Cloud point extraction and determination of Nickel (II) ions complex in real samples using New azo reagent

Review Article

Sarah Ali[1] & **Ibtehaj Raheem Ali**[2]
Department of Chemistry, College of Education for Women, University of Kufa, Iraq.
Correspondence: ibtihaj.alshirmani@uokufa.edu.iq
https://orcid.org/

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Abstract

New azo reagent (2-[Ceffodoxime azo]-4-Nitro -2-Phenol) (__CefAN) preparation by coupled reaction. The reagent was diagnosed by spectral methods using infrared (FTIR) and (UV. –Vis.) as an identification using(HNMR) spectrum and (C.H.N).This reagent use to determination nickel (II) ion as a pair ion complex that extracted and estimated according to the cloud point extraction(CPE) technique in enrichment layer ,after adjusting the optimal conditions and factors affected in efficiency extraction processes .The ion pare complex of nickel(II) is estimated according to the spectral method at λ_{max} =401 nm. The optimization of complexation and extraction conditions by (CPE) investigated as well as optimum pH =10, critical concentration of TX-100 was 0.2mL, heating time=80C°. Thermodynamic parameters of CPE for extraction process of the ion-pair association complex in Triton X-100 were also considered ΔH = 129.107264KJ ,ΔG=-56.4845 KJ and ΔS =525.75552J That referred endothermic reaction, withLimit of Detection (LOD=5.044μg mL^{-1}) and Limit of Quantitation (LOQ=50.044 μg mL^{-1}) ,Molar absorptivity (Ɛ= 3888.4$L.mol^{-1}.cm^{-1}$).The Stiochoimetry study to determination complex stricture that ratios of metal: reagent obtained are (1:1). Under the optimized conditions of a 10 mL sample gave preconcentration and enrichment factors are (50 and 10.1055) respectively with Sandell's sensitivity (1.4854 $x10^{-6}$μg cm^{-2}/0.001A.U). The calibration curve linear in the range of 1-8ppm with a correlation coefficient of 0.9972. The relative standard deviation for replicate determinations at (30) μg $10mL^{-1}$ level is58.63%. The proposed method applied for the determination of Nickel in fish, vegetables, plant leaves and water which gave satisfactory results.

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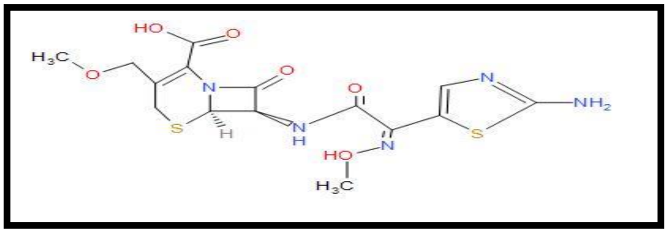
Public Interest statement

The research covered a study Cloud point extraction CPE methodology which is a is wide spread used for the applications of separating, Preconcentration and extracting of many medic mental or biological compounds as well as organic compounds and several metal ions as negative or positive and neutral complexes for aqueous solutions, More over this method is considered an easy, cheap and important technique and one of the application of green chemistry with high sensitivity which does not use organic solvents. Method of extraction and separation for Ni(II) cation from aqueous solutions was conducted by using organic reagent which is recently 2-[Ceffodoxime azo]-4-Nitro -2-Phenol) [CefAN] . In order to enrich, separate and estimate Ni2+ ion complexes. The organic reagent was diagnosed by spectral methods using infrared (FTIR) and (UV. –Vis.) as an identification using (HNMR) spectrum and (C.H.N).

Introduction

Cefpodoximeproxetil is a third-generation cephalosporin antibacterial drug substance including the infections of the respiratory and urinary tracts, skin and skin structure, and gonorrhea. It's appearances a white to light brownish – white powder, odorless or having a faint odor[1] . Cefpodoxime proxetil used in the treatment of due to disease use ceplible Gram-postive and Gram-negative bacteria[2]. Cefpodoxime is highly active against both Haemophilus influenzae and Moraxella catarrhalis including β-lactamase producing strains, with a minimum. inhibitory concentration for 90% of tested strains (MIC90) ⩽ 1 mg/L[3].pKa value of cefpodoxime is 3.22, and it's very slightly soluble in water ,soluble in acetonitrile and in methyl alcohol, slightly soluble in ether and freely soluble in dehydrate alcohol [4].Cefpodoximeproxetil have ammine group in its structure (as Shawn in figure1) that formationdizonume salt which inter the coupled reaction with a suitable phenol compound, that represented by3-Nitro phenol its structure (Shawn in figure 2). [5][6]



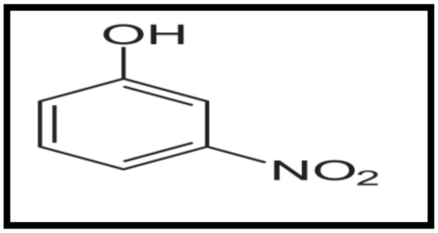


Figure 1:Structureof Ceffadoxe(cefpodoximeproxetil) **Figure2: structure of 3-Nitro phenol**

While3-Nitrophenol (*m*-nitrophenol) is a nitroaromatic compound[7]. It can be prepared from 3-nitroaniline, via diazotization reaction[8].

3-Nitrophenol is one of the isomers of mononitrophenol and is mainly used as an intermediate to prepare dyes, pigments, lumber preservatives, photographic chemicals and pesticides[9]. Some of the methods for its degradation are biotransformation[10], photocatalytic degradation and photooxidation[11].

3-Nitrophenol may be used in the preparation of 3-aminophenol. It may be employed as a weak acid in capillary isoelectric focusing (CIEF) method[12]. A general and efficient method for synthesis of both

symmetric and asymmetric aromatic azo compounds in one single step has been developed. The nitro compounds were reduced and the substituted anilines were oxidized by each other without any metal in the base condition. Various azo compounds with halogen, methyl and methoxy functional group were obtained by using available, cheap nitro compounds and substituted anilines[13].
nitroaromatic compounds have been considered resistant to attack because of the electron withdrawing properties of the nitro group[14]

Because of the importance of the drug compounds, a new azo reagent is prepared to study it interacts with the metal ions, such as Nickel (II) ion. Nickel is one of the most important industrial minerals. When increasing its concentration in the environment, it causes toxicity to plants, animals and the phenomena of nickel poisoning when low concentrations in food (40µg / Kg) are available. Exposure to high concentrations of nickel causes toxicity and cancer[15]. The average amount of nickel taken in the daily diet is about (200-300µg) [16],and the human body needs a small amount most of it is present in the pancreas and plays an important role in the production of insulin and lead to imbalance in the liver and nickel is present in the environment accompanied by oxygen or sulfur,[17] which is caused by volcanoes Nickel and its components in the occurrence of skin allergies known as nickel itching and most nickel and its salts do not cause poisoning, but is known to be carcinogenic and also affects the lungs and sinuses[18]**.**

Recently, procedures involving separation and pre concentration for the determination of Nickel in various matrices using spectrometric techniques, such as FAAS[19][20], ETAAS[21], ICP-OES[22]and ICP-MS[23][24], are well reviewed[25] These include electrochemical deposition[26], precipitation and coprecipitation[27], liquid–liquid extraction (LLE) [28], solid phase extraction[29]and liquid-liquid micro extraction[30]. Although, some of these methods of extraction have offered appropriate extraction efficiency and high pre concentration factor, but they have presented some drawbacks associated with sample contamination, relatively time consuming or high cost, rigid control of the conditions, require a large volume of organic solvents and relatively poor accuracy.

