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Cloud Point Extraction And Spectrophotometric Determination Of Cephalexin Drugs In Pharmaceuticals Preparation Through Complex Formation With Hg (II) And Ni (II).

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ABSTRACT

A simple, sensitive, rapid and extraction spectrophotometric method was developed for determination of parts per million levels of Cephalexin drugin pure form andPharmaceuticals preparation ,the method was based to determination of the drug cefalexin using Cloud point Extraction by formationchelating complexwith Hg (II) and Ni (II).Then the complex extracted with ethanol (CEX – Hg II) at pH (12) and (CEX– Ni II) chelating complex at pH (11)the optimum conditions were studied. Beer's law is obeyed in the working concentration range of (5-60) µg/mL of(CEX– Hg II) complex and (5- 65)µg/mL for (CEX– Ni II)complex The mole-ratio method has been used to determine the structure of chelate (CEX - Hg II) and (CEX– Ni II) found to be 2:1 L: M (Ligand: Metal). The Sandell sensitivity (S), molar absorptivity,, limits of detection (LOD) and quantification (LOQ) are calculated for both complex . The wavelength(398 nm) for (CEX – Hg II) and (708nm) for(CEX– Ni II). The method is applicable for the assay of the investigateddrugs in different dosage forms

Keywords: Antibiotic drug, Cefalexin, Nickel Ion, Mercury Ion, Cloud Point Extraction, chelating complex

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INTRODUCTION

Cephalosporins are β –lactam antibiotics obtained originally From a cephalosporium mold. These antibiotics have the same mechanism of action as the Pencillins, but differ in antibacterial spectrum Cephalosporins discovered by Bortzu in 1948 Cefalexin is the First generation of caphalosporins The chemical formula is C_{16} $H_{17}N_3O_4S$. H_2O . And the chemical structure for the drug(1) Cephalexin (CPX), (7R)-7-(D-α-amino-α- phenylacetamido)-3-methyl-3-cephem-4-carboxylic acid hydrateor (6R,7R)-7-{[(2R)-2-amino-2-phenylacetyl]amino}-3-methyl-8-oxo-5-thia-1-azabicyclo[4.2.0oct-2-ene-2 carboxylic acid hydrate (Figure 1) is a first generation**(2)**semi-synthetic derivative of cephalosporin, which is a powerful tool against gram-positive and gram-negative bacteria.

It exhibits a broad spectrum of activities, which have the ability to weakly bond with blood protein, therefore, have no metabolites, low toxicity and rapid absorptivity(3) the literature a large number of analytical methods have been proposed for the determination of cephalexin in pure form, in pharmaceutical preparations, and in biological fluids. But the problems with these methods are :they are expensive, time consuming and have environmental effect, mostly temperature dependence. These methods include a HPLC method, for estimation of cephalexin from formulations , flourimetry(4) and polarography,(5) high-performance liquid chromatography,(6) flow injection analysis,(7) densitometric method,(8) high-pressure thin layer chromatography(9) and reverse phase high-performance liquid chromatography(10)methods, for determination of cephalexin in pharmaceutical formulations, and in biological fluids. The present communication describes simple, sensitive, accurate, rapid and economical colorimetric-spectrophotometric method for the estimation of cephalexin in capsule dosage forms(11) for the determination of cephalexin and most reported methods involve multistep procedures, and have poor selectivity and sensitivities and narrow linear ranges. Spectrophotometry is considered as one of the most suitable analytical techniques for the analysis of pharmaceutical compounds, because of its low cost, simplicity, wide linear dynamic range and wide availability(12).is a first generation cephalosporin antibiotic , It is used in the treatment of susceptible infections of the respiratory tract, urinary tract, and skin(13). Cefalexin monohydrate Molecular weight: 365.4 gm Melting point: 326.8 0C Cefalexin is a white to faint yellow powder slightly soluble in water, insoluble in ethanol , chloroform and ether(14) . prepared complexes for β –lactam antibiotics with some metals, and they identify it by spectroscopic and physiochemical methods ,also they studied the reaction of these antibiotics with some transitional elements(15)

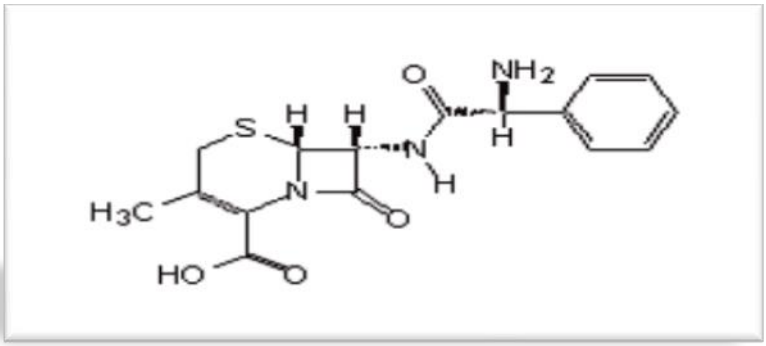

Figure (1): The structureofCefalexin

The cloud point procedure (CPE) is based on the following phenom- enon: an aqueous solution of some surfactant be comesturbid and separatesin to two isotropic phases if some condition such astemperature or pressure is changed or if an appropriate substance is added to the solution [16] The aim of present work was to develop simple, economical, rapid, precise ,accurate and ecofriendly method for determination of single drug by using Cloud Point Extraction

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EXPERIMENTAL PARTS

MATERIALS AND METHODS

Apparatus

- UV-Visible recording spectrophotometer (1986) Shimadzu Model (160A) (Japan) with a response time of 0.1s ,was used for spectrophotomatric determination A quartz cell of 5 ml internal volume and 1cm path length was used for absorbance measurements
- Hotplate Stirrer (Hotplate stirrer Model L-81 Labinco bv)
- Electric Balance (Sartorius, 4digitals, made in Germany)
- Oven (Memmert , maximum temperature 250,made in western Germany.)
- Water Bath **(**A thermostat water Bath, model Unitemp**)**
- Centrifuge (Triup International corp ,TRIU 800 Centrifuge ,made in Korea**).**
- PH-meter (model BP 3001).

Materials

- A pure grade of Cephalexine was obtained from Drug Industries and Midical Appliance (SID) Samarra/ Iraq
- All the chemical stock solution were prepared from analytical grade BDH

Preparation of Standard Solutions:

All glassware was used cleaned with distilled water and dried at 50˚C for 30 minute prior to use. Batch experiments were carried out in to ensure the reproducibility of results and the average value.

All metal used were of the highest purity and most solutions were prepared in distill water.

