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Spectrophotometric Method for Determination of lead , cobalt and cadmium Ions after preconcentration using Cloud Point Extraction techneque

A thesis Submitted to the council of College of Science- University of Babylon In Partial Fulfillment of the Requirements for the degree of Master in analytical Chemistry

By

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بأشراف

الأستاذ المساعد الدكتور احمد سعدون عباس وتوت

٢٠٢٢ م ١٤٤٤ هـ

يَرْفَعِ اللهُ الذّينَ آمَنوا مِنكُمْ
والذّينَ أوتُوا العِلْمَ دَرَجَاتٍ
واللهُ بِما تَعْمَلُونَ خَبِيرٌ

صدق الله العلي العظيم

سورة المجادلة (الآية١١)

DEDICATION

To my beautiful family, the safe base,

to my aunty who raised me and worked hard and sacrificed for me,

to my dear mother and father,

to my dear brother and sister, to my professors in the Department of Chemistry, to my brother and friend who supported me, Professor Nasr, to those with whom I was linked by lineage.

The fragrance of friendship. . Ward of love to the brothers brought me together in the field of work. . Dear colleagues, to every hand and template that walked with me the path of achievement, to be,,

I dedicate to you a summary of my scientific efforts

Ali Ihssan Hamza

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I also thank the Deanship of the College Science and the head of chemistry department for their efforts throughout the study period and for providing me with the basic requirements of the study.

I would like to thank all the staff at the College of Science / Department of Chemistry.

Finally, I would like to thank all my fellow researchers.

Supervisor *Certification*

I certify that this thesis was prepared under my supervision at the Department of Chemistry in College of Science at University of Babylon ;as A partial fulfillment of the requirements for the degree of Master in science of Chemistry, and this has never been published anywhere .

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In view of the available recommendations, I forward this thesis for debate by the examining committee.

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Sumary

The study was carried out based on one of the most important analytical methods for measuring trace elements in aqueous solutions, which is the cloud point extraction method (CPE), which relies on the methodology of pre-concentration of the analytical elements targeted by micellar systems that arise of non-ionic surfactants (Triton X-114) under certain conditions. 3-anilino -1-phenyl imino thiourea was used as a complexing agent for lead(II), cobalt and cadmium ions. ions were selected were determined in aqueous and real samples by applying the cloud point extraction method in determining these elements in different water sources and comparing the results of the method with other techniques to see the ability of the proposed method to determine these ions specifically. Starting from the spectroscopic investigation of [Pb(II) 3-anilino -1-phenyl imino thiourea] , [Co(II) -3-anilino -1-phenyl imino thiourea] and [Cd(II) 3-anilino -1-phenyl imino thiourea] complexes has been found the λmax at (560,550,525) nm respectively after applying The optimal conditions such pH, concentration of reagent, concentration of TritonX-114, temperature, incubation time, and centrifugation rate and time by using a UV-visible spectrophotometer. The optimal conditions for pH was (5,4.5.0) , concentration of reagent(1×10^{-3}) $mol.L^{-1}$, concentration of TritonX-114(0.6 , 0.8 , 0.4) respectively of 2.5%(volum/volum), temperature(50,45,55)^{o}C, incubation time(15,20,20)min and centrifugation time (20) and centrifugation rate (4000) rpm respectively. In addition, the analytical parameters for the determination of lead (II), cobalt(II) and cadmium ions were obtained using CPE method, and linear calibration curves were obtained at the ranges of (0.06-3),(0.3 -6.0) ,(0.012 -3.0)μg.mL-1 with limit of detection (LOD) (0.23,0.17 , 0.0091) μg.mL-1 and the limit of quantification (LOQ) (0.018, 0.49, 0.02) $\mu g.mL^{-1}$, a relative standard n deviation (RSD) for seven of samples, at (3.0, 6.2,3) $\mu g.mL^{-1}$ was (1.9%) with molar absorption coefficient (27951.28,5630.84, 9273) $L.mole^{-1}.cm^{-1}$ and stoichiometric ratio (M:L) (1:2) respectively. Also, the proposed method was applied successfully for the determination of trace amounts of Pb(II) , Co(II) and Cd(II) in real samples and the data obtained were compared statistically with flame atomic absorption spectroscopy (FAAS) (standard method) using t-test (1.65), F-test (1.08) for determining lead (II) , t-test (1.66), F-test (0.98) for determining Co(II) as these

values are less than of values for T,F critical two-tailed at p= 0.05, results indicate that there are no statistically significant differences at a 95% confidence level, so cloud point extraction method has precision and reliability and can be applied in determining lead (II) ,cobalt (II) and cadmium ions in the aqueous samples

