratio (D) of the Ni(II)–PAD ion-pair complex.

3-1-2: Effect of Triton X-100 Concentration

To improve the system's capacity for preconcentration, an effective CPE process should maximize extraction efficiency while maintaining a small volume of the surfactant-rich phase [27]. Figure 2 shows the results of examining the impact of Triton X-100 concentration over a range of 0.1–1.0 mL of 1% (w/v) surfactant added to 10 mL of an aqueous solution containing 20 µg mL^{-1} Ni^{2+} and 1×10^{-4} M PAD. Without centrifugation or cooling, phase separation was simple due to the surfactant-rich phase's high viscosity. The absorbance rose with surfactant volume up to a maximum of 0.7 mL (Figure 2), after which it sharply dropped due to an increase in the total phase volume and viscosity, which diluted the analyte and reduced sensitivity. In order to achieve the best extraction efficiency and preconcentration factor, a volume of 0.7 mL of 1% (w/v) Triton X-100 was chosen.

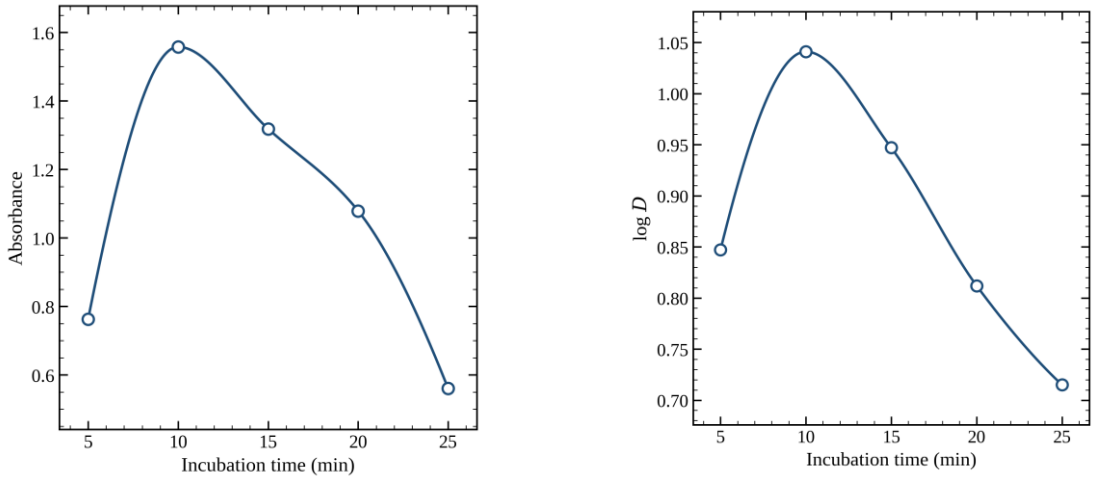


(a) **(b)**

Figure 2: effect of the Triton X-100 volume on (a) the stability of the Ni(II)–PAD complex (absorbance) and (b) the distribution ratio (D).

3-1-3: Effect of Heating Temperature and Incubation Time

The extraction was carried out in a thermostated bath at pH = 9 (set with a few drops of diluted ammonia), with = 1.5×10^{-3} M and 0.7 mL of 0.1% (w/v) Triton X-100, the latter forming the surfactant-rich phase, in order to achieve effective phase separation and preconcentration of the Ni^{+2} complex. The surfactant-rich phase was recovered by decantation after the system had equilibrated at 80 °C. It was then taken up in 5 mL of ethanol, and its absorbance was measured at λmax = 451 nm against a blank. The DMG method was used to determine D by measuring the residual Ni^{2+} [14]. After that, the impact of incubation time was examined over a range of 5 to 25 minutes; the findings are displayed in Figure 3.