- A stock solution of 1000 µg ml -1 or (1.4×10-3M) for the drug Cefalexine was prepared by dissolving 0.1g in minimum amount of water and diluted to mark with water in a 100 ml volumetric flask. Then 50 ml of the stock solution was diluted to100 ml with distilled water to Prepare 500 PPM solution.
- A solution of 1000 ppm of Hg +2 was prepared by dissolving 0.3399 gm of Hgcl2 in small amount of Water and complete the volume to 100 ml by using volumetric flask Then 50 ml of the stock solution was diluted to100 ml with distilled water to Prepare 500 PPM solution.
- A solution of 1000 ppm of Ni+2 was prepared by dissolving 4.0483 gm of Nicl2.6H2O in small amount of Water and complete the volume to 1000 ml by using volumetric flask Then 50 ml of the stock solution was diluted to100 ml with distilled water to Prepare 500 PPM solution.
- A 10% (v/v) of Triton X-114 was prepared by diluting 10 ml with water in a 100 ml volumetric flask
- A standard stock solution of sodium hydroxideNaOH (1M) was prepared by dissolving (4g) of the solid product in 100 ml of distill water Then 10 ml of the stock solution was diluted to100 ml with distilled water to Prepare 0.1M solution.

Interference Solutions of 1000 ppm

An amount of 1000 µg ml-1 stock solution of interferences is prepared by dissolving 0.1g of the different organic compound such as [Lactose, Starch, Arabic Gum, Glucose and Talc] and inorganic compound such as [0.2579g, 0.2500g] of Ca3(PO4)2 and CaCO3 respectively in distilled water and diluting them to the mark in 100 ml volumetric flask

Recommended CPE Procedure for Cefalexin Drug

Aliquots 10 ml of a solution containing known amount of Cefalexin drug was mixed with Ni+2or Hg +2ions Then pH was adjusted by using 0.1M NaOHand 10% (v/v) Triton x-100 for complex (CEX-Ni) and Tween20 for complex (CEX- Hg) The mixture was shaken for 1 min and left to stand in a thermo-stated bath at 50 oC, for 10 min. Separation of the phases was achieved by centrifugation at 3000 rpm for 10 min, with stirring at 5°C in ice bath the remaining of micellar phase was dissolved by ethanol, the measurements of absorbance of the complexes were followed by UV-visible spectrophotometer with used 1.0 cm quartz cell at

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λ max equal to 708.nm for CEX - Ni (II) complex and 398 nm for CEX- Hg (II) complex against blank which was prepared in the same way but without drug.

Preparation of Pharmaceutical Samples

Two types of pharmaceuticals for CEX namely capsules and syrup were obtained from the drugstores in Iraq and Amman . The powder of five capsules were mixed, homogenized, and the content of one capsule (0.58727g)which equivalent to 587.2 mg of active drug was dissolved in sufficient amount of water with continuous shaking and filtered. The filtrate solution was transferred into a 100 mL volumetric flask and diluted to mark with water.

solution contains5000µg mL-1 of CEX from which500µg mL-1 was prepared by dilution. 10mL containing different concentrations of the prepared sample solution were transferred to centrifugal tubes and each solution followed the recommended CPE procedure for Cefalexin and the content of drug was measured spectrophotometrically at λ max of 708 and 398nm. the pharmaceuticals for syrup As each (5ml) from drug contains (250mg) Cefalexin . Solution is prepared by taking (0.5ml) from syrup and dissolved in ethanol then solution is filtered and dilute in(50ml) volumetric flask by distilled water, so that it gives (500µg ml-1) from Cefalexin . The same procedure is applied for syrup , CPE procedure for Cefalexin and the content of drug was measured spectrophotometrically at λ max of 708 nm and 398 nm

Statistical Analysis

Excel 2010 (Microsoft Office R) was employed to carry out all statistical calculations

RESULTS AND DISCUSSION

Absorption Spectra

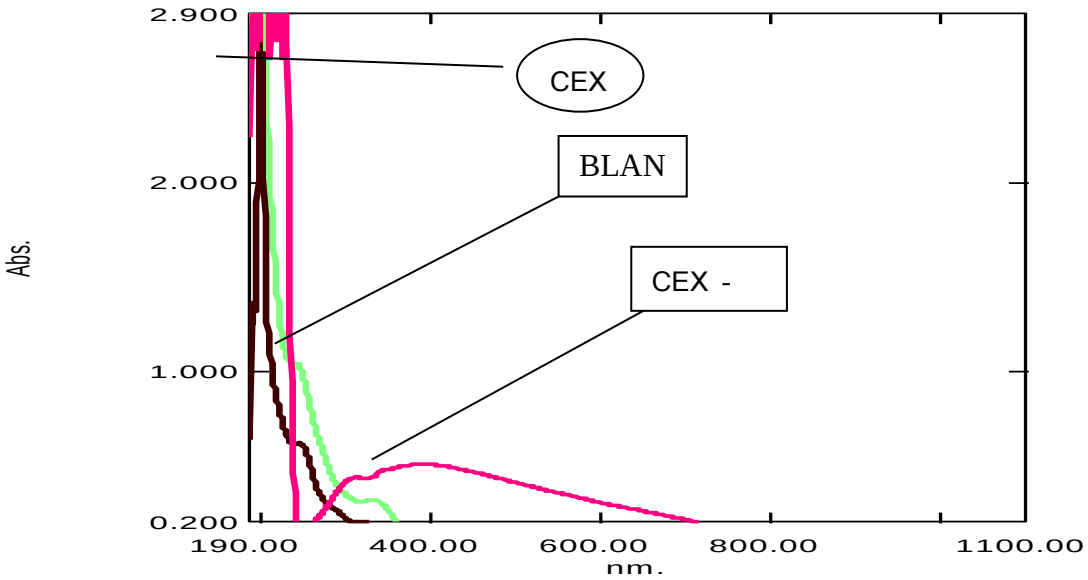

Figure 2: The absorption spectrum of the CEX - Hg (II)complex

In an attempt to ascertain the occurrence of reaction between two Complexes in the reaction system, an absorption maximum at 398 nm (Fig 2) and 708 nm (Fig 3) which was adopted of CPE for the drug . The absorption spectrum of the complex product formed was also recorded against the corresponding metal blank between 200 to 1100 nm before obtaining optimum conditions according to the recommended CPE procedure using a SHIMADZU, Double beam UV-Vis, model UV-1800 with 1.0 cm quartz cell. It was observed that the absorption maximum of the colored product complex of Cefalexin in 1.0 mL of 10% Tween 20 occurred 398 nm, giving the molar absorptivities of 3.5 ×102 L.mol-1.cm-1 for Cefalexin drug with

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mercury and 10% TX-100 occurred708 nm, giving the molar absorptivities of3.3 ×103L.mol-1.cm-1 for Cefalexindrug with nickel respectively . Thus the wavelength maximum at398 nm and 708nm for the Cefalexin complex product was used throughout this study for ppm amounts

