

PREPARATION OF NEW AZO REAGENT FOR EVALUATION OF NICKEL (II) IONS IN DIFFERENT SAMPLES USING CLOUD POINT METHOD

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Abstract

New azo reagent(2-[Ceffodoxime azo]-2-Doxycycline) preparation by coupled reaction. The reagent was diagnosed by spectral methods using infrared (FTIR) and (UV. –Vis.) as an identification using(¹H NMR) 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 affected factors in efficiency extraction processes. The ion pare complex of nickel(II) is estimated according to the spectral method at $\lambda_{max}=438\text{ nm}$. The optimization of complexation and extraction conditions by (CPE) investigated as well as optimum pH =9 , critical concentration of TX-100 was 0.5mL, heating time=85C°. Thermodynamic parameters of CPE for extraction process of the ion-pair association complex in Triton X-100 were also considered $\Delta H = 213.031102\text{KJ}$, $\Delta G=-63.1331372\text{ KJ}$ and $\Delta S =771.40849\text{J}$ That referred endothermic reaction. with Limit of Detection (LOD=1.0303 $\mu\text{g mL}^{-1}$) and Limit of Quantitation (LOQ=10.303 $\mu\text{g mL}^{-1}$) , Molar absorptivity ($\epsilon= 5609.8\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$). 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(20 and 17.6875) respectively with Sandell's sensitivity($1.5506 \times 10^{-6}\text{ }\mu\text{g cm}^{-2}/0.001\text{A.U}$) . The calibration curve linear in the range of 1-8 ppm with a correlation coefficient of 0.9999. The relative standard deviation for replicate determinations at (30) $\mu\text{g }10\text{mL}^{-1}$ level is 58.63%. The proposed method applied for the determination of nickel in fish, vegetables, plant leaves and water which gave satisfactory results.

Keywords: Cloud Point Extraction Determination Azo Preparation Identification thermodynamic Treatment purification Ni⁺²

1-Introduction

Because of the poison and pollution of most chemicals compound as a bad effect on environment and human health for that reason lead us to prepared a reagent from safety compound reagent to determine the heavy metals(Pandey and Madhuri, 2014) (Singh *et al.*, 2011) such as the drug(Wuana and Okieimen, 2011)(Stevens and Baker, 2009) to use it in green chemistry techniques (Clark and Macquarrie, 2008)(Poliakoff and Licence, 2007) as cloud point extraction (Paleologos, Giokas and Karayannis, 2005) to extracted Ni⁺² and Pb⁺² as a determined and treatment process add to enrichment and pre consternation method.

Cefpodoximeproxetil is a third-generation cephalosporin antibacterial drug substance including the infections of the respiratory and urinary tracts, skin and skin structure, and gonorrhoea. It's appearances a white to light brownish – white powder, odorless or having a faint odor(Frampton *et al.*, 1992) . Cefpodoxime proxetil used in the treatment of due to disease use ceplible Gram-positive and Gram-negative bacteria(Brittain, 2020). 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) $\leq 1\text{ mg/L}$ (Brittain, 2019).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 (Asnani *et al.*, 2012).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 by Doxycycline its structure

(Shawn in figure 1). (Rodríguez *et al.*, 2003)(Mosher, 1992)

While Doxycycline is an antibiotic used simply to treat certaininfection caused by bacteria and parasites(Acton, 2013) .Doxycycline is very helpful for bacterial pneumonia, pimples, chlamydia infection, early Lym disease, cholera and syphilis .It is very useful for therapy malaria when used with quinine and malaria

prevention(Kogawa and Salgado, 2012). Doxycycline can be used orally or intravenousl. Doxycycline is monohydrate and its pKa =3.3. its slightly soluble in water (Hellgren *et al.*, 1995). These antibiotics only treat bacterial infections, viral (including colds and flu). (Majewski, 2014) Doxycycline, a semisynthetic, second generation of tetracycline, the drug should be protected from light and its store in air- tight containers (Vranakis *et al.*, 2012). Because of the importance of the two 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(Sreekanth *et al.*, 2013). The average amount of nickel taken in the daily diet is about (200-300µg) (Hussain *et al.*, 2019),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,(Schaumlöffel, 2012) 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 (Bader, Edbey and Telgheder, 2014).