الخلاصه

أجريت الدراسة بالاعتماد على واحدة من أهم الطرق التحليلية لقياس العناصر النزرة في المحاليل المائية وهي طريقة استخلاص النقاط السحابية (CPE) والتي تعتمد على منهجية التركيز المسبق للعناصر التحليلية المستهدفة بواسطة أنظمة micellar. التي تنشأ من المواد الخافضة للتوتر السطحي غير الأيونية (Triton X-114) في ظل ظروف معينة. تم استخدام anilino -1-phenyl imino thiourea- ٣ كعامل معقد لأيونات الرصاص (II) والكوبالت والكادميوم. تم تحديد الأيونات في عينات مائية وحقيقية من خلال تطبيق طريقة استخراج نقطة السحب في تحديد هذه العناصر في مصادر المياه المختلفة ومقارنة نتائج الطريقة مع التقنيات الأخرى لمعرفة قدرة الطريقة المقترحة على تحديد هذه الأيونات على وجه التحديد. بدءًا من الفحص الطيفي لـ [Pb (II) 3-anilino -1-phenyl imino thiourea]،
[Co (II) -3-anilino -1-phenyl imino thiourea] و
[Cd (II) 3-anilino -1phenyl imino thiourea] تم العثور على مجمعات عند λmax عند (٥٢٥،٥٥٠،٥٦٠) نانومتر على التوالي بعد تطبيق الظروف المثلى مثل الرقم الهيدروجيني وتركيز الكاشف وتركيز TritonX-114 ودرجة الحرارة ووقت الحضانة ومعدل الطرد المركزي والوقت باستخدام الأشعة فوق البنفسجية المرئية مقياس الطيف الضوئي. كانت الظروف المثلى للرقم الهيدروجيني (٥،٤،٥،٠) ، تركيز الكاشف (١ × ١٠-٣) مول لتر -١ ، تركيز TritonX-114 (٠،٦ ، ٠،٨ ، ٠،٤) على التوالي ٢،٥٪ (حجم / حجم).) ودرجة الحرارة (٥٥،٤٥،٥٠) درجة مئوية ، وقت الحضانة (١٥،٢٠،٢٠) دقيقة ووقت الطرد المركزي (٢٠) ومعدل الطرد المركزي (٤٠٠٠) دورة في الدقيقة على التوالي. بالإضافة إلى ذلك ، تم الحصول على المعلمات التحليلية لتقدير أيونات الرصاص (II) والكوبالت (II) والكادميوم باستخدام طريقة CPE ، وتم الحصول على منحنيات المعايرة الخطية في نطاقات (٠،٠٦-٣) ، (٠،٣ -٦،٠) ، (٠،٠١٢ -٣،٠) ميكروغرام مل -١ مع حد الكشف (LOD) (0.23، 0.17، 0.0091) ميكروغرام مل وحد القياس الكمي (0.018) (LOQ، 0.49، 0.02) ميكروغرام مل ، الانحراف المعياري النسبي (RSD) لسبع عينات ، عند (٣،٠ ، ٦،٢ ، ٣) ميكروغرام / مل -١ كان (١،٩٪) مع معامل الامتصاص المولي (٢٧٩٥١،٢٨ ، ٥٦٣٠،٨٤ ، ٩٢٧٣) لتر مول -١. سم-١ ونسبة القياس المتكافئ (M: L) (١: ٢) على التوالي. أيضًا ، تم تطبيق الطريقة المقترحة بنجاح لتحديد كميات التتبع من Pb (II) و Co (II) و Cd (II) في عينات حقيقية وتمت مقارنة البيانات التي تم الحصول عليها إحصائيًا مع التحليل الطيفي

للامتصاص الذري للهب (FAAS) (الطريقة القياسية) باستخدام اختبار t (١,٦٥) ، اختبار F (١,٠٨) لتحديد الرصاص (II) ، اختبار t (١,٦٦) ، اختبار F (٠,٩٨) لتحديد Co (II) نظرًا لأن هذه القيم أقل من قيم تشير النتائج T، F الحرجة ثنائية الذيل عند $p = 0.05$ ، إلى عدم وجود فروق ذات دلالة إحصائية عند مستوى ثقة ٩٥٪ ، لذلك تتميز طريقة استخراج النقاط السحابية بالدقة والموثوقية ويمكن تطبيقها في تحديد الرصاص (II) ، والكوبالت (II) وأيونات الكاديوم في العينات المائية

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Abbreviations

Item	Description
AAS	atomic absorption spectrometry
AQ	aqueous phase
CPE	cloud point extraction
CCB	cocamidopropyl betaine
CPP	Cloud point preconcentration
CMC	Critical micelle concentration
CAPB	cocamidopropyl betaine
CPT	cloud point temperature
CVAFS	cold vapor atomicv fluorescence spectrometry
CPC	Cetyl pyridinium chloride
CMC	Critical micelle concentration
CHC	Critical hydrogen-bonding concentration
CP	Cloud point
CPE/GFAAS	Cloud point extraction/Graphite furnace atomic absorption spectrometry
CPT	Cloud point temperature
CVC	Critical vesicle concentration
DA	dimethyl arsenate
DDTP	O,O-diethyldithiophosphate
ETAAS	Electrothermal atomic absorption spectrometry
FAAS	Flame atomic absorption spectrometry
HLB	Hydrophilic-lipophic balance

HOCs	hydrophobic organic compounds
HPLC–UV	High preform liquid chromatography/ultraviolet
HPLC	High performance liquid chromatography
HPLC-ICP-MS	High performance liquid chromatography-inductively coupled plasma-mass spectrometry
ICPAES	inductively coupled plasma atomic emission spectrometry
ICPOES/MS	inductively coupled plasma optical emission spectrometry /mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
LLE	liquid- liquid extraction
MME	micelle - mediated extraction
MVT	micelle to vesicle transition
RPM	Round per miute
SPE	solid –phase extraction
SRP	surfactant rich phase
SDS	Sodium dodecyl sulphate
THF	Tetra hydro furan
UV-VIS	Ultraviolet-visible spectrometry