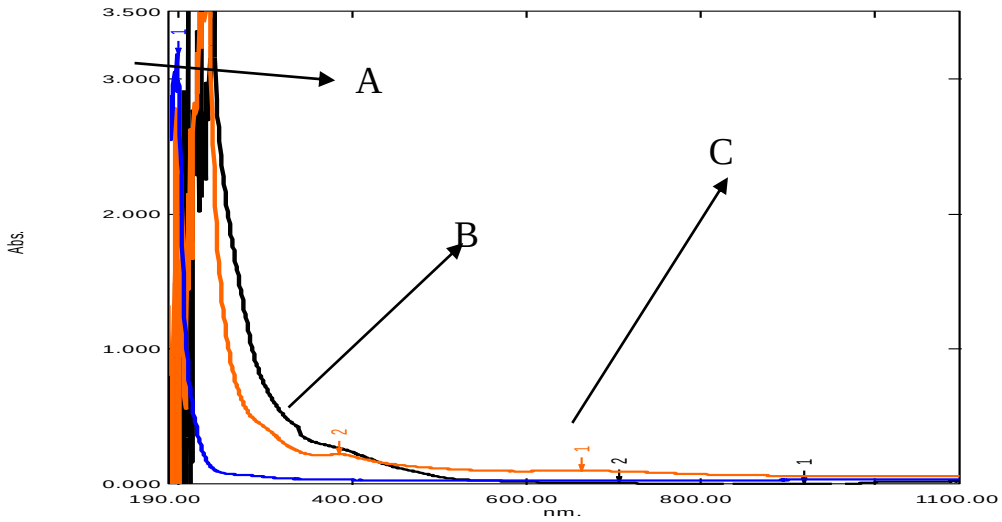

Figure 3: Absorption Spectra of (A) CEX Versus Distilled Water, and (B) metalVersus Distilled Water (C) Complexof the CEX - Ni(II) Against metal Blank

Optimization of CPE Methodology

A group of experiments has been conducted to study the effect of several variables that affect the extraction efficiency of the CPE and maximize the sensitivity of the detection system for drug under study using a classical optimization. The variables such as the concentration of metal ion , bestof pH ,best of buffer ,best of volume buffer, Surfactant amount, equilibration temperature and incubation time.

Effect of Hg (II) andNi (II) ion concentration:

The best concentration for Hg(II) andNi (II)ion is 500 µg /ml, it gave the maximum Absorbance figure (4) show that effect of concentration PPM on absorbance.

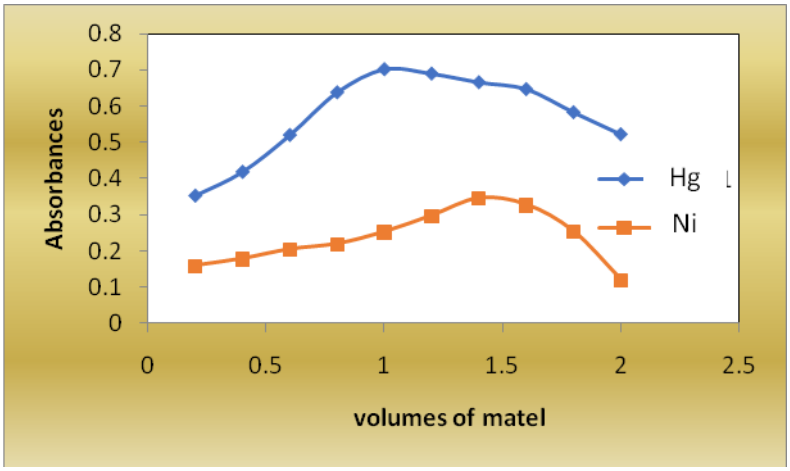

Figure 4: Effect of Optimum volumes Hg (II) andNi (II)ion conc. on absorbance of drug-metal complexes

Effect of pH

The pH plays a unique role on metal -chelate formation and subsequent extraction, and is proved to be a main parameter for CPE**(17)** to find the best acidic function of the ion extraction process different value

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of pH 1-13. The results are shown in Figure (5), the best separation was achieved at pH =11 for Ni(II) and pH=12 for Hg(II). show the value of absorbance intensity for the complexes drug-Hg and drug-Ni against the value of pH , the best values of pH recorded for the highest absorbance values were Plotting of the absorbance values versus the value of pH is shown in fig (5).

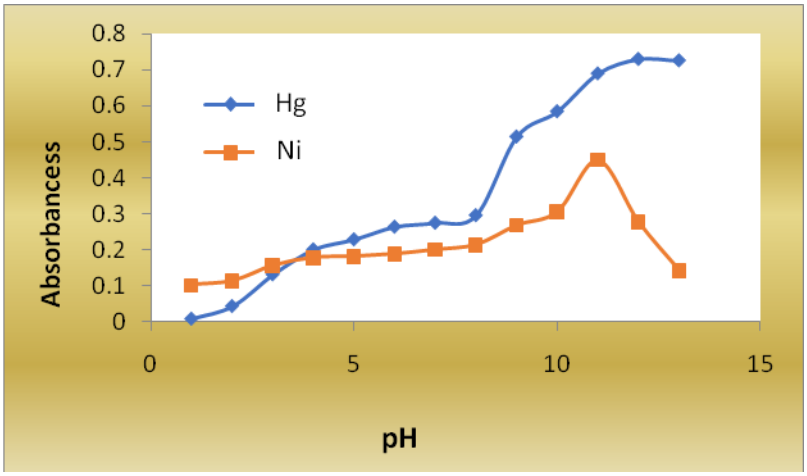

Figure 5: pH effect on the absorbance of drug- Hg (II)and drug -Ni (II)complexe

Effect of buffersolutions

the best values of buffer pH12 recorded for the highest absorbance values were ,The absorbance is measured the absorbance results are shown in table (1).for complexes (Hg+Cefalexin)

Table 1: buffer pH 12

Preparation buffer pH 12	Absorbance
Sodium hydrogen ortho phosphate	0.744
Potassium chloridebuffersolutions	0.540

And the best values of buffer pH 11 recorded for the highest absorbance values were ,The absorbance is measured the absorbance results are shown in table (2).for complexes(Ni+ Cefalexin)

Table 2: buffer pH 11

Preparation buffer pH 11	Absorbance
Sodium bicarbonate buffersolutions	0.535
Sodium hydrogen ortho phosphate	0.549

Effect of Volumes buffer solutions

Fig (6) show the value of absorbance intensity for the complexes drug-Niand drug- Hg against the value of buffer solutions , the best values of Sodium hydrogen ortho phosphatebuffer solutions recorded for the highest absorbance values , the best values of potassium chloridebuffer solutions recorded for the highest absorbance values

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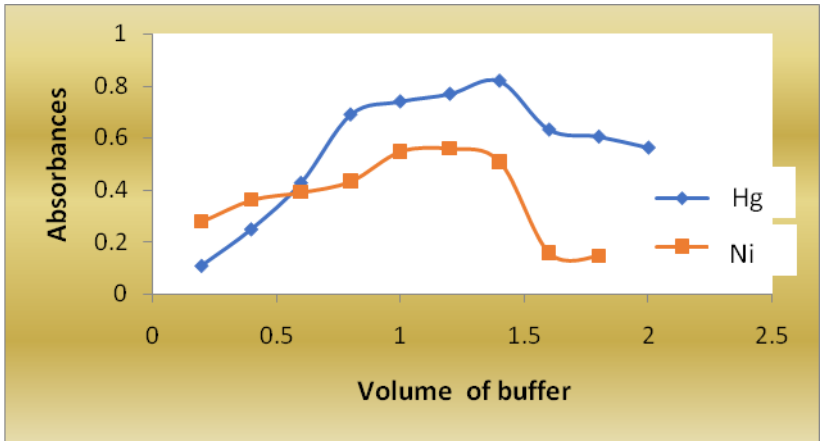

Figure 6: buffer of pH effect on the absorbance of drug- Hg (II)and drug -Ni (II)complexe

Effect Type of Surfactant with Each metal .