In the last decade, the separations and preconcentrations of metal ions, after the formation of chelates or ion-association complexes, by cloud point extraction (CPE) has been extensively used in analytical chemistry as an alternative preconcentration method to traditional extraction systems due to its efficiency, simplicity, low cost, commercially available surfactant, rapidity and safety[31]compared with majority of the above-mentioned procedures.Numerous reports have been published in the chemical literatures on the preconcentration of nickel, alone or with other elements, by CPE methodology prior to its determination using different instrumental techniques including flame atomic absorption spectrometry[32][33][34][35]electro thermal atomic absorption spectrometry[36][37][38]and inductively-coupled plasma atomic emission spectrometry[39][40].

UV-Vis. spectrophotometry is still the most attractive and popular method and employs in different fields of chemical analysis, especially in quality control due to availability of the instrument in many laboratories, the simplicity of analytical procedure, and satisfactory precision and accuracy. The combination of spectrophotometric detection with cloud-point extraction was first proposed by Watanabe and co-workers[41][42]who studied the extraction for the preconcentration of Mn[43] and Zn[44] in water samples after complexation with 1-(2-Pyridylazo)-2-naphthol (PAN) using poly ethylene glycol-mono-p-nonylphenyl ether(PONPE 7.5) as a micelle- mediated extracting[45]. This method (CPE) can be considered as treatment and purification method from pollutants such as nickel easy, cheap and safe, addition to determination, extraction method, enrichment and preconcentration.

2-Experimental

a. A Shimadzu double beam UV-Vis Spectrophotometer model UV-1700 (Japan) working at wavelength of 190-1100 nm (±0.3nm accuracy at D2 peak 656.1nm, 486.0 nm and ± 0.1 repeatability), spectral bandwidth of 1nm (190 to 900mm) equipped with 10-mm optical path cell was used for the scanning of absorption spectra of all reagents and complexes throughout this study. **b.** While absorbance measurements in the optimization study and detection of metals were done with Single beam (UV-Vis) Spectrophotometer, TRIUP International Corp - TRUV. 74, S (Italy). FTIR-8400S, Shimadzu (Japan) , CHN/O Analyzer,Nuclear Magnetic Resonance Spectrometer-HNMR (Central Dastcami laboratory – Iran).

2.1 Reagent and materials

All analytical grade reagents were used without further purification as received from different company. Doubled distilled water was used for diluting the standard, reagents and samples. The nonionic surfactant (Triton X-100) whose chemical structure is $C_8H_{17}C_6H_4(OC_2H_4)n$ with n equal to 9-10 and an average molecular weight of 625 g/mol, was purchased from Sigma (Sigma Ultra, >99.6%). (UK) and used without further purification. NaOH (99%), HCl (37%), $NaNO_2$;(99%) were purchased from CDH(UK),Ni(II) standard solution (1mg/mL) prepared by dissolving (0.2024g) of $NiCl_2.6H_2O$ (Merck, 99.97%) in50mL distilled water in volumetric flask. As well (2-[Ceffodoxime azo]-4-Nitro -2-Phenol) solution with 1×10^{-2} M concentration prepared by dissolving (0.2209g) in 0.5mL Triton X-100 and distilled water.A solution of 1% Dimethyl glyoxime (DMG) prepared by dissolve 1gm in 100 ml of ethanol. A solution of 4% potassium per sulphate ($K_2S_2O_8$) instantaneously prepared by dissolve 4 gm. in 100 ml of distillation water. All working solutions prepared by dilution appropriate volume.

2.2 Synthesisof (2-[Ceffodoxime azo]-4-Nitro -2-Phenol) (CefAN)as a copmlexing reagent

The reagents is prepared according to the procedure published elsewhere[46] by dissolving (3.77g 0.01 mole) of Cefpodoxime proxtel in a mixture containing 4 mL concentration HCl and 25 mL distilled water. After cooling this solution to 0 C°, 1.4 g of sodium nitrite dissolved in 10 mL, distilled water was added with maintaining the temperature at 0 C°. The mixture was set aside for 15 min for complete diazotizationprocess. Thereafter, the diazonium solution isadded drop by drop into beaker containing (1.418g, 0.01 mole) of 3-nitro phenoland 1.2 g sodium hydroxide dissolved in 150 mL ethyl alcohol with keeping temperature at 0 °C. After complete addition, the contents were left for two hours, then 150 mL of cooled distilled water was added, the pH of the solution adjusted to7 by HCl, a brown powder was precipitated and left for 24 h. The solid product was filtered off, washed with cold water, crystallized twice from hot absolute ethanol and dried over $CaCl_2$ to give yield Mp (135-136 ˚C) and chemical formula of $C_{21}H_{19}N_7O_9S_2$with Mw. (577.55 g. mol^{-1}). The reaction pathway can be shown schematically in Figure 3. The azo reagent synthesized in this work were identified by UV – Vis., FTIR, H^1 NMR that following study , and

C.H.N elemental analysis as shown in table (1). This reagent does not dissolve in water, but it dissolves in organic solvent such ethanol, chloroform, Acetone, DMF etc. To avoid for dissolving this reagent in organic solvents, it was prepared in 0.5% Triton X-100 before use.

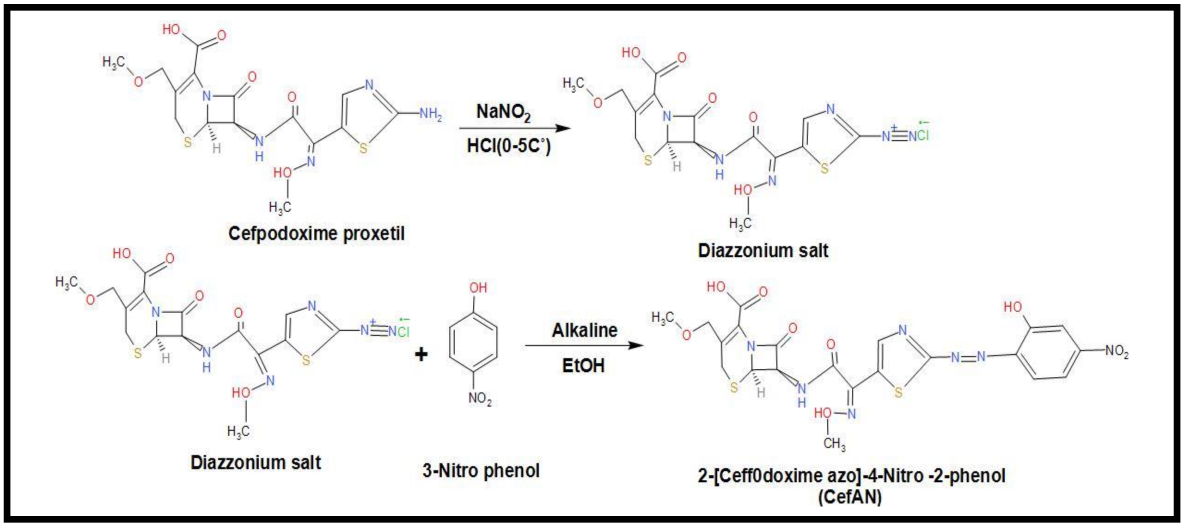


Figure3: Path way to preparation(2- [Ceffodoxime azo]-4-nitro-2-phenol)(CefAN) reagent.