Sumary

The study was carried out based on one of the most important analytical methods for measuring trace elements in aqueous solutions, which is the cloud point extraction method (CPE), which relies on the methodology of pre-concentration of the analytical elements targeted by micellar systems that arise of non-ionic surfactants (Triton X-114) under certain conditions. 3-anilino -1-phenyl imino thiourea was used as a complexing agent for lead(II), cobalt and cadmium ions. ions were selected were determined in aqueous and real samples by applying the cloud point extraction method in determining these elements in different water sources and comparing the results of the method with other techniques to see the ability of the proposed method to determine these ions specifically. Starting from the spectroscopic investigation of [Pb(II) 3-anilino -1-phenyl imino thiourea] , [Co(II) -3-anilino -1-phenyl imino thiourea] and [Cd(II) 3-anilino -1-phenyl imino thiourea] complexes has been found the λmax at (560,550,525) nm respectively after applying The optimal conditions such pH, concentration of reagent, concentration of TritonX-114, temperature, incubation time, and centrifugation rate and time by using a UV-visible spectrophotometer. The optimal conditions for pH was (5,4.5.0) , concentration of reagent(1×10^{-3}) $mol.L^{-1}$, concentration of TritonX-114(0.6 , 0.8 , 0.4) respectively of 2.5%(volum/volum), temperature(50,45,55)^{o}C, incubation time(15,20,20)min and centrifugation time (20) and centrifugation rate (4000) rpm respectively. In addition, the analytical parameters for the determination of lead (II), cobalt(II) and cadmium ions were obtained using CPE method, and linear calibration curves were obtained at the ranges of (0.06-3),(0.3 -6.0) ,(0.012 -3.0)µg.mL-1 with limit of detection (LOD) (0.23,0.17 , 0.0091) µg.mL-1 and the limit of quantification (LOQ) (0.018, 0.49, 0.02) $\mu g.mL^{-1}$, a relative standard n deviation (RSD) for seven of samples, at (3.0, 6.2,3) $\mu g.mL^{-1}$ was (1.9%) with molar absorption coefficient (27951.28,5630.84, 9273) $L.mole^{-1}.cm^{-1}$ and stoichiometric ratio (M:L) (1:2) respectively. Also, the proposed method was applied successfully for the determination of trace amounts of Pb(II) , Co(II) and Cd(II) in real samples and the data obtained were compared statistically with flame atomic absorption spectroscopy (FAAS) (standard method) using t-test (1.65), F-test (1.08) for determining lead (II) , t-test (1.66), F-test (0.98) for determining Co(II) as these values are less than of values for T,F critical two-tailed at p= 0.05, results indicate that

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chapter

one

introduction

1.Introduction

1.1 Cloud Point Extraction (CPE)

Water is an important and necessary resource for humans and their development [1]. Rapid industrial development has resulted in water pollution and deterioration of its quality [2], Industrial pollutants can cause severe damage to overall health and ecosystems [3]. Among the many industrial pollutants, lead is one of the most toxic and dangerous to human health [4]. The danger of lead is that it is highly toxic even in very low concentrations, in addition to its high stability, not biodegradable and the possibility of accumulation in living organisms during the food chain [5,6]. Exposure to lead causes many diseases: including cancer, anemia, mental retardation, kidney disease, nervous and reproductive system diseases [7]. Due to its harm to humans and the environment, it was essential to use effective techniques to determine the lead content in aqueous samples.

Cloud point extraction (CPE) is based on the phase behavior of non-ionic surfactants in aqueous solution , which exhibit phase separation after an increase in temperature or the addition of a salting out agent and use the centrifuge . Separation and preconcentration based on (CPE) are becoming an important and practical application of surfactant in analytical chemistry [8,9] . This method is also known as micelle-mediated extraction (MME) for its dependence on the formation of the micelle system to the preconcentration for the purpose of separation processes [10].

The classical liquid–liquid extraction and separation methods are usually time consuming and labor extensive and require relatively large volumes of high purity solvents. Of additional concern is disposal of the solvent used, which creates a severe environmental problem. Cloud point extraction (CPE) is an attractive technique that reduces the consumption of and exposure to a solvent, disposal costs and extraction time [11–18]. Cloud point methodology has been used for the extraction and

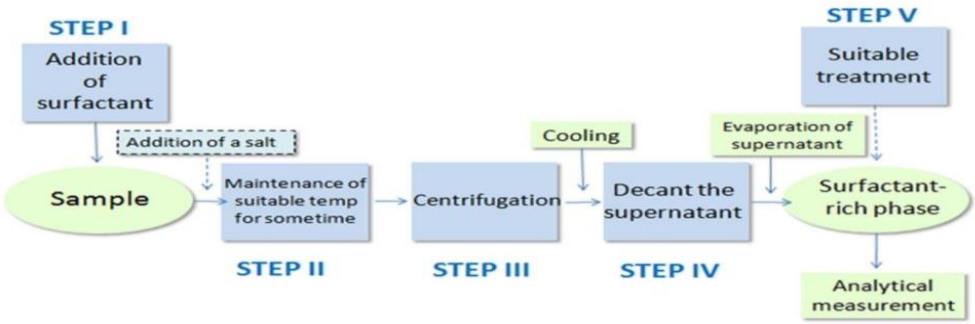
preconcentrations of metal ions after the formation of sparingly water soluble complexes[19,20].

In CPE, micelles are formed at a certain concentration of non-ionic surfactant after exposing the system to a temperature above a certain temperature called cloud point temperature (CPT) (21) . The temperature causes the system to become cloudy because the solution turns into two phases; the micellar and aqueous phases (22) .

The whole process in {CPE }includes the following steps :-

(1) Adding of salt out
(2) Adjustment pH of solution
(3) Incubation for clouding
(4) Centrifugation process
(5) Cooling
(6) Separation of SRP for analysis
(7) Pre-treatment of SRP
(8) Instrumental analysis procedure

The key steps in cloud point extraction are depicted in Fig.(1-1) . It is very difficult to remove aqueous surfactant phase from micellar phase which contained isolated analytes. However, the separation is followed by cooling in which viscosity of the micelles increases and then super-natant decanted, figure (1-1) shows all the above steps when applied to the sample (23).