The type of surfactant plays very substantial role in cloud point extraction process where each surface owns spectral properties depend on practical basis of Micelles .Aliquots of 10ml of a solution contains [1ml Cefalexin , 1.4ml Ni, 1.2ml buffer pH 11] for nickel metal and [1ml Cefalexin, 1ml Hg, 1.4 ml buffer pH 12] forMercury metal in 10ml volumetric flask and use different surfactant for each drug [Tween 20, Tween80, CTAP, SDS, Triton X-100, Triton X-114] at 500C for 10 min for complex incubation time then it centrifugeted at 3000 rpm for 10min , separated the surfactant- rich phase and dissolved in 1ml ethanol then measured by UV-Vis at **λmax** = 708nm for **Ni** and 398nm for Hg results shown in

Table 3: Effect of surfactant type on Absorbance

Surfactant	CTAB	SDS	Triton ×100	Tween 20	Tween 80	Triton ×114
Absorbance at λmax =708nm for Ni(II)	0.368	0.200	0.589	0.328	0.371	0.364
Absorbance at λmax =398nm for Hg(II)	0.298	0.398	0.270	0.835	0.214	0.123

It was observed that Triton ×- 100 which have maximum absorbance at 708 nm and Tween 20 which have maximum absorbance at 398nm is The best one For Further study as Shown in (Table 3) Plotting the absorbance values of the cloud point versus the type of surfactant is shown in Fig (7)

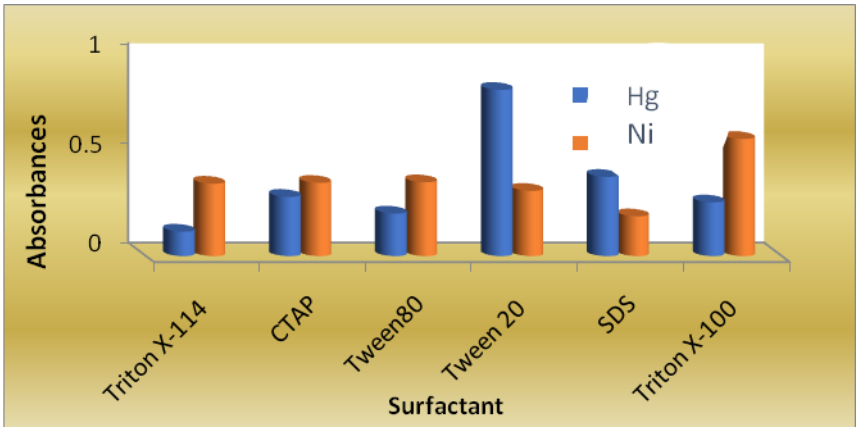

Figure 7 : Type of Surfactant forComplex (drug-Hg and drug-Ni)

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Effect ofVolumes surfactant

Type and concentration of surfactant are importantfactors, in cloud point extraction. Triton X-100 for Complex (drug-Ni)and Tween 20 for Complex (drug-Hg) was chosen as a proper surfactant due to its physic chemical characteristics, commercial availability , relativelylow price, low toxicity, it.s high Density in the surfactant rich phasetheTritonX-100 and Tween 20 volume rangewas examined0.2-1.8 ml and the results are shown in Figure 8.As can be seen the absorbance increasedwith the increase TritonX-100, therefore chose 1ml and 1.2ml for Ni(II)and Hg(II).

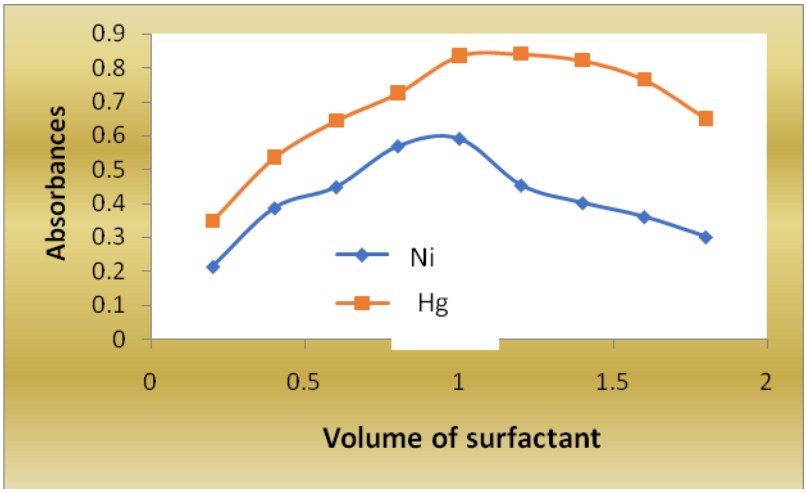

Figure 8: Effect of the TX-110 and Tween20 amount on absorbance of Complexes product [Conditions: For CEX : 50 µg mL-1, metal Ni and metal Hg

Effect of Equilibration Temperature and Incubation Time

The influence of these two parameters is considered of the most crucial steps in CPE, in order to ensure the efficient phase separation, which reflects certainly the magnitude of extraction efficiency of each target analyte. Figure (9)shows the variation on the absorption signal via varying the temperature between 60 to 40oC at 10 min incubation time for both complexes

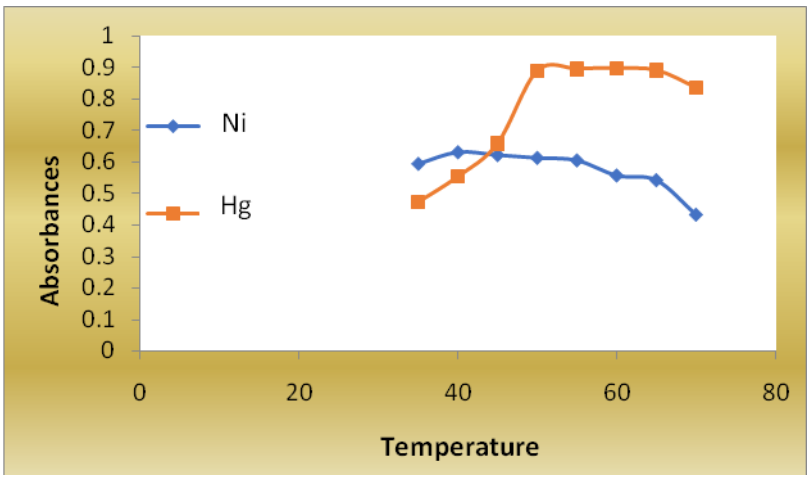

Figure 9: Absorbance Versus Temperature (A) for Ni(II) . (B) for Hg(II)

which proved that the maximum absorption signal of both target analysts was achieved at 40 and 60oC forComplexes (drug-Ni) and (drug-Hg) respectively because of high number of micelles formed in cloud point layer leading the entire transfer of the product into surfactant-rich phase that maximize the sensitivity. A significant decrease of the absorbance response was observed thereafter, probably due to the