Recently, procedures involving separation and pre concentration for the determination of Nickel in various matrices using spectrometric techniques, such as FAAS(Yebra, Cancela and Cespón, 2008)(Arrukh, 2012), ETAAS(Tsalev, 1995)(Meeravali and Kumar, 2012), ICP-OES(Bezerra, Bruns and Ferreira, 2006)((Society), 2006)and ICP-MS(Nollet and De Gelder, 2013)(Jackson *et al.*, 2018), are well reviewed(Soylak *et al.*, 2007) These include electrochemical deposition(Yeo, Choo and Yip, 2000), precipitation and coprecipitation(Komjarova and Blust, 2006), liquid-liquid extraction (LLE) (Amir *et al.*, 2012), solid phase extraction(Tuzen *et al.*, 2009) and liquid-liquid micro extraction(Lemos and dos Santos Vieira, 2014). 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(Chen and Teo, 2001)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(Şahin, Efeçinar and Şatıroğlu, 2010)(Silva, dos Santos Roldan and Giné, 2009)(M. Ghaedi, A. Shokrollahi, F. Ahmadi, H. R. Rajabi, and M. Soy lak, “Cloud point extraction for the determination of copper, 2008)(Silva, Matos and Lopes, 2015)electro thermal atomic absorption spectrometry (de Campos, dos Santos and Grinberg, 2002) (Abulhassani, Manzoori and Amjadi, 2010)(Brown *et al.*, 1981)and inductively-coupled plasma atomic emission spectrometry(Xu *et al.*, 2007)(Zhao *et al.*, 2012).

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(Goto *et al.*, 1977)(Watanabe and Tanaka, 1978) who studied the extraction for the preconcentration of Mn(Khammas, Jawad and Ali, 2014) and Zn(Ali and Jawad, 2013) 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(Jawad *et al.*, 2016).

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 (± 0.3 nm accuracy at D2 peak 656.1nm, 486.0 nm and ± 0.1 repeatability), spectral bandwidth of 1nm (190 to 900nm) 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).

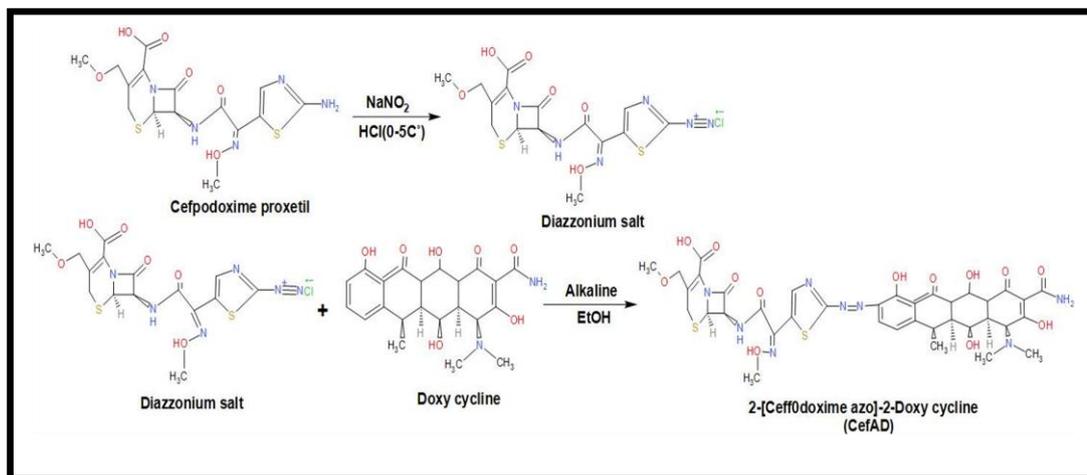
2.1-Reagent and Materials

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%), NaNO₂(99%) were purchased from CDH(UK), Ni(II) standard solution (1mg/mL) prepared by dissolving (0.2024g) of NiCl₂.6H₂O (Merck, 99.97%) in 50mL distilled water in volumetric flask. As well (2-[Ceffodoxime azo] 2-Doxycycline) 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 (K₂S₂O₈) instantaneously prepared by dissolve 4 gm. in 100 ml of distillation water . All working solutions prepared by dilution appropriate volume.