Figure(1-1) Key steps in cloud point extraction.

CPE method has several advantages, including: a simple experimental procedure (as it combines in only one stage the process of formation of complex and micelles) [24], capacity to undertake a variety of analyzes [25], inexpensive [26], with high enrichment factor, most importantly, it uses a low-toxic surfactant (less consumption of organic solvents) [27], so it's an environmentally friendly technique and causing little pollution [28], i.e. CPE is consistent with the principle of green chemistry that pushes towards preserving the environment by reducing the environmental impact chemicals and their products [29].

There are many Advantages for Cloud Point extraction [30] :-

1) Requires a very small amount of relatively non-flammable and non-volatile surfactants.

2) Safety,

3) No use of cartridges and special equipments, hence inexpensive.

4) Because of the very small volume fraction of the surfactant rich phase, the analytes can be highly concentrated, thus enhancing the sensitivity of chromatographic analysis.

5) Easy disposal of surfactants,

6) Low toxicity,

7) No use of organic sovents as in LLE, hence ecofriendly.

8) Simple procedure .

1.2 surfactant :-

Some compounds, like short-chain fatty acids, are amphiphilic or amphipathic, i.e., they have one part that has an affinity for nonpolar media and one part that has an affinity for polar media. These molecules form oriented monolayers at interfaces and show surface activity (i.e., they lower the surface or interfacial tension of the medium in which they are dissolved). In some usage surfactants are defined as molecules capable of associating to form micelles. These compounds are termed surfactants, amphiphiles, surface-active agents, tensides, or, in the very old literature, paraffin chain salts.

The most commonly used term, surfactant, was originally registered as a trademark for selected surface-active products[31] and later released to the public domain.[32] Soaps (fatty acid salts containing at least eight carbon atoms) are surfactants. Detergents are surfactants, or surfactant mixtures, whose solutions have cleaning properties. That is, detergents alter interfacial properties so as to promote removal of a phase from solid surfaces.

The unusual properties of aqueous surfactant solutions can be ascribed to the presence of a hydrophilic head group and a hydrophobic chain (or tail) in the molecule. The polar or ionic head group usually interacts strongly with an aqueous environment, in which case it is solvated via dipole–dipole or ion–dipole interactions.[33,34]

Figure (1-2) below shows the hydrophobic (head) and hydrophilic (tail) parts in surfactant molecule [35].

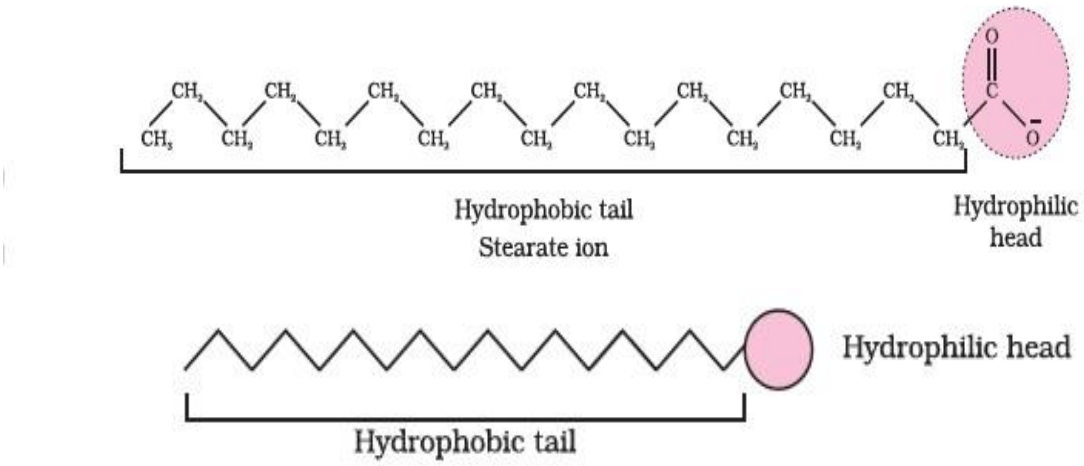


Figure (1-2): the hydrophobic and hydrophilic parts of the structure

1.2.1 Principles of surfactant-mediated extraction and CPE

In CPE, the role of extraction solvent is played by the micellar (surfactant-rich) phase originating from a homogenous surfactant solution that is added to the sample. In aqueous dispersion media, the surfactant aggregate orientates its hydrocarbon tail towards the centre to create a non-polar core. Hydrophobic compounds, which include many bioactive compounds, present in the aqueous solution are isolated and partitioned in the hydrophobic core of the micelles [36,37]. With a decrease in the number of polyethoxylate groups (Ethylene Oxide number) or an increase in the alkyl carbon number, intermicellar attractive forces increase and the cloud point decreases [38].