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instability or dissociation of product at higher temperature than optimal. 40 and 60°C were selected and used as optimal in the general CPE procedures of both analytes. Figure (10) illustrates the study of varying of incubation time from 5 to 45 min at optimum temperatures of both analytes. It was found that the incubation times of 25 and 30 min were quite enough for the maximum absorption signal of Complexes (drug-Ni) and (drug-Hg) in the product extraction respectively

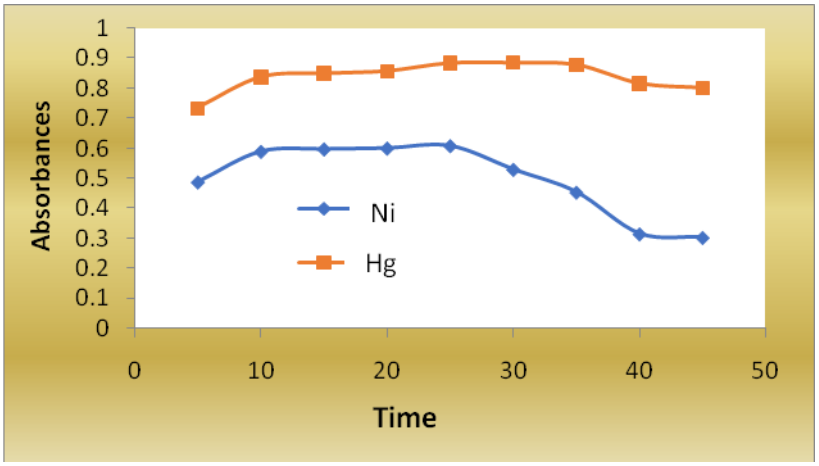

Figure 10: Absorbance Versus Time (A) for Ni (II). (B) for Hg (II)

Order of Additions

The effect of order for additions of the metal on the absorbance of each analyte by the general CPE was tested. Fig (11) shows that the best order of addition is the number 2 and 3 fortarget analytes due to giving a highest absorption signal among the others. The absorbance is measured and the absorbance results are shown in table (4)

Table 4: Data of Absorbance to Order Additions

No	Order Additions	Absorbance at λmax =708nm for Ni (II)	Absorbance at λmax=398nm for Hg(II)
1	D+ M+B+T	0.630	0.893
2	M+D+B+T	0.721	0.414
3	D+B+M+T	0.271	0.948
4	M+B+D+T	0.536	0.757

Plotting of the absorbance values versus the order additions is shown in Fig (11)

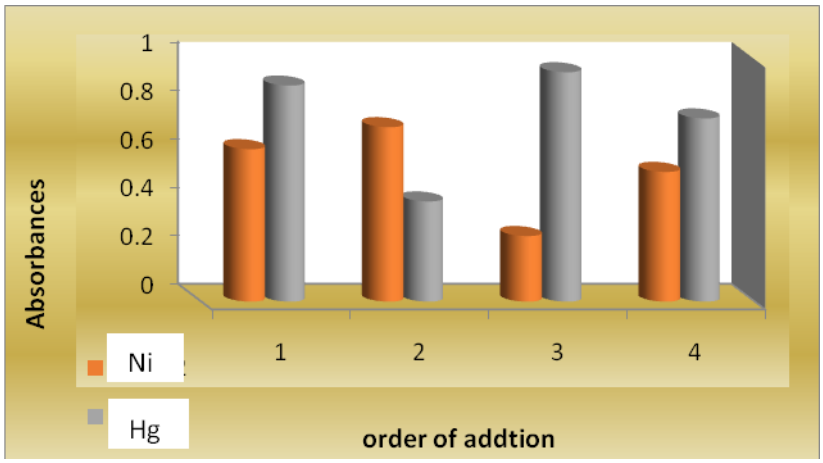

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Figure 11 : Effect of Order Additions. (A) For Ni . (B) For Hg

Effect of organic solvents

Different organic solvents are examined to evaluate their effects on the intensity of the resulting complex and the data are shown in(Table 5).

Table 5: Data of Absorbance to Solvents

No	Solvents	Absorbance at λmax = for Ni	Absorbance at λmax = for Hg
1	Water	0.718	0.947
2	Ethanol	0.565	0.809
4	Acetonitril	0.213	0.216
6	Chloroform	0.157	0.312
7	Acetyl aceton	0.026	0.134
8	Dimethy formamide	0.515	0.367
9	Dimethy phthalate	0.382	0.289
10	Dimethy malonate	0.310	0.245

Plotting of the absorbance values versus the solvent is shown in fig (12)

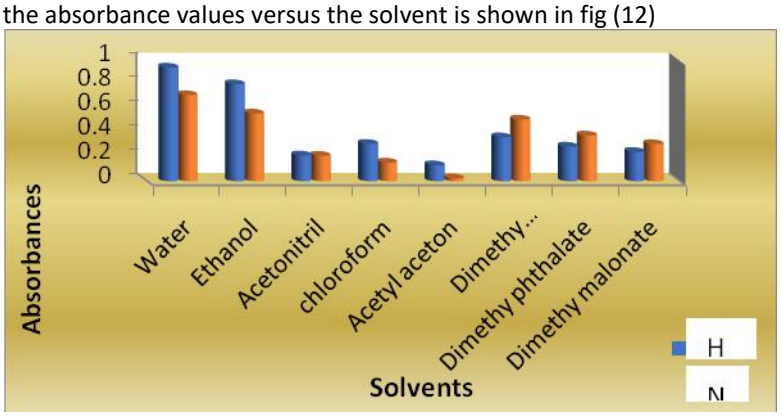

Figure 12 : Effect of Solvents . (A) For Ni . (B) For Hg

It has been shown that water is the optimum solvent , economically , sensitivity method ,cheap price, to provide and nontoxic. This solvent is fixed in subsequent experiment

Effect of Interference

The effect of some foreign organic compounds and Inorganic compounds, which often found in environmental , were studied by adding 1ml of (100ppm) Equal amounts organic compounds, Inorganic compounds to 1ml of (100ppm) of complex . The color was developed following the recommended procedure described earlier

Table 6: Effect of Interference

100ppm interference	Absorbance at λmax = for Ni	Absorbance at λmax = for Hg
With out	0.721	0.947
Lactose	-0.011	0.034

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Starch	0.151	0.219
Arabic Gum	0.123	0.324
Glucose	0.367	0.098
Ca3(PO4)2	0.159	0.167
CaCO3	0.283	0.0087

It was observed that the table 6 were not interfering with the determination at levels found in complex form.