2-3:Elemental Analysis CHN/O

Table1: Elemental Analysis of CefAN

Compound : $C_{21}H_{19}N_7O_9S_2$

Element	%C	%H	%N	%S
calculated	43.66	3.31	16.98	11.1
Found	41.89	3.01	15.76	9.71

2.4- Recommended Procedure

For cloud point extraction, aliquots of 10 mL of the standard or sample solution containing the analyte (5.1116×10^{-5} M, 30µg) $NiCl_2.6H_2O$, at pH = 10 and Triton X-100 (0.2 mL of 0.1% w/v) were allowed to heated for 10 min in a thermo stated bath at 80C°. Separation of the two phases were occurred immediately where the surfactant-rich phase became a highly viscous (without need of centrifugation and cooling) and settled down at the bottom of the tube making the aqueous phase easily separation by decantation. Later, the surfactant-rich phase in the tube was dissolved in 5 mL of ethanol and the absorbance of the resulting solution was measured at λ_{max} =401 nm in a 1cm cell against a reagent blank prepared a same way and determination the remain Ni^{+2}in aqueous solution by spectrophotometric method dimethylglyoxim (DMG)[47] to calculate extraction ratio D. and Calibration curve was obtained by plotting the absorbance versus the concentration of Ni^{2+} under the established optimized conditions. as show in Figure 4.

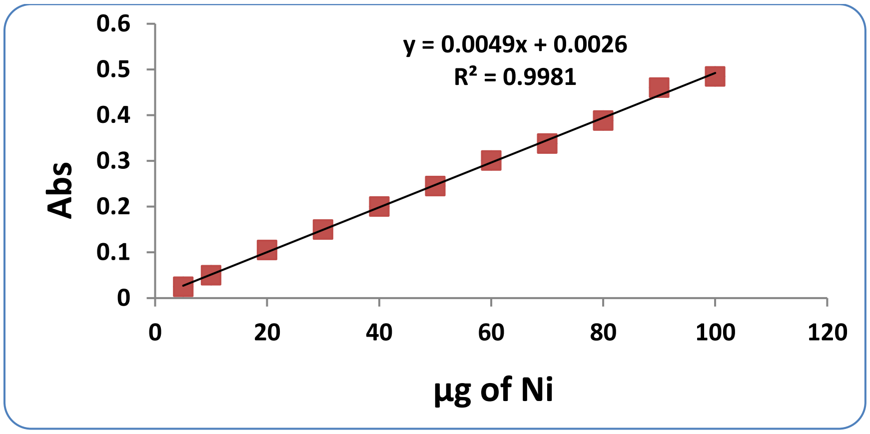


Figure 4 : Calibration curve for spectrophotometric determination of Ni (II).

2.5.Preparation of Samples

A duplicate sample (fish or plant) solution was prepared by transferring approximately 5 g of dried sample into a250 mL conical flask and adding 10 mL of concentrated HNO_3. The contents of the flask were heated on an electric hotplate until the volume was reduced to 2-3 mL. After cooling, a further of 10 mL of concentrated HNO_3, 5ml of concentration $H_2SO_4$and 4mL H_2O_2 were added and the content reheated to boiling until the volume became 2-3 mL, then 10 mL of water were added until colorless solution was obtained indicating of the oxidation of organic matter. The content was cooled and transferred into 100 mL volumetric flask and diluted to the mark with distilled water after filtration. An aliquot of 5 mL of sample solution isput in 25 mL conical flask and treated with 1 mL of 5% potassium iodide solution with continuous shaking and then filtered. The filtrated was transferred into 10 mL volumetric flask and diluted to the mark with water. The nickel (II) was determined according to the recommended procedure. The blank solution was prepared in the same manner without analyte.

2.6. Antibacterial activity

The invitrobiological screening effects of the compounds were tested against the bacteria staphylococcus aurous, Escherichia coli and by the well-diffusion method[48] using Muller-Hinton agar as medium[49]. Wells of 6 mm in diameter were made in the agar plates by using sterile cork borer, then agar surfaces were inoculated with each bacterium. The tested compounds were dissolved in distilled water (D.W) to obtain a solution (29,116,289 ppm) concentration. The plates were incubated at 37 C° overnight; the zones of inhibition formed were measured in mm. Each experiment was performed in triplicate and the average of the three ages of the three determinations was recorded.

3-Results and Discussion

3.1Absorption Spectrum of Prepared (CefAN) Reagent and Ion Pair Association Complexe[Ni; (CefAN)] Extracted by Cloud Point Extraction(CPE)

The UV - visible spectrum of the synthesized CefAN reagent that dissolved in Triton X-100 (0.5 mL of 0.1% w/v) were composed of two main maximum absorption peaks Shaw in (Figure 4).The formation of ion-association complex between Ni $^{+2}$ and CefAN in basic, medium at pH=10, critical concentration of TX-100 was 0.2mL, heating time=80C° critical concentration of CefAN was (2x10^{-4}M), under the conditions of CPE procedure is also achieved via an emergence of a new absorption maxima at λ_{max} of 401 compared with that found with the reagent CefAN alone which gave an absorption maxima at λ_{max} of 360 nm as depicted in the spectra (a) and (b) of (Figure 5).

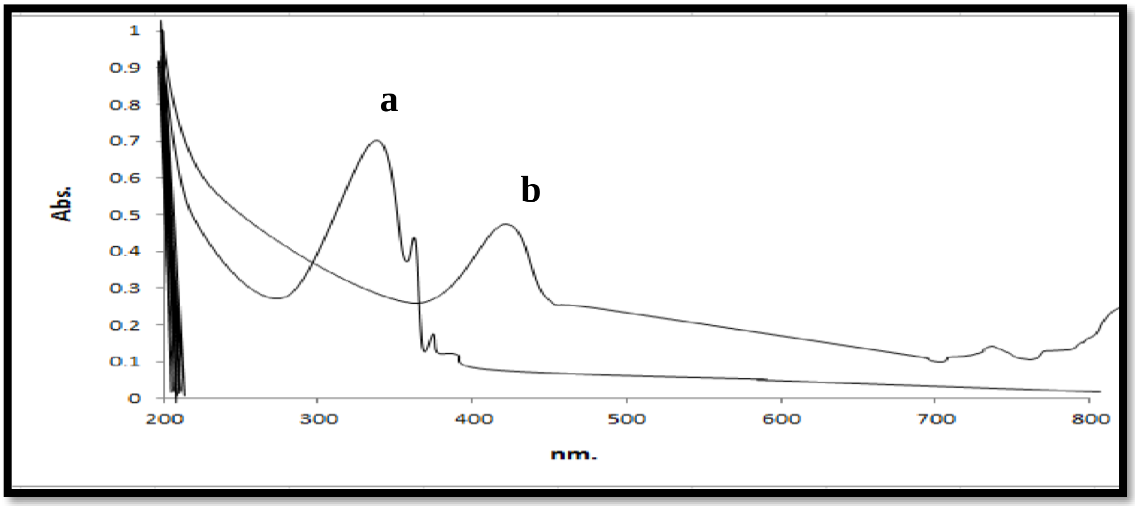


Figure (5) UV-Vis spectra of 1x10^{-4} M CefAN in diluteTX-100 was 0.2mL surfactant .and (5.1116x10^{-5} M of Ni^{+2})

Absorption spectra of (a) CefAN (b) Ni $^{+2}$

3.2 FT-IR Spectrum of CefAN Reagent

The FTIR spectrum of [**CefAN**] was obtained for compound sample prepared as KBr disk by using a Shimadzu FT-IR spectrophotometer series 8400S. Figure (6) and Table (2) summarize the IR data of the synthesized **CefAN**. The occurrence of the azo bands at (1514.17-1462.09cm^{-1}) confirmed that a synthesized dye containing azo group in the solid state[50][51][52].