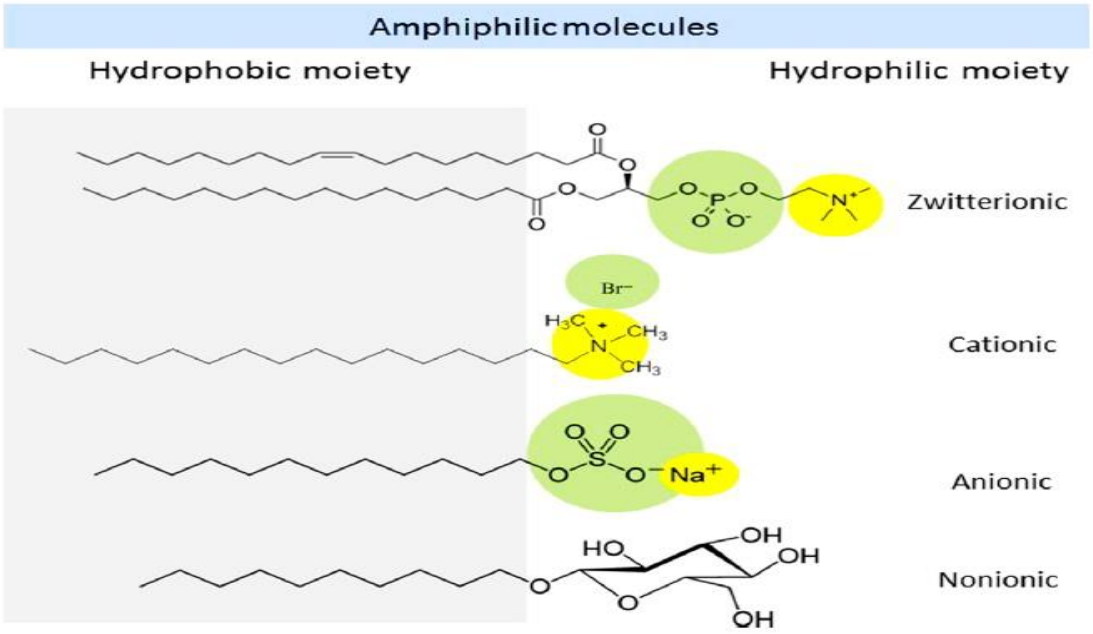
In the aqueous solution, the unique structure of the surfactant allows for sparingly soluble or water insoluble substances to be solubilized because they can associate and bind to the micellar assembly. The interactions between the surfactant and analyte may be electrostatic, hydrophobic or a combination of both. When a surfactant solution is heated over acritical temperature, the solution easily separates into two distinct phases: one contains the surfactant at a concentration below or equal to the CMC, and the other is a surfactant-rich phase. The hydrophobic compounds initially present in the solution and bound to the micelles are extracted into the surfactant-rich phase. This

phenomenon is particularly obvious for polyoxyethylene surfactants and can be attributed to the two ethylene oxide segments in the micelle, which repel each other at low temperature when they are hydrated and attract each other when the temperature increases because of dehydration.[39-41]

1.2.2 Classification of Surfactants

The structure of the surfactant is distinguished by its amphibious nature [35], as it has a hydrophobic tail group (non-polar) and a hydrophilic head group (polar) [42]. The main groups in surfactants give an important advantage in the process of self-assembling monomers and forming various structures such as the assembly of micelles [43].

Surfactant can be classified into four broad categories: anionic, cationic, amphoteric and nonionic . that illustrated in (Figure 1- 2) below [44]



Figure(1-3) : Categorization of surfactants

1.2.2.1 Anionic Surfactants

Particularly, the functional head group in this type of surfactant carries a negative charge at dissociate in the aqueous solution. Because it is finally ionized to a

hydrophilic radical and cation. The cation commonly, is an alkaline ion (Sodium ion, potassium ion or a quaternary ammonium) [45, 46]. Functional the head groups are sulfonate group, phosphate group, sulfate group, and carboxylate group, have this class a straight tail of saturated or unsaturated aliphatic chains ranging in length from (C12- C18). Its solubility varies depending on the unsaturated bonds it possesses [47]. Due to their low cost and easy production process, anionic surfactants have wide applications, and are one of the most commonly used types among other surfactants [48], among which is sodium dodecylbenzene sulfonate (SDBS) which is used to cleanse organic food from microbes [43]. Figure (1-4) represents the groups of some common anionic surfactants and their chemical composition [49].

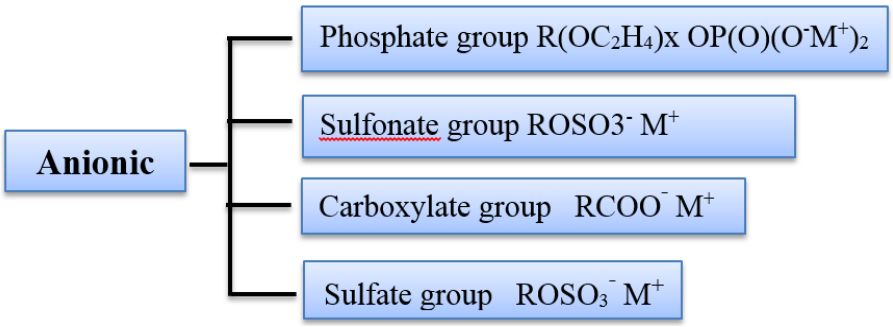


Figure (1-4): Main types of anionic surfactants groups
R=hydrophobic or tail group, M^+ =counter cation.[49]

1.2.2.2 Cationic surfactants:

Cationic surfactants have a positively charged hydrophilic head group [50], may be permanent or dependent on the pH of the solution. In water, these compounds dissociate into an amphibious and anionic part. Anions are usually halogens while the cationic part consist from quaternary ammonium. Compounds of quaternary ammonium the most common categories of cationic surfactants, often called quaternary ammonium salts and only formed in the presence of anion as shown in Figure (1-5) [51].