Selected Optimum Conditions

After the study of the effect of different physical and chemical conditions on the absorbance intensity of the colored product,

Table 7 : The optimum conditions for the determination of Cefalexin

Optimum	Concentrations	Range selected	Optimum quantities of complex (CEX--Ni)	Optimum quantities of complex (CEX-Hg)
ƛ max(nm)	----	190-1100	708nm	398nm
Effect of volume of metal ion required	500 ppm	0.2-2.0 mL	1.4mL	1mL
Effect of PH	0.1M(NaoH)	1-14	11	12
Buffer pH	----	------	Sodium hydrogen ortho phosphate	Sodium hydrogen ortho phosphate
Effect of volume of Buffer	----	0.2-1.6mL	1.2mL	1.4mL
Effect of volume of surfactant required	10%(v/v)	0.2 – 1.8mL	Tx-110 1mL	Tween 20 1.2 mL
Effect of time heating	----	5-60 min	25 min	30 min
Effect of temperature	------	35-70°C	40°C	60°C
Cefalexin solution required	500 ppm	5-65 ppm 5-60ppm	1mL	1 mL

the optimum conditions for the proposed procedure were summarized in (Table 7) and were used in all subsequent experiments.

Preparation of Calibration Curve in CPE

Amount of 10ml solution is prepared containing increasing concentration of drug Cefalexin by taking [1.4ml Ni, (5-65) µg ml-1Cefalexin ,1.2ml buffer pH 11and 1ml 10%(v/v)Triton X-100] and for Hg metal take [(5-60) µg ml-1Cefalexin, 1.4 ml buffer pH 12 , 1ml Hgand 1.2 ml 10%(v/v) Tween 20]then it is completed to the mark by distilled water, are mixed ,heated at optimum temperature in the thermostat water bath at optimum incubation time , to form cloud point then aqueous phase is separated by centrifugation at 3000 rpm for 10min ,1ml ethanol is added to the surfactant-rich phase to dissolve it then is measured by UV-Vis at λmax = 708 nm for nickel and at λmax = 398nm for mercury , triplicate manner The absorbance measurements are illustrated in table 8 and table 9

Table 8 : The absorbance measurements of standard solutions of complex (CEX-Ni)

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Conc. ppm	Mean Absorbance	RSD%	Found	Recovery%
5	0.297	0.5831	4.7938	95
10	0.336	0.7874	8.8144	88
15	0.401	0.2493	15.5154	103
20	0.447	0.8066	20.2577	101
25	0.501	0.3046	25.8247	103
30	0.542	0.3690	30.0515	100
35	0.592	0.2925	35.2061	100
40	0.636	0.4159	39.7422	99
45	0.689	0.2513	45.2061	100
50	0.72	0.1388	48.4020	96
55	0.776	0.2232	54.1752	98
60	0.841	0.1815	60.8762	101
65	0.878	0.1972	64.6907	99

The calibration curve was . Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (C EX- nickel) as shown in Fig (14)

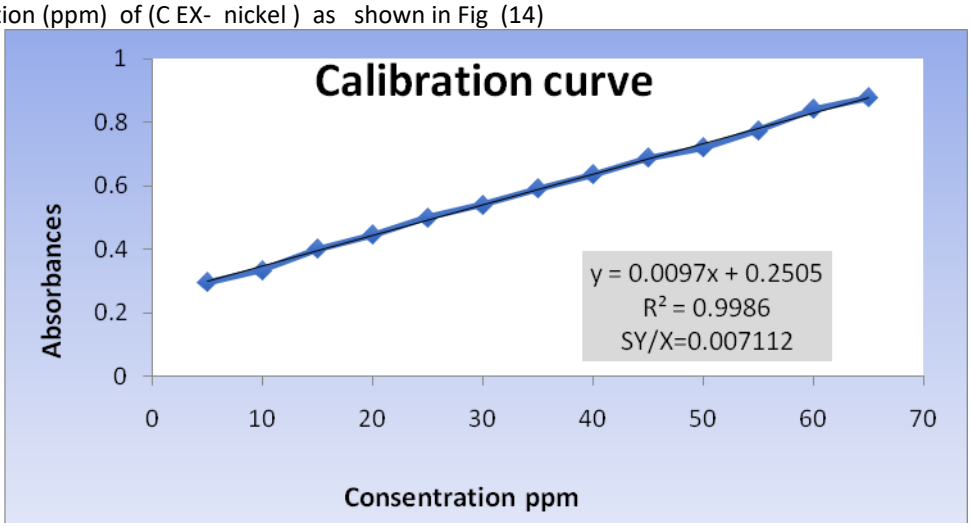

Figure 14 : (Cefalexin + Ni) Calibration Curve

Table 9 : The absorbance measurements of standard solutions of complex (CEX- Hg)

Conc. ppm	Mean Absorbance	RSD%	Found	Recovery%
5	0.134	1.2925	3.7348	74.69
10	0.248	0.4032	10.0331	100
15	0.334	0.2994	14.7845	98
20	0.437	0.3963	20.4751	102
25	0.517	0.3868	24.8950	99
30	0.632	0.4186	31.2486	104
35	0.714	0.1400	35.7790	102
40	0.806	0.3282	40.8618	102
45	0.882	0.2267	45.0607	100
50	0.952	0.3787	48.9281	97
55	1.047	0.1654	54.1767	98

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60	1.151	0.0868	56.9226	99

The calibration curve was . Plotting the mean absorbance values of the cloud point versus the concentration (ppm) of (CEX- Mercury) as shown in Fig (15)

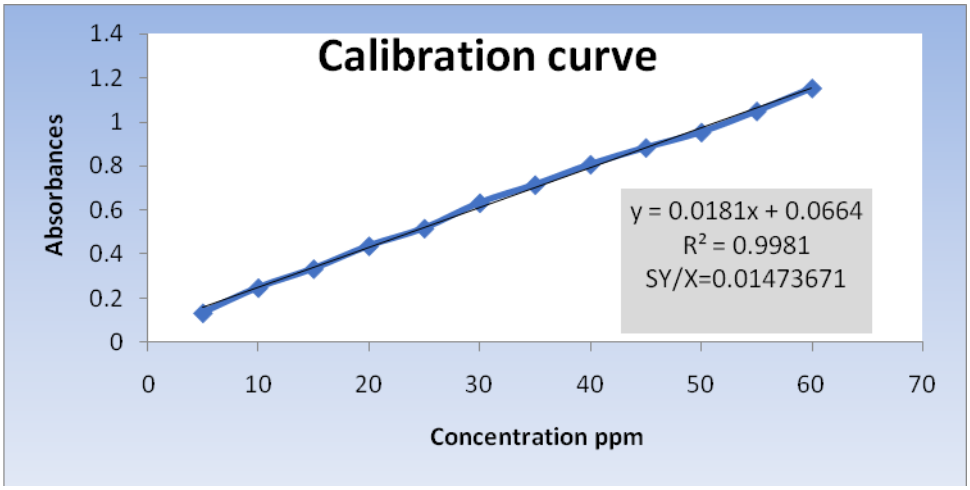

Figure 15 : (Cefalexin +Hg) Calibration Curve

Optical characteristics Features of the calibration curve.