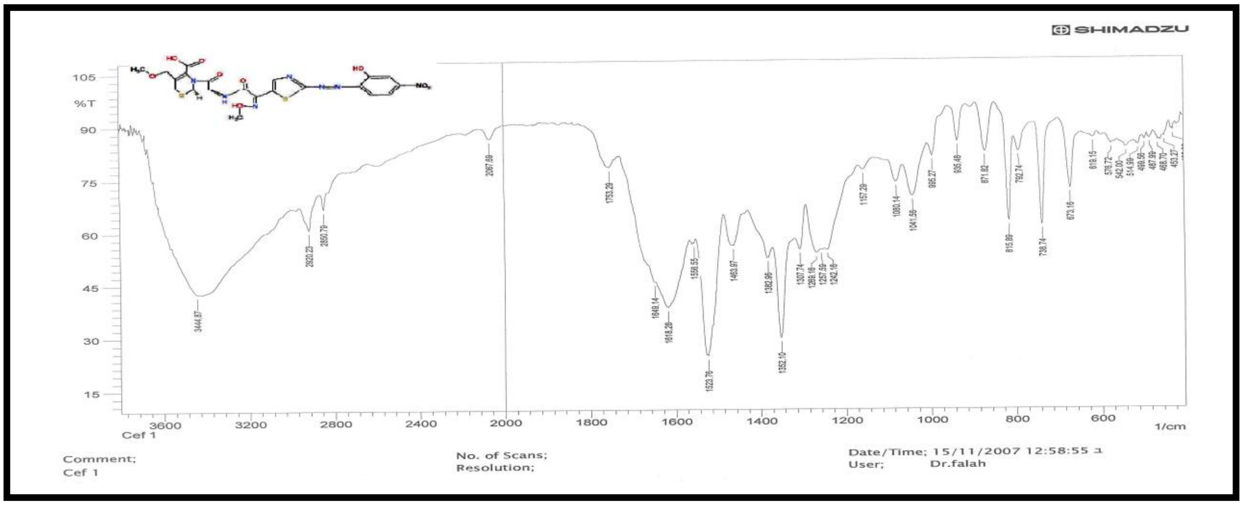


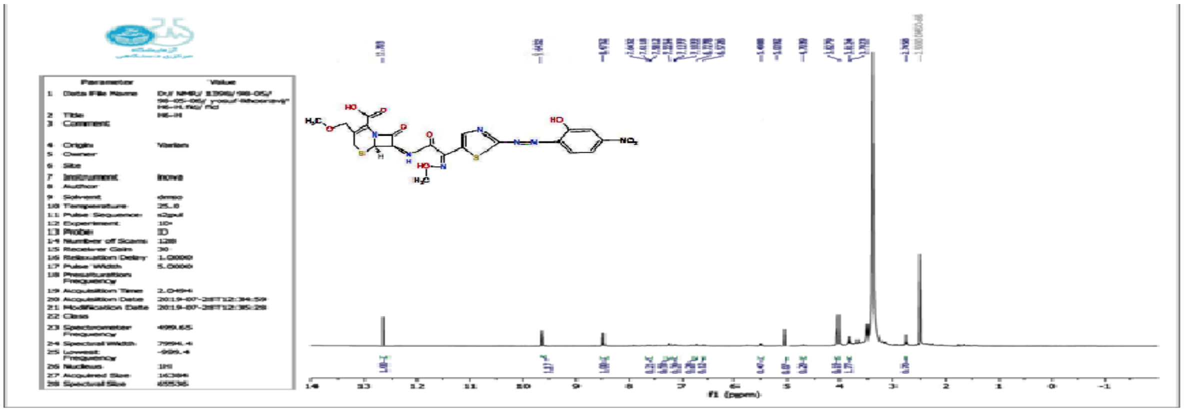
Figure 6: IR spectrum (KBr) of CefAN.

Table 2 IR data (400-4000cm-1) of CefAN

IR bands	Wave number (cm^{-1})
O-H stretching (m, ν)	3415
C-H aromatic (w, ν)	2945
C-H aliphatic(w)	2945
C=N (s, ν)	1604
N=N(m ,ν)	1456
C=C(m, ν)	1382
C-O(m, ν)	1057
C-N(m, ν)	1209
C-S(m, ν)	1043
C-H (s, δ, bend aromatic)	2945
C=O	1618

(s= strong, m= medium, w= weak, ν= stretching, δ= bending)

3.4NMR Spectra of CefAN Reagent



respectively (A6) were also identified by using 1H-NMR appearing of (2.74) for (2H) (CH_2-S) 3,6-dihydro-2H-1,3-thiazine (3.81) (3H) for (CH_3-O-)methoxy methane (3.82) for (3H) (CH_3-O-N=)formaldehyde O-methyl oxime (4.70) for (2H) (O-CH_2-)methoxy ethane (5.03) for (1H) (S-CH-N)3,6-dihydro-2H-1,3-thiazine (5.49) for (1H) (N-CH-C=O)azetidine (6.5-7.8) for (1H)Aromatic (8.47) for (1H) (NH-C=O)formamide (9.64) for (HO-)Hydroxyl (12.70) for (1H) (HO-C=O)Hydroxyl .

3.6 Optimization of The Parameters for Cloud Point Extraction of Nickel(II) Efficiency by CPE

many of factors impact the CPE procedure to determination of Ni(II) such as pH investigated there are (Triton X-100, CefAN, Ni^{+2}) concentration, heating time and equilibrium temperature by using classical optimization.

3.6.1 Effect of pH_{ex} on Extraction Efficiency by Cloud Point Extraction by CPE

The results in Figure (3.6.a and3.6.b) shows that optimum value for pH_{ex} is pH=10 which give higher distribution ratio (D). The pH lower than optimum value of aqueous layer effect to may be protonated the nitrogen atom in thiazol ring in reagent molecule and occupy the lone pair electron caused to minimizing the complexation ability and decrease the distribution ratio (D),in the other hand the pH higher than optimum value caused to decrease distribution ratio (D) by produced stable species of Ni^{+2} ions in aqueous solution cannot be extracted in addition of produced ion pair complex contain hydroxyl ion (OH^-) as anion which is more stable in aqueous phase and minimizing**.**

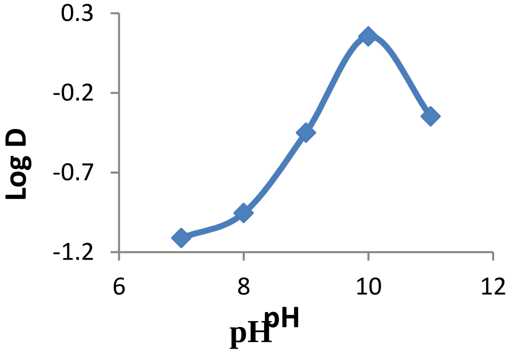


Figure 3.6.a : Effect of pH_{ex} concentration on formation and stability of species extracted.