2.2-Synthesis of 2-[Ceffodoxime Azo]-2-Doxycycline (CefAD) as a complexing reagent.

The reagents is prepared according to the procedure published elsewhere(Langová-Hnilíčková and Sommer, 1969)by dissolving (3.77g 0.01 mole) of Cefpodoxime proxetil 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 diazotization process. Thereafter, the diazonium solution is added drop by drop into beaker containing (4.44g , 0.01 mole) of Doxy cycline and 1.2 g sodium hydroxide dissolved in 150 mL ethanol alcohol with keeping temperature at 0 °C. After complete addition , the contents was left for two hours , then 150 mL of cooled distilled water was added, the pH of the solution adjusted to (7) by HCl , a brown powder was precipitated and left for 24 h. The solid product was filtered off, washed with cold water, recrystallized twice from hot absolute ethanol and dried over CaCl₂ to give yield. Mp (154-155 °C) and chemical formula of C₃₇H₃₉N₈O₁₃S₂ with Mw. (869.9g mol⁻¹) . The reaction pathway can be show schematically in Figure 2 .The azo reagent synthesized in this work were identified by UV – Vis., FTIR, H¹ NMR that following study , and C.H.N elemental analysis as shown in table (1). This reagent does not dissolved 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.



Figure(1) Path way to preparation (2-[Ceffodoximeazo]-2-Doxycycline)(CefAD) reagent

2-3:Elemental Analysis CHN/O

Table 1 :Elemental Analysis of CefAD

Compound : C₃₇H₃₉N₈O₁₃S₂

Element	C%	H%	N%	S%
calculated	51.08	4.51	12.88	7.37
Found	48.98	3.78	11.39	7.21

2.4- Recommended Procedure

For cloud point extraction, aliquots of 10 mL of the standard or sample solution containing the analyte (5.1116x10⁻⁵ M, 30µg) NiCl₂.6H₂O, at pH = 9 and Triton X-100 (0.5 mL of 0.1% w/v) were allowed to heated for 10 min in a thermostated bath at 85⁰C. 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} =438 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)(Marczenko, 1986) (Bahram and

Khezri, 2013) 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.

2.5-Preparation of Samples

A duplicate sample (fish or plant) solution was prepared by transferring approximately 5 g of dried sample into a 250 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 10 mL of concentrated HNO_3 , 5 mL of concentration H_2SO_4 and 4 mL 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 is put in 25 mL conical flask and treated with 1 mL of 5% potassium iodide solution with continuous shaking and then filtered. The filtrate 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 invitro biological screening effects of the compounds were tested against the bacteria staphylococcus aureus, Escherichia coli and by the well-diffusion method (Magaldi *et al.*, 2004) using Muller-Hinton agar as medium (Toama, Issa and Ashour, 1978). 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

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

The UV - visible spectrum of the synthesized CefAD reagent that dissolved in Triton X-100 (0.5 mL of 0.1% w/v) were composed of two main maximum absorption peaks shown in (Figure 4). The formation of ion-association complex between Ni^{+2} and CefAD in basic, medium at pH=9, critical concentration of TX-100 was 0.5 mL, heating time=85°C critical concentration of CefAD was (2×10^{-4} M), under the conditions of CPE procedure is also achieved via an emergence of a new absorption maxima at λ_{max} of 438 compared with that found with the reagent CefAD alone which gave an absorption maxima at λ_{max} of 429 nm as depicted in the spectra (a) and (b) of (Figure 2).