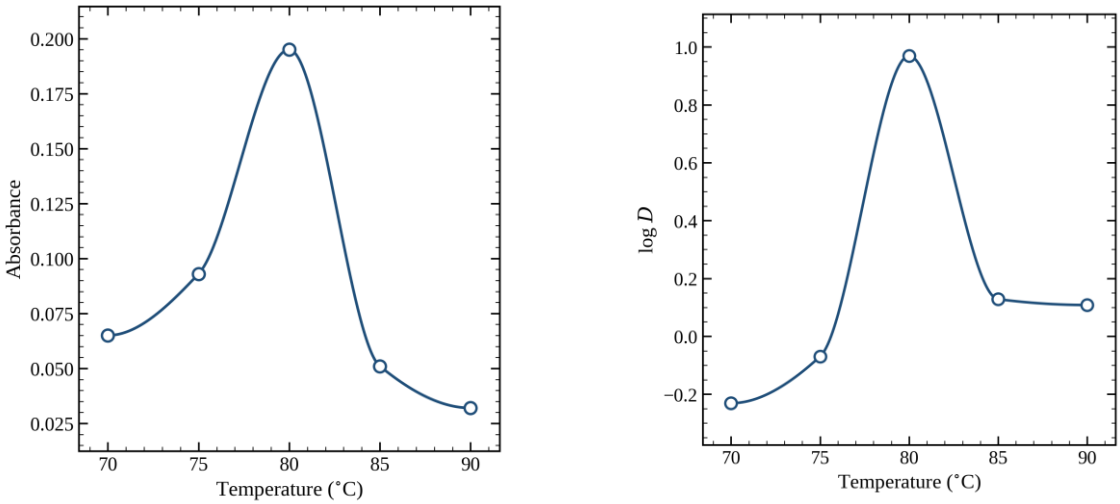


(a) **(b)**

Figure 3: effect of the incubation time (5–25 min at 80 °C) on (a) the absorbance and (b) the distribution ratio (log D) of the ion-association complex.

It was discovered that a 10-minute heating time produced the best extraction efficiency. The kinetics of the process are reflected in the heating time: the ion-pair complex formation and extraction peak at 10 minutes. While longer times decrease efficiency by favoring the opposite reaction that is, by increasing the rate of dissociation and decreasing the amount of complex extracted shorter times prevent the system from reaching equilibrium. Thus, a 10 minute incubation period was adequate to achieve the highest absorbance of the nickel complex.

Since it controls both the reaction's completeness and the effectiveness of phase separation, the equilibration temperature is an equally important factor. Temperatures between 70 and 90 °C were used to extract 20 µg of Ni^{2+} from a 10 mL aqueous solution. The optimal outcomes were attained at 80 °C, where the abundance of micelles generated at the cloud-point temperature encourages the hydrophobic complex's full transfer into the surfactant-rich phase, maximizing sensitivity. Figure 5 illustrates how the separation efficiency drastically decreases above 80 °C due to thermal breakdown of the ion-association complex. As a result, all subsequent experiments used an equilibration temperature of 80 °C.



(a) **(b)**

Figure 4: effect of temperature on (a) the extraction efficiency (absorbance) and (b) the distribution ratio (log D) of the ion-pair complex.

3-1-4: Effect of PAD Concentration and Stoichiometry

By extracting 20 µg of Ni^{+2} from 10 mL of aqueous solution using PAD concentrations between 1×10^{-1} and 2×10^{-3} M, dissolved in water containing 0.7 mL of Triton X-100, the relationship between absorbance and reagent amount was investigated. Following extraction using CPE at pH = 9 and a 10-minute heating period at 80 °C, the surfactant-rich phase was separated by decantation, dissolved in 5 mL of ethanol, and measured at λmax = 451 nm. As shown in Table 1, the DMG method was used to determine the residual Ni^{+2} in order to obtain D.

Table 1: effect of the PAD concentration on the distribution ratio (D) and the extraction efficiency (E%) of Ni(II) by CPE.

[L] reagent concentration (M)	D	E%
0.0001	33.50704	97.10204
0.0005	62.63636	98.42857
0.0010	52.26087	98.12245
0.0015	1959	99.94898
0.0020	12.72549	92.71429

A PAD concentration of 1.5×10^{-3} M produced the most stable, easily extractable complex and the highest distribution ratio, while 1×10^{-4} M produced the lowest efficiency. The ion-pair complex is not fully formed below 1.5×10^{-3} M, which inhibits its transfer into the surfactant phase. The mass-action effect causes the complex to partially dissociate above this concentration, resulting in a negative deviation in the absorbance.

The dependence of log D on log was used to calculate the stoichiometry of the extracted complex [15]. The complex extracted into the surfactant-rich phase has a Ni^{2+} :PAD ratio of 1:1, as indicated by the plot's near unity slope (Figure 6).

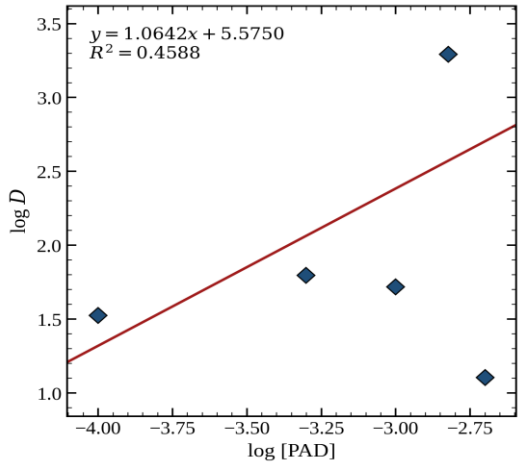


Figure 5: plot of log D versus log [PAD] used to establish the stoichiometry of the extracted complex.