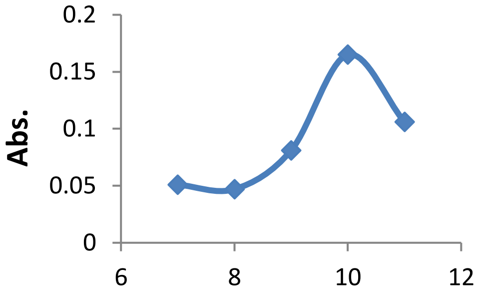


Figure 3.6.b : Effect of pH_{ex} on distribution ratio (D).

3.6.2 Stoichiometry and Effect of CefAN Concentration on Extraction Efficiency by CPE

The variation of absorption signal as a function of (2-[Ceffodoxime) azo]-4-Nitro -2- phenol) CefAN amount was investigated extracted 30µg Ni^{+2} from 10mL aqueous solution by different concentrations($1x10^{-5}$M,$5x10^{-5}$M,$1x10^{-4}$M, $2x10^{-4}$M, $3x10^{-3}$M, $5x10^{-4}$M, $7x10^{-4}$M)of (CefAN) dissolved in (water with 0.5 mL Triton X – 100) by CPE method at pH =9 after adjusted by drops of dilution ammonium solution and (0.2 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation rich- phase by 10 min. heating time in thermostat at temperature 80 C^0 after that separation cloud point layer (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 401 nm against blank prepared a same way, determination the residual Ni^{+2}in aqueous solution by spectrophotomatric method di methylglyoxim (DMG) to calculate distribution ratio D. The results were illustrated in Figures (3.6.3 a. and 3.6.3 b) .

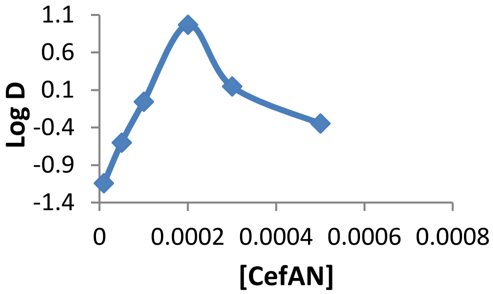


Figure 3.6.2 a : Effect of organic reagent concentration on formation and stability of extracted species.

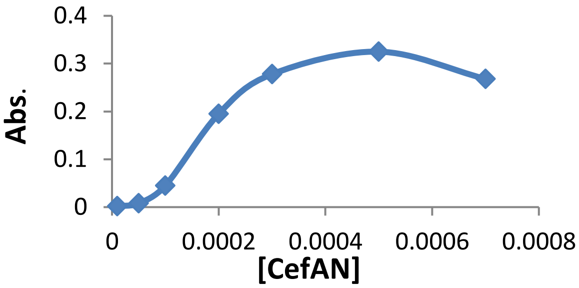


Figure 3.6.2 b : Effect of organic reagent concentration on extraction efficiency.

The results have shown that the concentration of $2x10^{-4}$ M (CefAN) provides a high distribution ratio (D) with highly stable ion pair complex formation extractable into surfactant, whereas at $7x10^{-4}$M gave lowest extraction. At lower than $2x10^{-4}$M CefAN, unstable ion-pair complex was formed which led to depress the extraction into surfactant. Whilst, at higher than of $2x10^{-4}$M more dissociation of complex might occur resulting in a negative deviation in the absorbance values because of mass action.

It is another hand reported the analysis of the dependence log D vs (log C_{CefAN}) by preparing it 10 mL aqueous solution contain 30 **µg** of Ni^{+2} and $2x10^{-4}$ M CefAN as a comlpexation reagent that formation ion par association complex [Ni^{+2}; CefAN] that extracted by CPE method in pH =10 after adjusted by drops of dilution ammonium solution and (0.2 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation enrichment phase by 10 min. heating time at temperature 80 C^0 after that separation cloud point (rich-phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 401nm against blank prepared a same way and determination the remain Ni^{+2}in aqueous solution by spectrophotometric method dimethylglyoxim (DMG) to calculate extraction ratio D. permits determination of the stoichiometry in the extracted complex[53]The slope on the Figure 3.6.2.C log D vs. (log [CefAN]) coordinates is nearly equal to one, indicating the ion pair complex with Ni^{+2}: CefAN ratio 1:1 is extracted into the surfactant-rich phase.

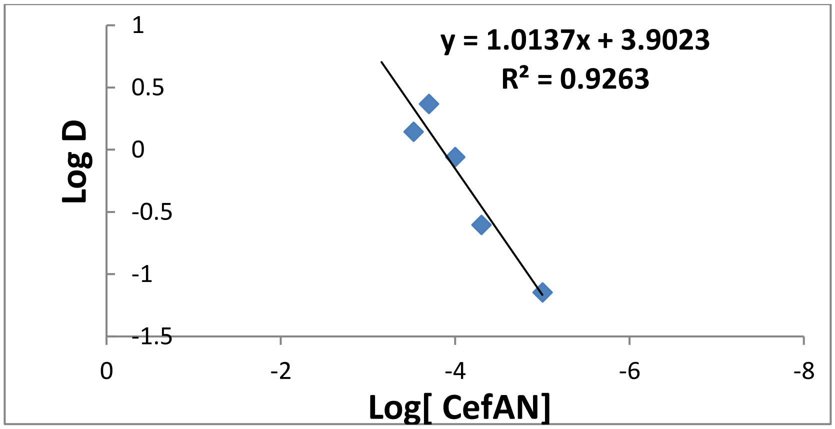


Figure 3.6.2.C: log D vs (log [CefAN])

3.6.3Kinatic Study and Effect of Heating Time on Extraction Efficiency by CPE

The import phase separation and preconcentration of an analyte efficiently, optimal time are very crucial parameters for complete reaction for that the incubation time were examined due to the reaction completion and efficient separation of the phases, 30μg Ni $^{+2}$ ion in 10mL aqueous solution was extracted at varied and different incubi time (70 C°,75 C°,80 C°,85 C°,90 C°) in thermostat and [CefAN] =2x10^{-4} by CPE method at pH =10 after adjusted by drops of dilution ammonium solution and (0.2 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation rich- phase at temperature 80 C^{0} after that separation cloud point layer (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 401 nm against blank prepared a same way, determination the residual Ni^{+2}in aqueous solution by spectrophotomatric method dimethylglyoxim (DMG) to calculate distribution ratio D the results were demonstrated in Figures 3.6. 3a,b

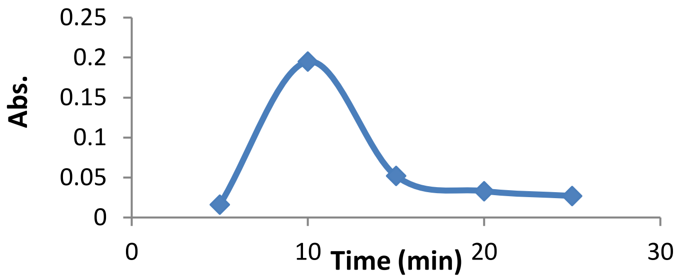


Figure 3.6.3 a: Effect of incubi time on efficiency.