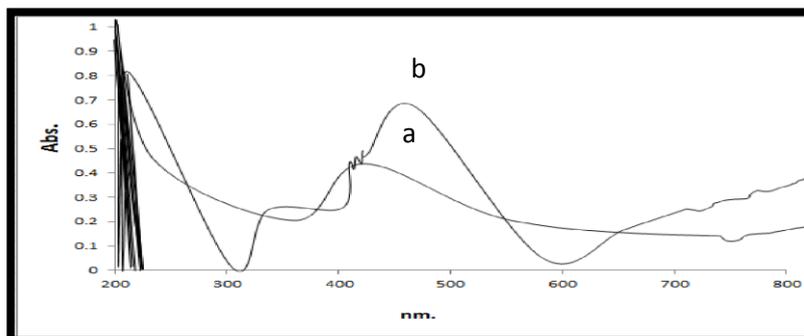


Figure (2) UV-Vis spectra of 2×10^{-4} M CefAD in dilute TX-100 was 0.5 mL surfactant and (5.1116×10^{-5}) M of Ni^{+2})

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

3.2 FT-IR Spectrum of CefAD Reagent

The FTIR spectrum of [CefAD] was obtained for compound sample prepared as KBr disk by using a Shimadzu FT-IR spectrophotometer series 8400S. Table (2) summarize the IR data of the synthesized CefAD. The occurrence of the azo bands at ($1514.17-1462.09 \text{ cm}^{-1}$) confirmed that a synthesized dye containing azo group in the solid state (Nakamoto, 2006) (AL-Adilee, Abass and Taher, 2016) (Ali, 2006).

Table 2: IR data (400-4000cm⁻¹) of CefAD

IR bands	Wave number (cm ⁻¹)
O-H stretching (m, v)	3415
C-H aromatic (w, v)	2945
C-H aliphatic(w)	2850
C=N (s, v)	1618
N=N(m, v)	1463
C=C(m, v)	1382
C-O(m, v)	1080
C-N(m, v)	1352
C-S(m, v)	1041
C-H (s, δ, bend aromatic)	2949
C=O	1604.77

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

3.4NMR Spectra of CefAD Reagent

the diagnosis with H NMR spectrum gave the following results: methylcyclohex-1-enol(2.17) (3H) for (CH₃-N-tri methylamine (2.17-2.54) for (1H,2H) (CH)not Aromatic (3-3.5) for (3H,2H,1H) (CH₃-O-CH₂-)methoxy ethane (OH-CH-)4-hydroxycyclohexanone (4.22) for (3H) (CH₃-O-N=)formaldehyde O-methyl oxime (4.37) for (1H) (CH-C=O)3-(di methyl amino)-2,6-dioxocyclohexanecarboxamide(4.93) for (1H) (S-CH-N)(S)-1-methyl-3-(methyl amino)azetidn-2-one (5.03) for (1H) (N-CH-C=O)(S)-1-methyl-3-(methyl amino)azetidn-2-one (7-7.5) for (1H)Aromatic (7.41) for (2H) (NH₂)(8.49) for (1H) (NH)(12.54,1263) for (1H) (OH)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, CefAD 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

10 mL aqueous solution contain 30 µg of Ni⁺² and 2x10⁻⁴ M CefAD as a complexation reagent that formation ion pair association complex [Ni⁺²; CefAD] that extracted by CPE method in deferent pH (7,8,9,10,11) after adjusted by drops of dilution ammonium solution and (0.5 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation enrichment phase by 10 min. heating time at temperature 85 C⁰ after that separation cloud point (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 438 against blank prepared a same way and determination the remain Ni⁺²in aqueous solution by spectrophotometric method dimethylglyoxim (DMG) to calculate extraction ratio D. The results were illustrated in Figures(3).