Table 10.shows the main featuresof the calibration curve and measuring the absorbance at nm and nm

Table 10 : Optical characteristic features of calibration curve

Parameter	Complex (Cefalexin + Ni)	Complex (Cefalexin + Hg)
Color of product	Yellow	Green
Wave length λ max (nm)	708nm	398nm
Concentration rang (µg ml-1)	(µg ml-1)	(µg ml-1)
Regression equation	y=0.0097 x +0.2505	y=0.0181x +0.0664
Correlation coefficient(r)	0.9992	0.9990
Correlation coefficient (r2)	0.9986	0.9981
Variation coefficient (%)	99.86	99.81
Limit of Detection (µg ml-1)	0.92783	0.49723
Limit of Quantitation(µg ml-1)	3.09278	1.65745
Sandell's sensitivity (µg cm-2)	0.1030	0.9920
Slope (m)	0.0097	0.0181
Intercept (C)	0.2505	0.0664
Molar absorptivity(L.mol-1.cm-1)	3.3×103	3.5 ×102

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Composition of product	2:1	2:1
C.L for slope (b±tSb) at 95 %	0.0097± 2.3×10-4	0.0181±5.3×10-4
C.L for intercept (a±tSa) at 95 %	0.2505± 9.2×10-3	0.0664±0.01983
C.L for Conc.15 µg ml-1at 95%	15.5154±2.4×10-4	0.334±2.4×10-3
C.L for Conc. 30 µg ml-1at 95%	30.0±4.9×10-3	0.632±6.5×10-3
C.L for Conc. 60µg ml-1at 95%	60.8±3.9×10-3	1.151±2.4×10-3

Stoichiometric Determination of Color complex :

Continuous Variation Method (Job`s method)

A series of (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9) ml of (5×10 -4) mol L-1of the solution that containCefalexinwas pipetteinto each of 10mlvolumetric flask then (0.9,0.8,0.7,0.6,0.5,0.4,0.3,0.2,0.1) ml of (5× 10-4) mol L-1of metal the absorbance of the solution was measured by UV-Vis Spectrophotometerat λ max 708nm and 398nm the stoichiometric ratio betweenCefalexin with metal 2:1 results are shown in the Table(11)

Table 11 : The continuous variation method of Cefalexin with metal (nickel and mercury) complex.

V D mL	V M mL	CD / CT	Absorbance at λ=708 for Color compound(Ni-CEX)	Absorbance at λ=498 for Color compound(Hg-CEX)
0.1	0.9	0.1	0.034	0.0861
0.2	0.8	0.2	0.072	0.103
0.3	0.7	0.3	0.093	0.121
0.4	0.6	0.4	0.115	0.136
0.5	0.5	0.5	0.131	0.149
0.6	0.4	0.6	0.126	0.123
0.7	0.3	0.7	0.102	0.111
0.8	0.2	0.8	0.081	0.093
0.9	0.1	0.9	0.054	0.076

Plotting the value of absorbance versus the CD / CT is shown in Fig (16)

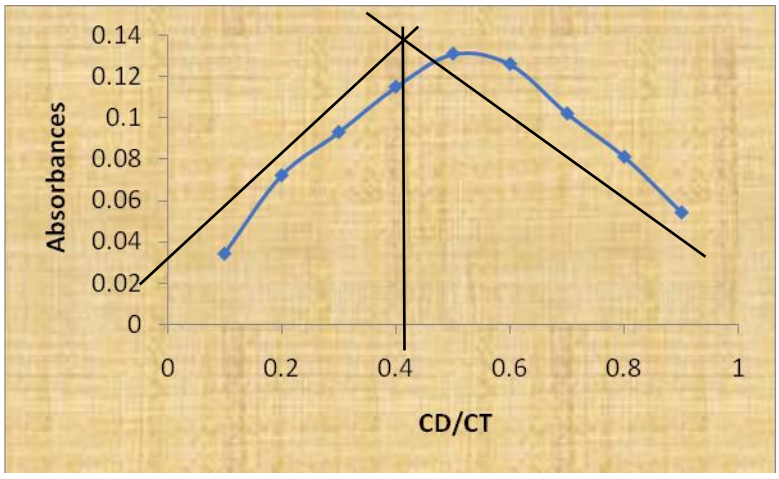

Figure 16: Continuous variation method plo

CD: values of the compound (Cefalexine)
CM: The values of the metal (Nickel).
CT: Total (CM+ CD)

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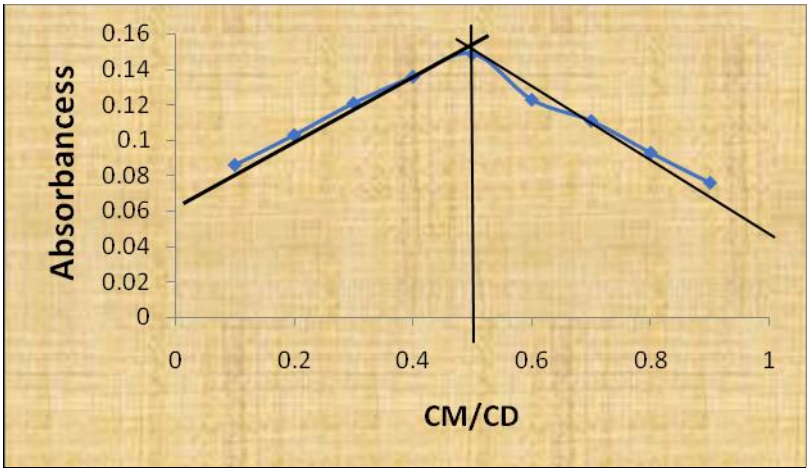

Figure 17: Continuous variation method plot.

CD: values of the compound (Cefalexine)
CM: The values of the metal (mercury)
CT: Total (CM+ CD)

Mole – Ratio Method

Aliquots of 10 mL solution containing (5×10-4) molL-1 of (1mL)Cefalexine and increasing concentrations (5×10-4) mol L-1 of (0.5,1,1.5,2,2.5,3,3.5,4,4.5,5) mL of (Hg) mercury and (Ni) nickel(2.5×10-5—2.4×10-4)mol L-1 metal . The absorbance of the solutions were measured by UV-Vis spectrophotometer versus blank at λ max= 708nm and 498nm the stoichiometric ratio between 2:1 results are shown in the Table (12).

Table 12: The Mole - Ratio Method of the Cefalexine with mercury and nickel

CL	CL / CM	Absorbance at λ=708 for Color compound(Ni-CEX)	Absorbance at λ=498 for Color compound(Hg-CEX)
2.5 ×10-5	0.5	0.023	0.065
5×10-5	1	0.064	0.148
7.5×10-5	1.5	0.098	0.196
1×10-4	2	0.142	0.231
1.25×10	2.5	0.163	0.245
1.5×10-4	3	0.171	0.243
1.75×10-4	3.5	0.185	0.246
2×10-4	4	0.192	0.247
2.25×10-4	4.5	0.198	0.248
2.5×10-4	5	0.206	0.249

Plotting the value of absorbance versus the CL / CM is shown in Fig (18)

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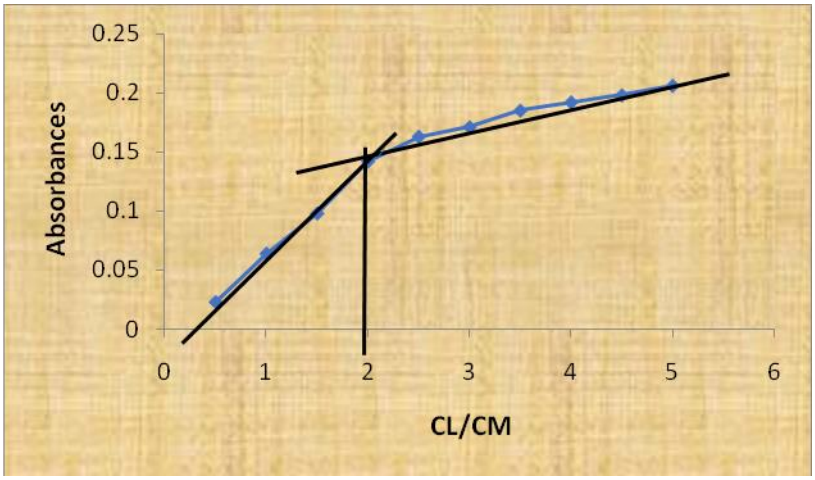

Figure 18: Mole - Ratio plot ofCefalexineand Nickel complex.