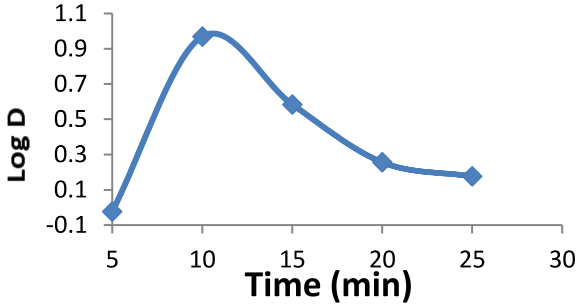
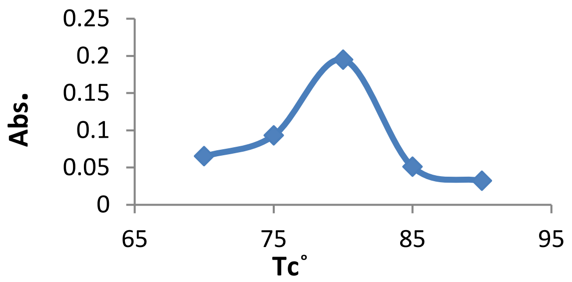


Figure 3.6.3 b: Effect of incubi time ion pair complex extraction formation on association and D value.

The results show 10 min. is the optimum heating time to reach higher extraction efficiency whereas heating time represent the kinetic side of extraction method at this time reaching maximum ion pair association complex formation and extraction, any time less than optimum not allow to reach the favorable equilibrium, so that any heating time more than optimum heating time effect to decrease extraction efficiency because increase backward direction of equilibrium that is mean increase the rate of dissociation and decreasing in ion pair complex extracted**.** It was also observed that the incubation time of 10 min is sufficient for the maximum absorbance of Nickel complex**.**

3.6.4 Thermodynamic study and Effect of Temperature on Extraction Efficiency by CPE

10mL aqueous solution contain 30 μg Ni $^{+2}$ and 2x10^{-4} M of CefAN at different temperature.The temperature was varied from 70 to 90 C° in a search of optimum value. It was shown that the highest absorbance signals at the temperature 80 C° achieving quantitative extraction (Figure 3.6.4 a and 3.6.4b). High temperatures are not suitable to procedure the CPE because higher temperatures cause instability of complex due to thermal decomposition of ion- pair association complex. In this study, 80 C° is chosen as the optimized temperature for subsequent experiments.



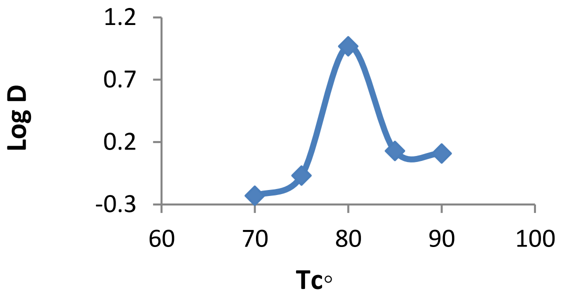


Figure 3.6.4a : Effect of temperature on Figure 3.6.4 b : Effect of temperature
extraction efficiency After calculated. on ion pair formation and stability.

Thermodynamic study

Thermodynamic parameters and the equilibrium extraction constants (K_{ex}) for the extraction of [Ni^{+2} :CefAN] ion pair complex during the CPE using Triton X-100 as a extracting agent at various temperatures were determined. The equilibrium constants (K_{ex}) at the selected temperature were calculated from equation below .These thermodynamic equilibrium constants (K_{ex}) is actually represents all equilibrium constants that affect the separation process such as complex transportation constant from aqueous phase to surfactant phase, aggregation micelles constant with increasing temperature, association constant of the complex and distribution constant of the surface between aqueous phase and surfactant (micelles) phase.and the results are shown in Table **3** and Figure 3.6.4c.

$$K_{ex} = \frac{D}{[Ni+2][CefAN]}$$

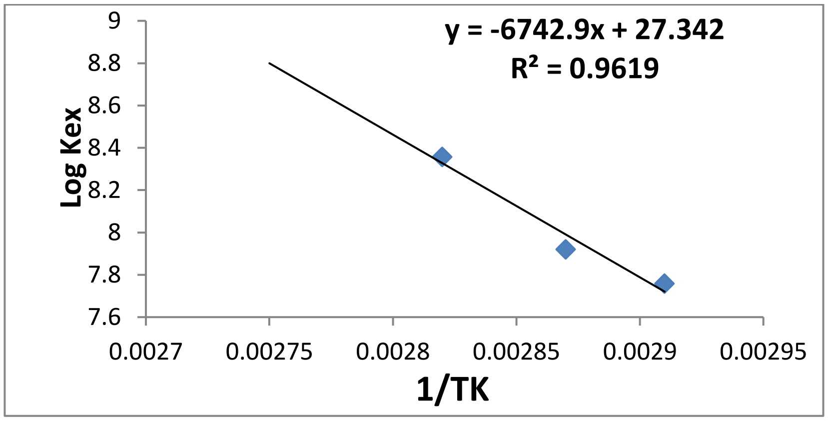


Figure 3.6.4c : Effect of temperature on extraction constant

From the slope of straight line from the plot of log K_{ex} versus 1/T (Figure **3.6.5c**) and relations below[54]. calculated thermodynamic parameters demonstrated in Table 3

$$\textbf{Slope} = \frac{-\Delta Hex}{2.303\ R}$$

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

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

Table 3: Thermodynamic data for extraction Ni^{+2} ion

TC°	TK°	ΔHexKJ	ΔGex KJ	ΔSex J
70	**343**	**129.107264**	**-50.9505**	524.94956
75	**348**		**-52.7672**	522.62759
80	**353**		**-56.4845**	525.75552
85	**358**		**-55.6421**	516.0595
90	**363**		**-56.2787**	510.70496

Enthalpy value ($\Delta H_{ex)}$ found to be 129.107264 kJ mol^{-1} while the values of Gibb's free energy (ΔG_{ex}) were obtained at different temperatures. Once these two parameters are obtained, the values of entropy ($\Delta S_{ex)}$ were also calculated. The results revealed that the extraction of ion-pair complex is easy and thermodynamically favorable due to the small value of ΔH_{ex} which leads to dehydration of micelles (i.e decrease the value of ΔH_{solv} and increase ΔH_{hyd}) and resulting in increasing the phase-volume ratio thus extraction efficiency enhancement.

.

$$\Delta H_{ex.} = \Delta H_{solv.} - \Delta H_{hyd.}$$

It was also noted form **Table 3** that the values of ΔG_{ex} approximately decreased with temperature. The negative values of **Δ**G_{ex}indicate that the ion-pair complex solubilization process is also spontaneous andthermodynamically favorable[55].The reaction of extraction by CPE is endeothermic reaction and positive value of ΔS_{ex} mean the reaction is entropic inregine.

3.6.5 Effect of Electrolyte Salts on Extraction Efficiency by CPE

It is known that the behavior of some electrolyte salt solutions lie in increasing the extraction percent during CPE process due to their act in increasing the dehydration of surfactant-rich phase.(Table 4) reveals that some of electrolyte salts such as group (I) and (II) metals as chlorides and nitrates were selected on the bases that these anions do not compete the anionic of complex and behave variably in increasing the distribution ratio of ion association complex under extraction by CPE.