3-1-5: Thermodynamic Study

The following equation was used to calculate the equilibrium extraction constants (Kex) for the [Ni^{+2}: PAD] ion-pair complex at various temperatures using Triton X-100 as the extractant. The transfer of the complex from the aqueous to the surfactant phase, the temperature-dependent aggregation of micelles, the association of the complex, and the partitioning of the surfactant between the two phases are all included in Kex, an overall constant that represents all of the equilibria controlling the separation. Figure 7 and Table 2 present the corresponding findings.

$K_{ex} = D / (Ni^{+2} \cdot [PAD])$

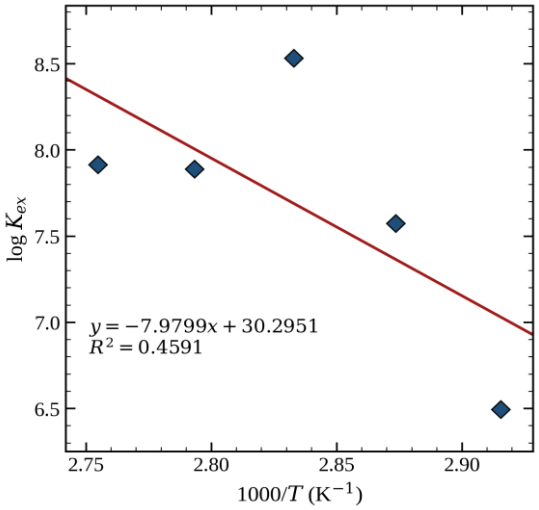


Figure 6: van't Hoff plot of log K_{ex} versus 1000/T for the extraction of the Ni(II) complex.

The thermodynamic parameters were obtained from the slope of the linear plot of log K_{ex} against 1/T (Figure 7), using the relations given below [16]; the results are listed in Table 2.

Slope = $-\Delta H_{ex} / (2.303\ R)$

$\Delta G_{ex} = -\ R\ T\ \ln K_{ex}$

$\Delta G_{ex} = \Delta H_{ex} - T\Delta S_{ex}$

Table 2: thermodynamic parameters for the extraction and removal of the Ni^{+2} ion.

ΔH_{ex} (kJ)	T (K)	ΔG_{ex} (kJ)	ΔS_{ex} (J)
0.15126	343	-0.2863	124.78066
	348	-42.6485	145.4165
	353	-57.6472	163.7350
	358	-54.05576	151.41628
	363	-54.9974	151.9248

The Gibbs free energies (ΔGex) and entropies (ΔSex) were calculated at each temperature, and the enthalpy of extraction (ΔHex) was determined to be 0.15126 kJ mol-1. The extraction is easy and thermodynamically favorable, as indicated by the small positive value of ΔHex. This is linked to micelle dehydration (a decrease in ΔHsolv and an increase in ΔHhyd), which increases the phase-volume ratio and improves efficiency. Endothermic reactions are thus the result.

Table 2 demonstrates that as the temperature rises, ΔGex becomes more negative. While the positive values of ΔSex suggest that the process is entropy-driven, the negative values of ΔGex verify that the solubilization of the ion-pair complex into the micellar phase is thermodynamically favorable and spontaneous [17].

3-1-6: Effect of Interfering Ions

Table 4 presents the findings of an investigation into the impact of various salts containing potentially interfering metal cations on the extraction of Ni^{2+} by CPE. Under the CPE conditions, these cations generally had no discernible impact on the formation of the Ni^{2+} ion-pair complex; when they did, it showed up as a small drop in the distribution ratio. In practice, the determination of Ni^{2+} is unaffected by these cations because their concentrations in environmental samples are low.

Table 3: effect of foreign cations on the formation and extraction of the Ni(II) complex by CPE.

E%	D	Abs 445	Abs 451	Interferent
82.81633	4.819477	0.171	1.747	$MnCl_2 \cdot 2H_2O$

98.42857	62.63636	0.018	1.998	$FeCl_2$
85.06122	5.693989	0.149	1.564	$CoCl_2$
93.02041	13.32749	0.071	1.989	$CuCl_2$
85.16327	5.740028	0.148	0.870	$Pb(NO_3)_2$

3-1-7: Effect of Electrolyte Salts

The impact of electrolytes made up of group I and group II metal nitrates and chlorides was investigated; these salts were selected because their anions do not compete with the complex being studied during the CPE process. The ionic activity of the solution is somewhat reduced by the presence of such electrolytes. Table 5 provides an overview of the Ni^{2+} extraction results.

Table 4: effect of electrolyte salts on the extraction and removal of Ni^{+2} by CPE.