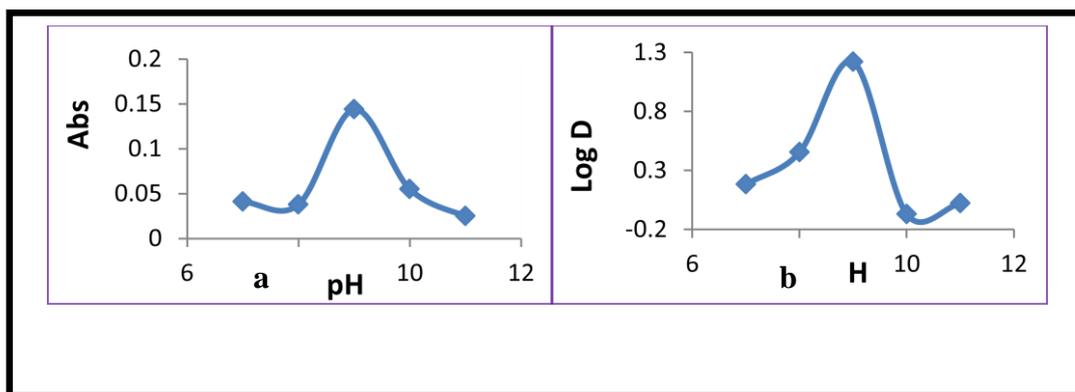


Figure 3:a : Effect of pH_{ex} on formation and stability of complex extracted by CPE.

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

The results in Figure (5) shows that optimum value for pH_{ex} is pH=9 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.

3.6.2: Stoichiometry and Effect of CefAD Concentration on Extraction Efficiency by CPE

The variation of absorption signal as a function of (2-[Ceffodoxime) azo]-2-Doxycycline) CefAD amount was investigated extracted 30µg Ni⁺² from 10mL aqueous solution by different concentrations(1x10⁻⁵M,5x10⁻⁵ M,1x10⁻⁴M, 2x10⁻⁴M, 3x10⁻³M, 5x10⁻⁴M, 7x10⁻⁴M)of (CefAD) 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 (0.5 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 85 C⁰ 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} = 438 against blank prepared a same way, determination the residual Ni⁺²in aqueous solution by spectrophotometric method di methylglyoxim (DMG) to calculate distribution ratio D. The results were illustrated in Figures (4) .

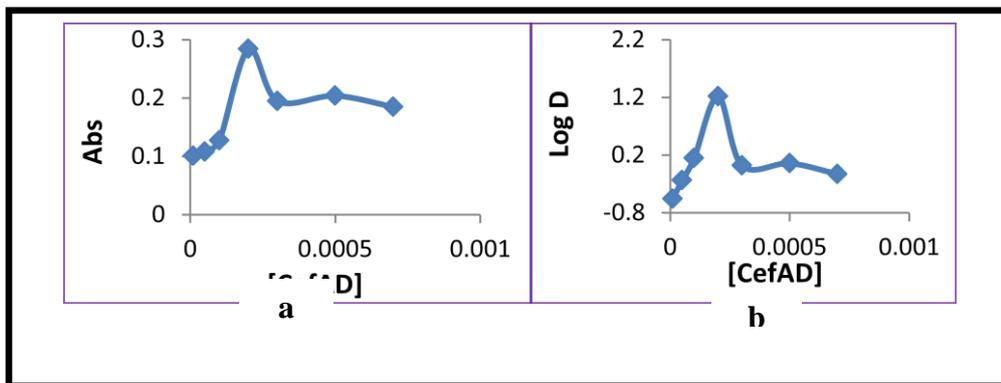


Figure 4: a : Effect of organic reagent concentration on formation and stability of extracted species. b : Effect of organic reagent concentration on distribution ratio (D).

The results have shown that the concentration of 2x10⁻⁴ M (CefAD) provides a high distribution ratio (D) with highly stable ion pair complex formation extractable into surfactant, whereas at (7x10⁻⁴M) gave lowest extraction. At lower than 2x10⁻⁴ M CefAD, unstable ion-pair complex was formed which led to depress the extraction into surfactant. Whilst, at higher than of 2x10⁻⁴M 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_{CefAD}) by preparing it 10 mL aqueous solution contain 30 µg of Ni⁺² and 2x10⁻⁴ M CefAD as a complexation reagent that formation ion pair association complex [Ni⁺²; CefAD] that extracted by CPE method in pH =9 after adjusted by drops of dilution ammonium solution and (0.5 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation enrichment phase by 10 min. heating time at temperature 85 C⁰ after that separation cloud point (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at λ_{max} = 438 against blank prepared a same way and determination the remain Ni⁺²in aqueous solution by spectrophotometric method dimethylglyoxim (DMG) to calculate extraction ratio D . permits determination of the stoichiometry in the extracted complex(Doroschuk *et al.*, 2004)The slope on the Figure 5 log D vs (log [CefAD]) coordinates is nearly equal to one, indicating the ion pair complex with Ni⁺²: CefAD ratio 1:1 is extracted into the surfactant-rich phase.