30µg Ni^{+2} ion in 10mL aqueous solution was extracted according to cloud point method in the presence 0.01M of some electrolytes in aqueous solutions and follow the procedure detailed in general method. The results were shown in Table 4:

Table 4 : Effect of electrolytes on extraction efficiency of Ni^{+2}

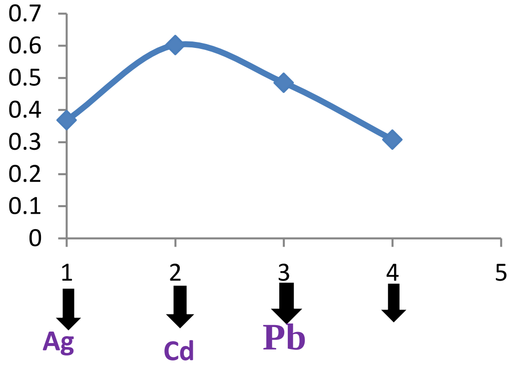
Electrolyte	Abs. at λ_{max} =401nm	E%
LiCl	**0.438**	99%
NaCl	**0.311**	97.4%
$CaNO_3$	**0.209**	95%
$NaNO_3$	**0.126**	94.3%
KNO_3	**0.109**	96.6%

NH_4NO_3	**0.289**	96%

The results appear the presence of electrolyte salts in aqueous phase effect to increase extraction efficiency because the electrolyte causing contribute in increasing the ionic activity in solution of aqueous phase as well as destroy the hydration shell of metal ion Ni^{+2} and this effect change and ionic radius with higher charge density giving higher effect.

3.6.6Effect of Interferences on Extraction Efficiency by CPE

30µg $Ni^{+2}$was extracted from 10mL aqueous solutions according to cloud point technique in the presence 0.01M of some interferences metal cation by following general procedure the results were demonstrated in Figure 3.6.6a,b:



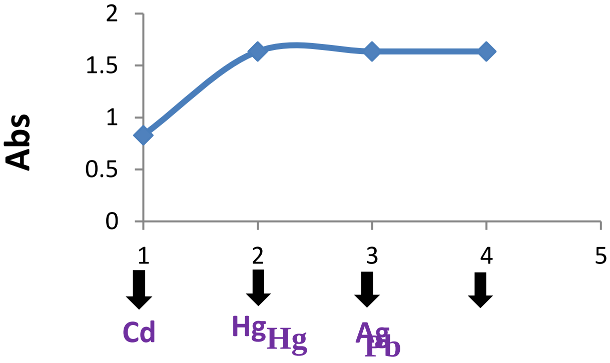


Figure 3.6.6 a: Effect of interferences on Figure 3.6.6 b : Effect of interferences onion pair association complex formation.extraction efficiency and D value.

some salts which contain both cations and anions on the extraction of Ni^{+2} by CPE. As a rule cations such as Cd^{+2} , Hg^{+} , Ag^{+} and Pb^{+2} cannot give an evidence for the interfere with Ni^{+2} because they are work in the optimum condition for Ni^{+2} thereby cannot form cation complexes capable to compete with interested metal cations complex, in the formation of ion-pair complexes under the conditions of CPE that leading to depress the distribution ratio markedly of interference cation extraction.

3.6.7 Calibration curve and Effect of Nickel Ion Concentration on Extraction Efficiency by CPE

Extracted different concentrations of metal ion Ni^{+2} (10 µg,20 µg,30 µg,40 µg.50 µg,60 µg,70 µg,80µg) from 10mL aqueous solutions contain 2x10^{-4} M CefAN as a comlpexation reagent that formation ion par association complex [Ni^{+2}; CefAN] that extracted by CPE method at pH =10 after adjusted by drops of dilution ammonium solution and (0.2 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation rich-phase by 10 min. heating time in thermostat at temperature 80 C^0 after that separation cloud point layer (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 401nm against blank prepared a same way, determination the residual Ni^{+2}in aqueous solution by spectrophotomatric method dimethylglyoxim (DMG) to calculate distribution ratio D. The results were illustrated in Figures 7 and obtaind the figures of merit which statistically from this calibration curve show as table 5.

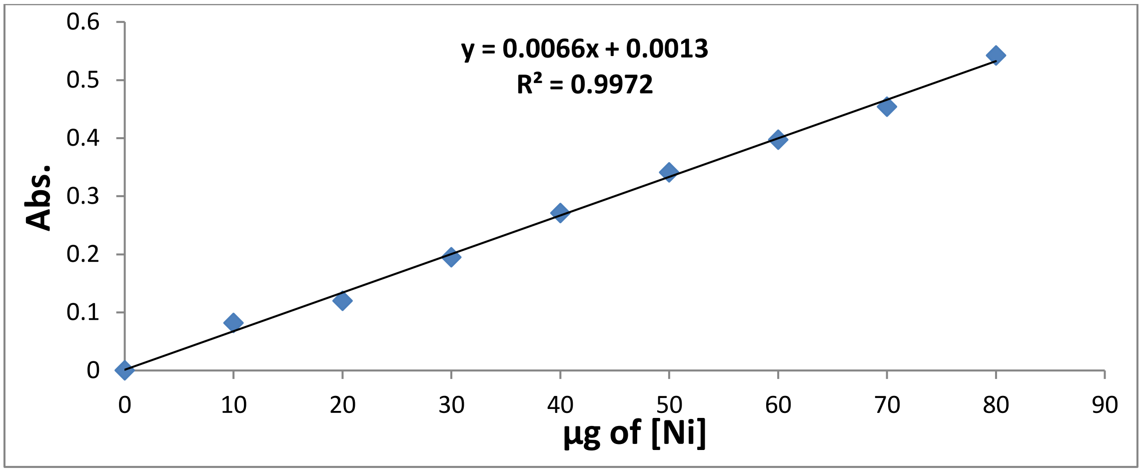


Figure 7: calibration curve and Effect of nickel ion concentration on formation and stability of species extracted.

The results show 30µg Ni(II) in 10mL aqueous solution is the optimum concentration giving higher extraction efficiency because the concentration of metal ion is one of effective thermodynamic parameters and the increasing of metal ionNi^{+2}in aqueous solution help to increase the rate of forward direction and the higher effect appear at 30µg which is giving maximum concentration as well as any concentration more than optimum effect to decrease the extraction efficiency by effect of increasing the rate of back ward direction of thermodynamic equilibrium which is much increase dissociation and decrease the concentration of species formation according to mass action law.

Table 5: Figures of merit for the determination of Ni^{+2} by the proposed methods

Parameter	Value
λ_{max} (nm)	401
Regression equation with CPE procedure	y = 0.0066x - 0.0013
Correlation coefficient(r)	0.9972
Coefficient of determination (R^2)	100%
Concentration range (µg mL^{-1}),(ppm)	(10-80), (1-8)
Limit of Detection (µg mL^{-1}) ,(ppm)	(5.044),(0.5044)
Limit of Quantitation (µg mL^{-1}), (ppm)	(50.44),(5.044)
Sandell's sensitivity (µg cm^{-2}/0.001A.U)	(1.4854×10^{-6} µg cm^{-2})
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	3888.4
Composition of complex (M: L)	1:1
RSD% (n=9)at 30 µg mL^{-1}	58.63
Preconcentration factor	50
Enrichment factor	**10.1055**

3.6.9 Application

The regression line was used to estimate the analyte concentration in the samples selected which appears justified on statistical basis. The proposed method was applied for the determination of nickel (II) in various samples including plants (vegetable), fish and water selected randomly from different areas of the Karbala City (middle of Iraq) in order to test its applicability and reliability. For this purpose, an aliquot of each sample prepared according to recommended procedure was preconcentrated following CPE for nickel determined spectrophotometry. The results of the proposed method for each type of the selected samples were compared with flame atomic absorption spectrometry (FAAS) in our laboratory. The results are summarized from **Table 6**.