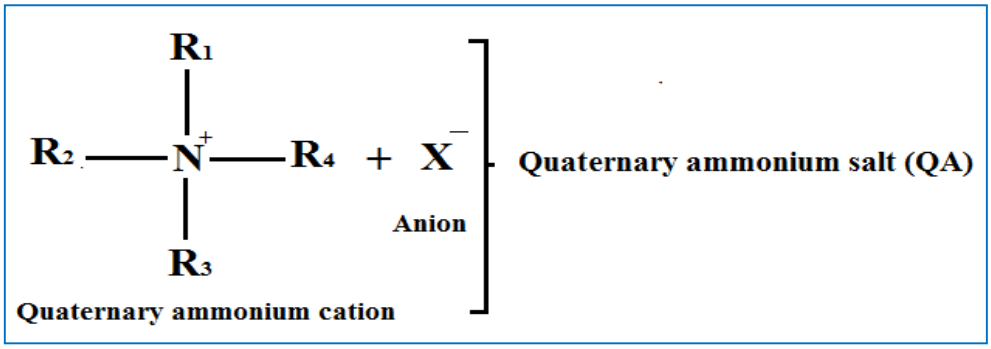


Figure (1-5): General structure of cationic surfactants, (R) Alkyl or aryl groups or hydrogen atoms and (X^-) Anions needed to form salt.

On the other hand, nitrogen compounds - primary, secondary and tertiary amines - are which carry positive charge depending on the pH of the aqueous solution and belongs to this category [52]. Figure (1-6) commonly compounds from this class [50].

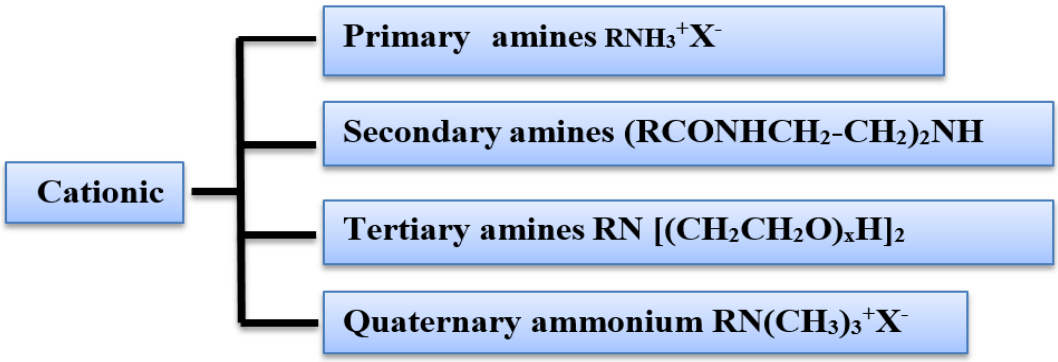


Figure (1-6): Main types of Cationic surfactants groups
(R) Hydrophobic or tail group, (X) counter ion.

Most cationic surfactants usually contain straight alkyl chain (s) with lengths between 8 and 24 carbon atoms. Compared to non-anionic and anionic surfactants, cationic surfactants are more toxic, expensive and in spite of that essentially irreplaceable in some specific industrial applications such as textile softening, textile industry, biocides, road construction, anti-corrosion, disinfectants, biocides, emulsifiers, hair conditioning, and cosmetic industry [53, 54].

1.2.2.3 Zwitterionic surfactants:

Zwitterionic surfactants are characterized by having two different charges on the molecule depending on the pH in the solution [55]. The hydrophilic head group is relatively large and shows more affinity with water and it can be used with other classes of surfactants to change the properties of solubility and micelle size [56]. The molecule's two charges increase the importance of it in giving distinctive properties to micelle as the ability to withstand reaction conditions such as high temperatures and a high salinity environment, allowing them to enter oilfield applications, usually, the positive charge of the main groups in a molecule is a positive quaternized ammonium ion or a phosphonium ion, and can be the groups are negatively charged such as a carboxylate, sulfonate or sulfonate. In addition, the tail group (hydrophobic) for surfactants of this type have a straight chain as in the cocamidopropyl betaine (coco betaine) as shown in Figure (1-8) or a long chain of alkyl groups such as a fatty acid [57].

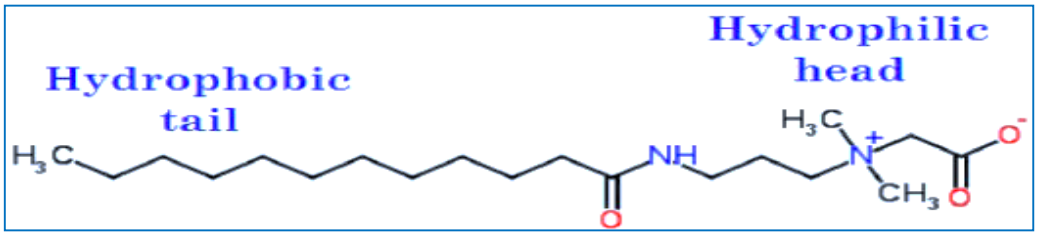


Figure (1-7): Chemical structure of the amphoteric surfactant shows the presence of the two charges on the molecule, (cocamidopropyl betaine (CAPB)).

Therefore the main type of zwitterionic is showing in figure (1 -7) below[58].