CL: concentration of the metal (Nickel)
CM: concentration of the compound (Cefalexine)

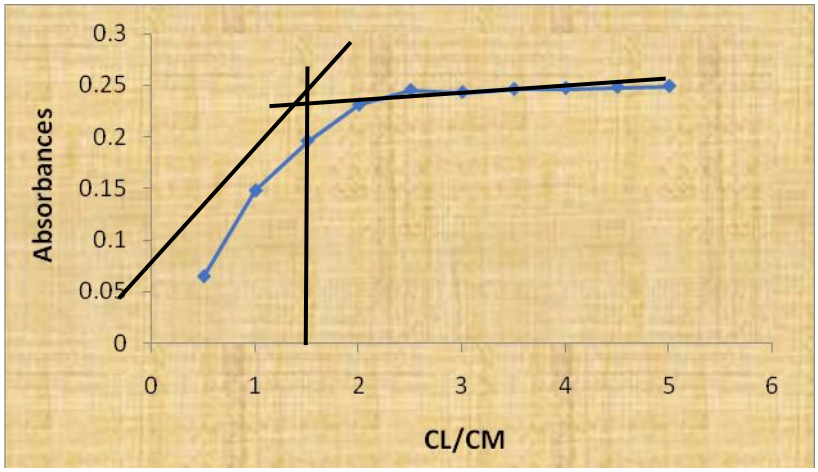

Figure 19: Mole - Ratio plot of Cefalexine and mercury complex.

CL: concentration of the metal (mercury)
CM: concentration of the compound (Cefalexine)

Applications of the Cloud Point Extraction on Pharmaceuticals.

CPE has been applied on pharmaceutical Cefalexin , the manufacture company [Novartis] that contains (587.27 mg) from Cefalexin .The results are good and of high reliability in the analysis of samples in the pharmaceutical preparation. The results are summarized in the table (13) for CEX .

Table 13 : Data for Determination CEX with nickel in the Pharmaceutical Preparation Capsule (Cefalexin) by CPE.

Amount of CEX / µg ml-1	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery%	Average Recovery%	Erel%	Average Erel%
15	0.394	0.6715	14.7938	98.6	99.5	-2	-0.66
35	0.590	0.1514	35	100		0	

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45	0.687	0.2206	45	100		0	

[*]= Average of Five

The proposed method is also applied on syrup Cefalexin the manufacture company is [Novartis]. As each (5ml) from drug contains (250mg) Cefalexin , we get good and high reliability results that are summarized in the table (14) for CEX by CPE.

Table 14: Data for Determination CEX with nickel in the Pharmaceutical Preparation Syrup (Cefalexin by CPE

Amount of CEX / µg ml-1	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery%	Average Recovery%	Erel%	Average Erel%
15	0.402	0.3933	15.6185	104	101	4.1233	1.6798
35	0.583	0.2712	34.278	97		-2.962	
45	0.700	0.2258	46.3492	102		2.9782	

Table 15: Data for Determination CEX with mercury in the Pharmaceutical Preparation Capsule (Cefalexin) by CPE.

Amount of CEX / µg ml-1	Mean absorbance	Relative stander deviation (RSD)	*Found	Recovery%	Average Recovery%	Erel%	Average Erel%
15	0.309	0.7236	14.5082	96	100	-3.278	0.4598
35	0.713	0.2217	35.7237	102		2.0677	
45	0.902	0.1108	46.1657	102		2.5904	

Table 16: Data for Determination CEX with mercury in the Pharmaceutical Preparation Syrup (Cefalexin) by CPE

Amount of CEX / µg ml-1	**Mean absorbance**	**Relative stander deviation (RSD)**	***Found**	**Recovery%**	**Average Recovery%**	**Erel%**	**Average Erel%**
15	**0.322**	**0.4910**	**14.1215**	**94**	**99**	**-5.856**	**-0.4522**
35	**0.698**	**0.2265**	**34.8950**	**99**		**-0.3**	
45	**0.920**	**0.1718**	**47.1602**	**104**		**4.80**	

Stability constant of reaction product

The conditional or apparent stability constant of the2:1 (Drug and metal) product was evaluated and described as shown Complete founding the stability constant [K] colored product Formed imputation of (metal :drug) as followed:

A series of solution were prepared containing three different concentration of metal and Cefalexin(2:1) and the concentration (5×10-4) molL-1 for each (nickel with Cefalexin) and the concentration (5×10-4) molL-1 for(mercury withCefalexin) when Formed imputation under this Condition easily to Hydrolysis and the Intensity Absorption was very low Another series of solution was prepared containing three deferent concentration of metal and Cefalexin but with abundance of the metal (the best concentration) The complex was prepared with no decomposition express of the intensity absorption Am and application the relationship the value degree of decomposition can be calculated as follows (α):

$$\alpha = \frac{A_m - A_s}{A_m}$$

Stability constant [K] as follows ; and calculated the

$$S + R \rightarrow SR$$

$$\alpha c \quad \alpha c \quad (1-\alpha)c$$

$$K = \frac{[SR]}{[S][R]}$$

$$K = \frac{(1-\alpha)c}{(\alpha c)(\alpha c)} = \frac{1-\alpha}{\alpha^2 c}$$

Where: K; stability constant

C; the concentration of the product complex .and it equivalence the concentration of Cefalexin are shown in Table (17)

Table 17: Stability Constant of the complex(Ni+ Cefalexin)

Vol of Cefalexin	Absorbance at λ= 708nm				K (Average) (l.mol-2)
	As	Am	α	K (l.mol-2.)	
0.3	0.195	0.198	0.01515	8.5×106	
0.5	0.342	0.349	0.02005	4.8×106	
0.7	0.521	0.525	0.00761	3.4×107	1.5×107

Table 18: Stability Constant of the complex(Hg+ Cefalexin) formed

Vol of Cefalexin	Absorbance at λ 398nm				K (Average) (l.mol-2)
	As	Am	α	K (l.mol-2.)	
0.3	0.203	0.205	9.75×10-3	2×107	
0.5	0.435	0.438	6.84×10-3	4.2×107	
0.7	0.564	0.568	7.04×10-3	4×107	3.4×107

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