E%	D	Abs 445	Abs 451	Electrolyte
86.59184	6.458143	0.134	0.664	NH_4NO_3
91.69388	11.03931	0.084	0.987	LiCl
87.81633	7.207705	0.122	0.874	NaCl
93.83673	15.22517	0.063	1.255	KNO_3
85.46939	5.882022	0.145	0.841	$NaNO_3$

Table 5 demonstrates that while the absorbance somewhat decreased as the complex's ionic activity decreased, the majority of electrolytes had very little effect on the micellar phase containing the complex. At 0.2 M, sodium nitrate showed the most pronounced effect. Since the complex is formed in an alkaline medium, the addition of electrolyte tends to prevent the formation of the anionic species by lowering its thermodynamic formation constant. In general, the presence of electrolyte salts had a measurable impact on the extraction of Ni^{2+}.

3-2: Analytical Figures of Merit

Plotting the absorbance against the concentration of Ni^{2+} for a series of 10 mL standard solutions containing 0.1–20 µg mL^{-1} of Ni^{2+} under optimal conditions allowed for the construction of a calibration curve (Figure 8). Table 6 provides a summary of the analytical figures of merit obtained from this curve.

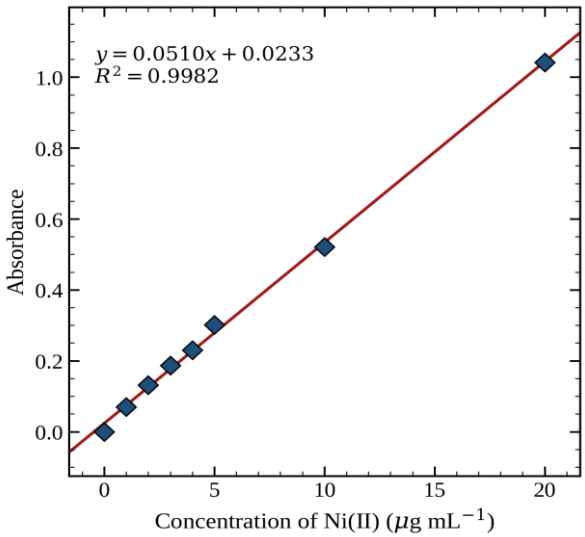


Figure 7: calibration curve for the determination of Ni(II) under the optimized conditions.

Table 5: analytical figures of merit for the determination of Ni^{+2} by the proposed method.

Parameter	Ni(II)
λ_{max} (nm)	451
Regression equation	y = 0.051x + 0.0233
Correlation coefficient (r)	0.9982
Coefficient of determination (R^2)	99.8%
Linear range (µg mL^{-1})	0.1–2

Limit of detection (µg mL^{-1})	0.09
Limit of quantitation (µg mL^{-1})	0.9
Composition of complex (M : L)	1:1
Preconcentration (enrichment) factor	14.29

3-3: Application to Real Samples

The chosen samples' nickel content was estimated using the regression equation. In order to evaluate the method's applicability and reliability, it was then used to determine and remove nickel (II) from a variety of samples, including fish and vegetables gathered from different parts of Najaf City (central Iraq). Every sample was preconcentrated by CPE, prepared in accordance with the suggested protocol, and subjected to spectrophotometric analysis. Table 6 summarizes the results that were validated by comparing them with those obtained in our laboratory using flame atomic absorption spectrometry (FAAS).

Table 6: nickel(II) concentrations (ppm) determined in different samples from Najaf City (central Iraq).

No.	Sample	Proposed method (ppm)	FAAS method (ppm)	$\bar{X}$	S^2	t_{cal} (n=4)	t_{crit} (95%, DF=4)	P-value
1	Local fish (Euphrates River)	1.33	1.38	15.7096	1.069	0.079	2.776	0.94
2	Aubergine	2.28	2.35					
3	Cucumber	2.10	2.18					
4	Zucchini	0.028	0.06					

The means obtained by the proposed method and FAAS were compared using a paired t-test (Table 6). The calculated t-value (0.079) was lower than the critical t-value (2.776), and the corresponding p-value (0.94) was greater than 0.05. Therefore, no statistically significant difference was observed between the proposed method and FAAS, confirming the accuracy and reliability of the proposed analytical procedure.

4- CONCLUSIONS

Using the cloud point extraction method and the azo reagent PAD, a quick and easy spectrophotometric method for determining, separating, and removing nickel (II) has been developed. The reagent is easily prepared and forms a stable 1:1 ion-pair complex with Ni^{2+} that is effectively retained in the cloud point (surfactant-rich) layer once the ideal conditions of reagent concentration and pH have been established.

The thermodynamic analysis demonstrated that the extraction is endothermic, entropy-driven, and spontaneous, and that the cloud-point phase's formation is crucial to the process. The method demonstrated its suitability for both the determination of nickel and the treatment and purification of water contaminated with this ion when applied to fish, vegetable, and water samples. The results were in good agreement with FAAS.

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