Table 6: Concentrations of Ni^{+2} (µg G^{-1}) in different Samples of The Karbala City (Middle of Iraq).

NO.	Sample name	Proposed method µg/10ml	FAAS method µg	Paired t –test				
				X_d	**S_d**	**t_{cal} (n=5)**	**$t_{crit.}$at 95% DF=5**	P- value
1	**Local fish from the Euphrates River**	**1.548815**	**0.07008**	**91.6973**	**1197.6436**	**-4.14305**	**2.571**	34.6070267
2	**Water from the Euphrates River**	**0.359342**	**-0.14412**					
3	**Aubergine**	**0.976184**	**2.945**					
4	**Cucumber**	**0.509342**	**2.1792**					
5	**zucchini**	**1.196184**	**0.033**					

All statistical results performed by the paired t- test for comparison of means between the proposed and FAAS methods for all samples (**Table 6**) . have revealed that all p values [$P(t_{cal.}<t_{crt.})$two tailed] based on the 5% critical values of 2.57 (t critical two tailed) were more than the t calculated values indicating acceptance of null hypothesis (Ho). and this means that the proposed method under study is better than the method FAAS.

note:Measurement of FAAS for aqueous solutions only without a cloud point, so the water did not give a result with FAAS.

3.6.10 Antibacterial activity studies

Biological activity studies of the drugs and its reagents complexes have been tested for invitro growth inhibitory against S.aureus, , E.coli and by well–diffusion method. Their zones of inhibition against different bacteria are presented in table 7 and its statistical presentation is shown in figure 8. The results show that the drugs and complexes display more potency and the reagent has less potency against same pathogenic bacterial strains under same experimental conditions. The mechanism of action of antimicrobial agents can be discussed under five headings, (1)inhibition of cell wall synthesis, (2)inhibition of cell membrane function, (3)inhibition of protein synthesis[56], (4)inhibition of folate metabolism and (5)inhibition of nucleic acids synthesis. The differences in antimicrobial activity are due to the nature of metal ions and also the cell membrane of the microorganisms.

All statistical results performed by the paired t- test for comparison of means between the potency of cefpodoxime proxetil and reagent (CefAN) for all samples (**Table 7**) have revealed that all p values [$P(t_{cal.}<t_{crt.})$two tailed] based on the 5% critical values of 2.776 (t critical two tailed) were more than the t calculated values indicating acceptance of null hypothesis (Ho) which The observations of antimicrobial activity prove that compounds exhibit antimicrobial propertiesdrugs show more inhibitory effects than the parent reagent.

Table 7: Antibacterial activity data (zone of inhibition in mm) of the drugs ,reagent metal complexes

Bacteria **Compounds**	G(+Ve)					G(-Ve)				
	Staphuloccous .aureus mm.				Paired t –test	E.coli mm.				Paired t -test
1-Ceffadoxe	15	11	12	14	X_d=14.5	23	13	15	20	X_d=13.25
2- reagent (CefAN)	**20**	**8**	**13**	**17**	S_d=15.1661	**18**	**8**	**12**	**15**	S_d=19.5829
3- Nickel compleX				**16**	t_{cal} =-0.5449				**18**	t_{cal} = 1.4381
					n=4					n=4
					$t_{crit.}$at 95% =2.776					$t_{crit.}$at 95% =2.776
					DF=5					DF=5
					P- value= 3.8943					P- value= 4.4252

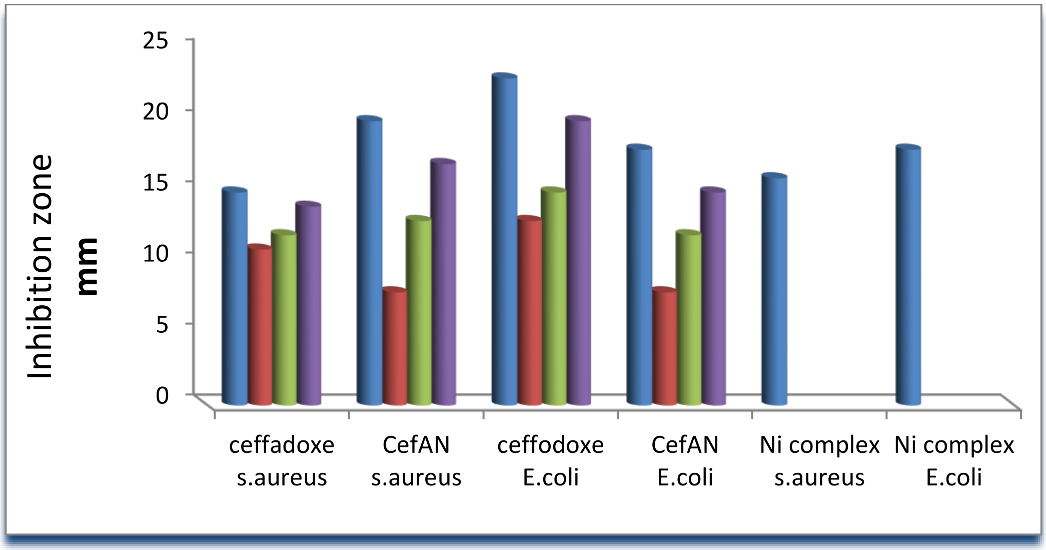


Figure 8 : Statistical representation for antibacterial activity of Drugs , CefAN reagent and its Ni^{+2} complexes

4 Conclusion

For the best of our knowledge, the separation and pre-concentration of Nickel as ion-association complex into surfactant rich phase in a single-step extraction compared to metal chelates for that a new essay for the extraction and detection of $Ni^{+2}$was examined using a new synthesized organic reagent, from drugs to reducing pollution by organic reabent for the first time, in an attempt to extent the analytical capability of CPE technique in conjugation with spectrophotometry. The results we have attained are promised that enough to continue ahead to carry out further works that concerning the development more sensitive methods in this field. Though there has been a marked improvement in most analytical data and good extraction efficency with a good bilogical and inviromantal aplication , the thermodynamic study is still needs much work to investigate the effect of other parameters such as the variation of surfactant amount added and the concentration of the reacting species abreast with temperature for the formation an ion-association complex to fully understand the mechanism of solubilisation of this type of molecules in micelles. However, it should be borne in mind that the implementation of metal cation as hared one like Nickel analysis by CPE is not as straightforward as anticipated as with metal ions in the form of chelates due to the limitations such as interfering species, because precepetation of another metal cation whith this prepared reagent in the optemum condtion for Nickel, surfactant type and electrolytic salts which can expect be solved in future works. These limitations have been alleviated to some extent by careful optimization of the chemical variables in this study. However, the proposed procedure permits to increase the popularity of UV-Vis spectrophotometric technique after CPE beside the solvent-free extraction for metal cations from complex matrices which proved to be fairly simple, sensitive, precise and accurate thereby it might be considered as an alternative for atomic spectrometric techniques.

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Author Bionote

Sarah Ali Mohammed Reda, a postgraduate student, with a master's degree in research stage specializing in analytical chemistry Supervised by Asst. Prof. Dr. Ibtehaj Raheem Ali, in Department of Chemistry, College of Education for Women, University of Kufa, Kufa, Najaf, Iraq.

Authorship and Level of Contribution

The authors equally contributed in the research, writing and revision of the paper.

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