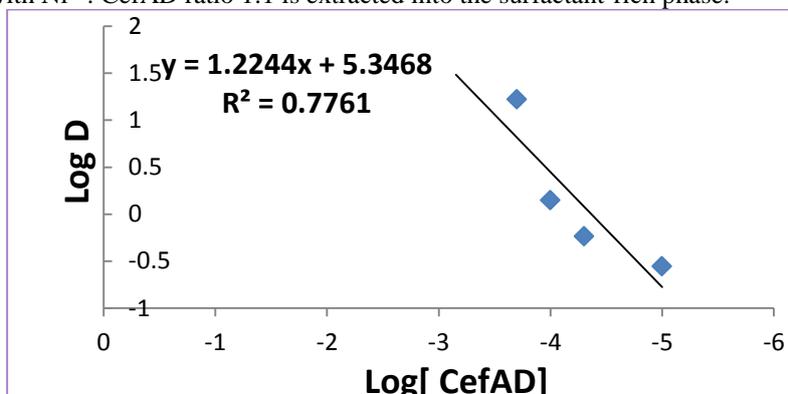


Figure 5: log D vs (log [CefAD])

3.6.3 Kinatic 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 [CefAD] =2x10⁻⁴ by CPE method at pH =9 after adjusted by drops of dilution ammonium solution and (0.5 mL of 0.1% w/v) of Triton X-100 as a surfactant that formation rich- phase at temperature 85 C° 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} = 438 against blank prepared a same way, determination the residual Ni⁺² in aqueous solution by spectrophotometric method dimethylglyoxim (DMG) to calculate distribution ratio D the results were demonstrated in Figures(6).

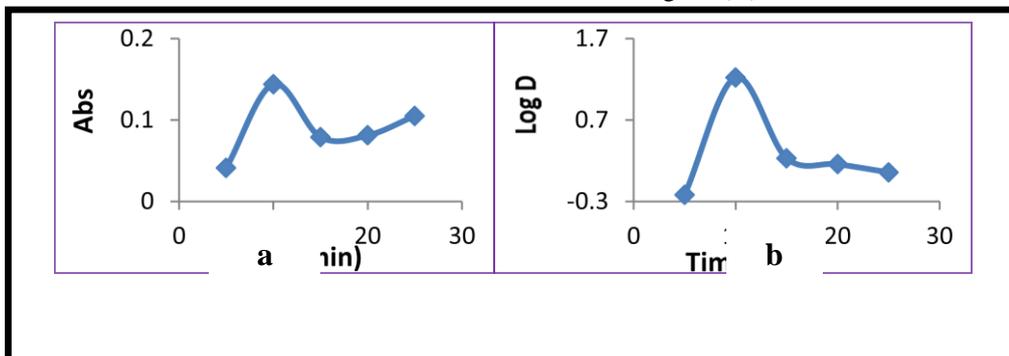


Figure 6: a : Effect of incubi time on formation on extraction efficiency; and b : Effect of incubi time ion pair complex association and D value.

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 CefAD 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 85 C° achieving quantitative extraction (Figure 7). 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, 85 C° is chosen as the optimized temperature for subsequent experiments.