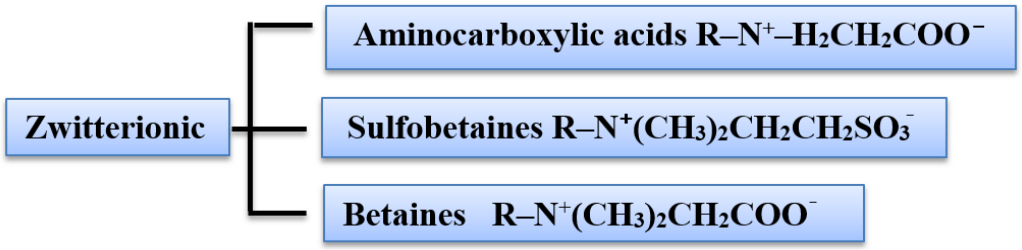


Figure (1-8): Main types of Zwitterionic surfactants groups

(R) Hydrophobic or tail group .

1.2.2.4 Nonionic surfactants:

Non-ionic surfactants are characterized by the fact that it no charges and have a head group hydrophilic [44]. However, when are disintegrated in some aqueous solutions may appear a charge such as tertiary amine oxides depending on the pH for solutions. Polyethylene oxides are also nonionic surfactants, they carry a positive charge in acidic media, and it is appearing in cationic form. Carboxylic acids with a long chain are considered uncharged in neutral aqueous media, but they appear as anionic in alkaline solutions [44]. Figure (1-8) appear some important non-ionic surfactants [47].

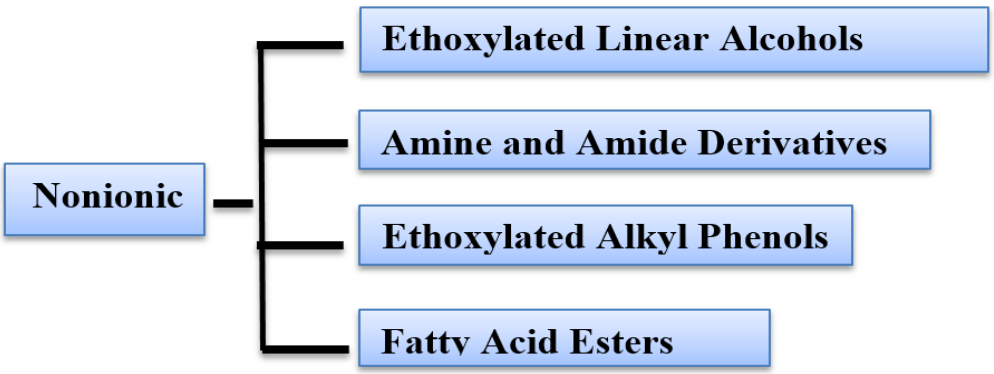
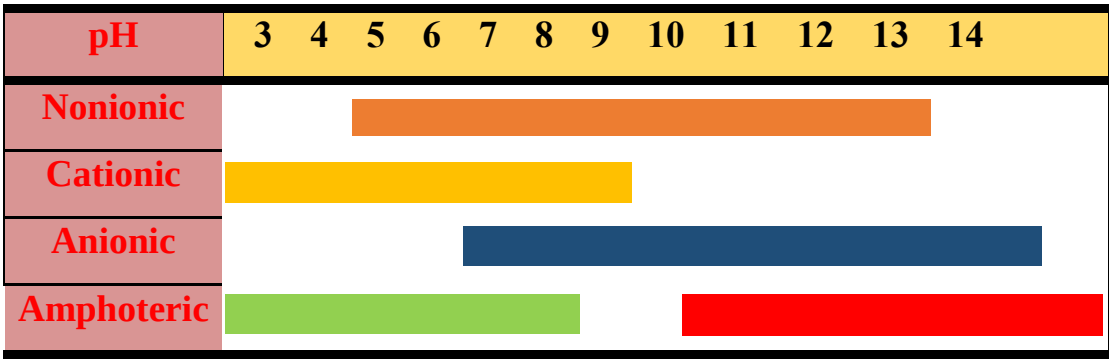


Figure (1-9): The main types of non-ionic surfactants

Due to low toxicity non-ionic surfactants [58], this type is considered one of the most popular surfactants and It is characterized by good foaming, as well as it has low values of critical micelle concentration compared to the other classes [59]. Being non-charged compounds and thus are different from other surfactant classes, they have great compatibility with other types and are an ideal choice for creating micellar mixtures with different properties that can be incorporated in many applications, industrial products, etc. such as their use as emulsifiers or wetting agents and in the production of household cleaners [60].

The weak anionic groups show a limited solubility in an alkaline medium only, and it is observed that the cationic groups (weak) possess solubility in acidic solutions, while the pH ranges of the solubility for non-inionic groups have a wide range, the reason for this is the possibility of forming hydrogen bonds, while amphoteric groups with positive and negative charges have solubility at low and high pH ,Table (1-1) shows the appropriate pH values for each type [61].

Table (1-1): Surfactant Solubility Ranges.



1.2.3 Physicochemical properties of surfactants

Fundamental property of surface active agents is that monomers in solutions tend to form aggregates, called micelles or in other words, in aqueous solution, molecules having both polar and non polar regions form aggregates called micelles. [62] In a micelle, polar heads form an outer shell in contact with water, while non polar tails are sequestered in the interior. Hence, the core of a micelle, being formed of long nonpolar tails.[63]