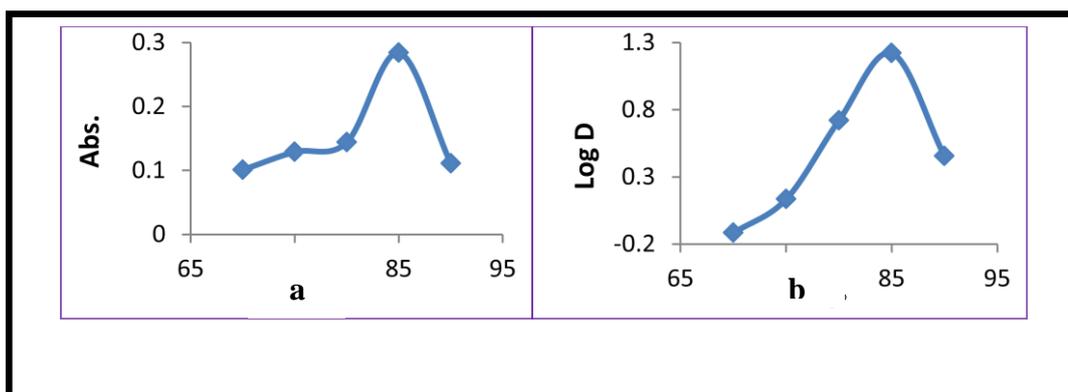


Figure 7: a : Effect of temperature on ion pair formation stability; b : Effect of temperature extraction and efficiency after calculated.

Thermodynamic study

Thermodynamic parameters and the equilibrium extraction constants (K_{ex}) for the extraction of [Ni⁺²:CefAD] 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(8).

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

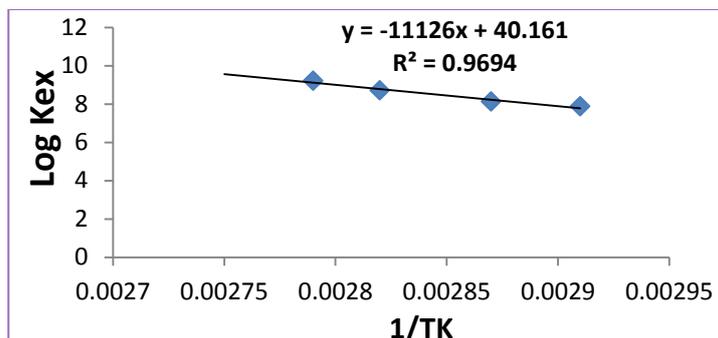


Figure 8 : Effect of temperature on extraction constant

From the slope of straight line from the plot of log Kex versus 1/T (Figure 8) and relations below(Atkins, 1994). calculated thermodynamic parameters demonstrated in Table 3:

$$\text{Slope} = \frac{-\Delta H_{ex}}{2.303 R}$$

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

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

Table 3: Thermodynamic data for extraction Ni⁺² ion

TC°	TK°	H _{ex} KJΔ	G _{ex} KJΔ	S _{ex} JΔ
70	343	213.031102	-51.7032411	771.82024
75	348		-54.1274124	767.69688
80	353		-58.8645583	770.24266
85	358		-63.1331372	771.40849
90	363		-58.6843174	748.52418

Enthalpy value (ΔH_{ex}) found to be 213.031102kJ 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 (ΔS_{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 from **Table 3** that the values of ΔG_{ex} approximately decreased with temperature. The negative values of ΔG_{ex} indicate that the ion-pair complex solubilization process is also spontaneous and thermodynamically favorable(Ali, Khammas and Jawad, 2012) .The reaction of extraction by CPE is endothermic 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} =438nm	D	E%
LiCl	0.572	32.7078	98.3%
NaCl	0.287	16.6471	96.3%
CaNO ₃	0.389	10.0982	89%
NaNO ₃	0.117	12.0319	91.6%
KNO ₃	0.164	9.3448	95.6%
NH ₄ NO ₃	0.172	8.6774	93.6%

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.

3.6.6 Effect 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 table(5).

Table 5 : Effect of Interference on extraction efficiency of Ni^{+2}

Interferences	D	E %	Log D
Hg ⁺²	0.63043	86.6	0.20036
Cd ⁺²	6.5	38.6	0.81291
Ag ⁺¹	0.70454	41.3	-0.1521
Pb ⁺²	1.52101	60.3	0.18213

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 2×10^{-4} M CefAD as a complexation reagent that formation ion pair association complex [Ni^{+2} ; CefAD] that extracted by CPE method at pH =9 after adjusted by drops of dilution ammonium solution and (0.5 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 85 C⁰ after that separation cloud point layer (rich- phase) by decantation and dilute by ethanol at 5 mL volumetric flask and absorption measured at $\lambda_{max} = 438$ against blank prepared a same way, determination the residual Ni^{+2} in aqueous solution by spectrophotometric method dimethylglyoxim (DMG) to calculate distribution ratio D. the figures of merit which statistically from this calibration curve show as table 6.

Table 6: Figures of merit for the determination of Ni^{+2} by the proposed methods

Parameter	Value
λ_{max} (nm)	438
Regression equation with CPE procedure	$y = 0.0096x - 0.0019$
Correlation coefficient(r)	0.9999
Coefficient of determination (R ²)	100%
Concentration range (µg mL ⁻¹),(ppm)	(10-80), (1-8)
Limit of Detection (µg mL ⁻¹),(ppm)	(1.0303),(0.10303)
Limit of Quantitation (µg mL ⁻¹), (ppm)	(10.303),(1.0303)
Sandell's sensitivity (µg cm ⁻² /0.001A.U)	(1.5506 x10 ⁻⁶ µg cm ⁻²)
Molar absorptivity (L.mol ⁻¹ .cm ⁻¹)	5609.8
Composition of complex (M: L)	1:1
RSD% (n=9) at 30 µg mL ⁻¹	58.63
Preconcentration factor	20
Enrichment factor	17.6875

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 ion Ni^{+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.

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 7**.

Table 7: Concentrations of Ni⁺² (5g/100ml) in different Samples of The Karbala City (Middle of Iraq).

Samples	Proposed method $\mu\text{g}/10\text{ml}$	FAAS method μg	Paired t –test				
			Xd	Sd	tcal (n=5)	tcrit.at 95% DF=5	P- value
Local fish from the Euphrates River	1.57666	0.07008	70.8062	114.671132	10.297239-	2.571	10.70846
Water from the Euphrates River	0.396021	- 0.14412					
Aubergine	0.508924	2.945					
Cucumber	0.580967	2.1792					
zucchini	0.477741	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 7**) . have revealed that all p values [$P(t_{\text{cal.}} < t_{\text{crit.}}$) 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 (H_0).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 **Ceffadodoxe** , **Doxy cycline** 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 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, (4)inhibition of folate metabolism and (5)inhibition of nucleic acids synthesis(Salih, Salimon and Yousif, 2012). 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 , doxycycline and reagent (CefAD) for all samples (**Table 8**) have revealed that all p values [$P(t_{\text{cal.}} < t_{\text{crit.}}$) 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 (H_0) that mean the preparation reagent have mor action inhbtion than its druge but which the observations of antimicrobial activity prove that compounds exhibit antimicrobial the parent reagent drugs more inhibitory effects than properties drug show.

Table 8: Antibacterial activity data (zone of inhibition in mm) of the drugs ,reagent metal complexes

Bacteria Compounds	G(+Ve)				Paired t -test	G(-Ve)				
	Staphulococcus .aureus mm.					E.coli mm.				
1-Ceffadodoxe	15	11	12	14	$X_d=16.75$ $S_d=18.7912$ $t_{\text{cal}}=1.2234$ $n=4$ $t_{\text{crit.at 95\%}}=2.776$ $DF=5$ $P\text{- value}=4.3348$	23	13	15	20	$X_d=15.5$ $S_d=39.2908$ $t_{\text{cal}}= 0.5075$ $n=4$ $t_{\text{crit.at 95\%}}=2.776$ $DF=5$ $P\text{- value}=6.2682$
2-Doxy cycline	35	18	22	24		25	12	14	18	
3- reagent (CefAD)	24	10	15	18		26	9	11	16	
4- Nickel complex	16					18				

4-Conclusions

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 Ni²⁺ was examined using a new synthesized organic reagent, from drugs to reducing pollution by organic reagent for the first time, in an attempt to extend 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 efficiency with a good biological and environmental application, 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 hard 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 pre-precipitation of another metal cation which this prepared reagent in the optimum condition 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.

5- References

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