1.2.4. Properties of Nonionic Surfactants

Non-ionic surfactants are characterized by good solubility in water due to the ability of the hydrophilic part to establish hydrogen bonds with water molecules, while their viscosity decreases when the solution is heated as a result of the destruction of those relatively weak bonds, where it easily ends up forming a cloud state can be separated into two phases, the cloud point (CP) degree of non-ionic surfactants

changes when using some additives, for example but not limited to the use of added salts to improve separation efficiency. These additives lead to a change in the activity of the cloud state of the non-ionic surfactant , so it is necessary to monitor these changes in the presence of these different effects on micelle process for non-ionic surfactants [64, 65] . The effect of non-ionic surfactants with CPT is a distinctive and useful property compared to other types of surfactants, and for this reason, it can be used in many uses as suspensions, as a dilution agent in many pharmaceutical preparations, and as emulsifiers or ointments, a foaming agent, as a detergent, and can be used as a solubility enhancer [66] . In CPT, Triton X-114 is the most used non-ionic surfactant due to its physical and chemical properties that set it apart from other compounds, such as having low CPT values ranging from 23-25 ° C in addition to a high density of SRP [67] Figure (1-9) represents the chemical structure of Triton X-114 [68]

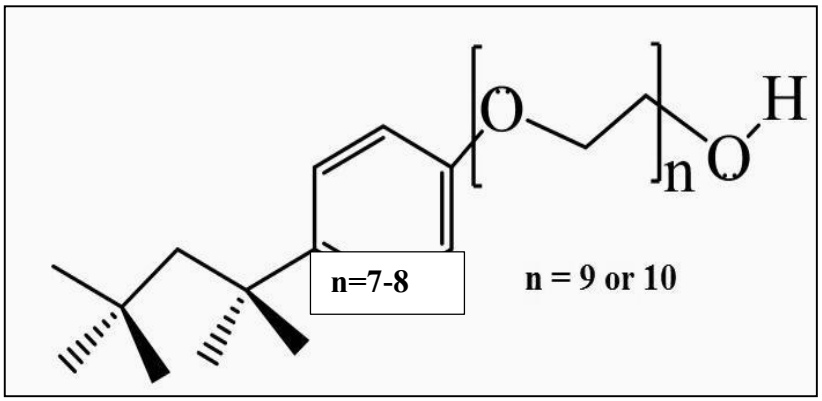
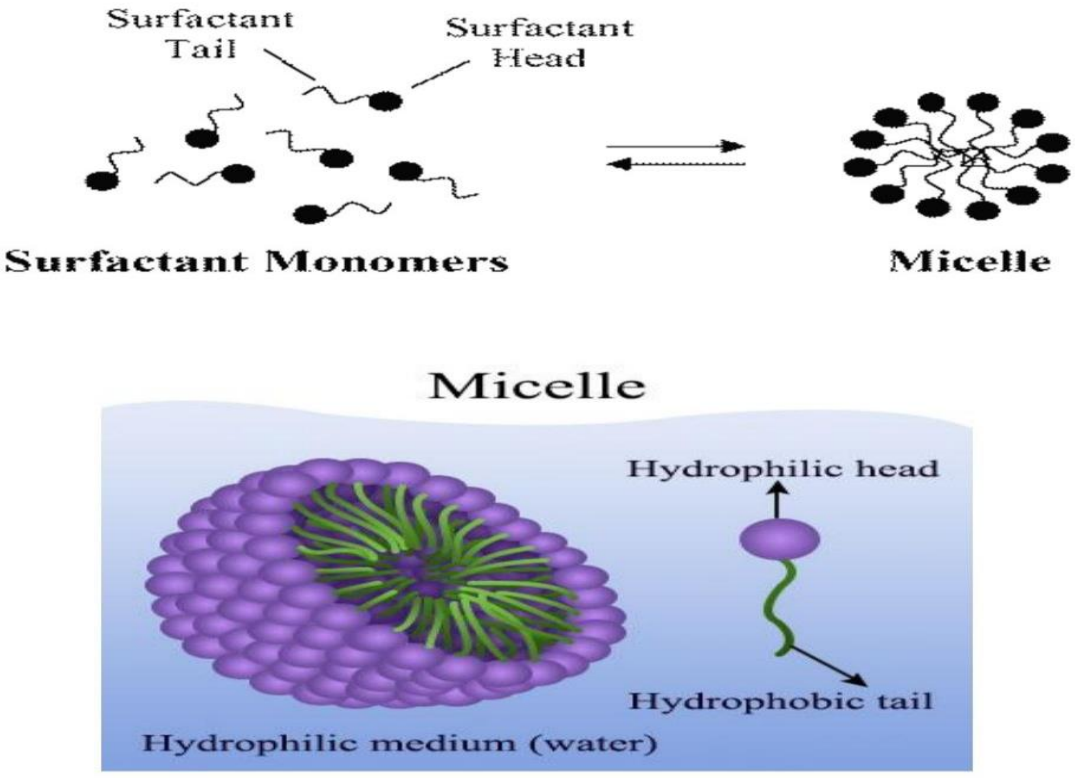


Figure (1-10): Chemical structure of Triton X-114.

1.3 Formation of Micelles

Surface active agents are known by their ability to assemble their molecules (monomers) in aqueous solutions to form micelles depending on their amphibious properties in having hydropholic heads and hydrophobic tails [69] . This assembly have a spherical structure [70] . Although the spherical suggestion of the micelles structure, in reality their structure depend on the relative volume and area occupied by the head and tail groups of surfactant particles forming the micelle structure, Figure (1-10) shows the structure of micelle formation [71] . Generally, among the most important

micellar properties at which micellar formation begins, there are two properties; CMC and the average number of microscopic surfactant molecules formed, which is known as the aggregation numbers (Nagg) [72] . The critical assemblage concentration is called the critical micellar concentration (CMC) is one of the properties of the surfactant, and is defined as the point at which the surfactant begins to reach its optimum state when the critical concentration has reached and the formation of a monolayer adsorption from the monomers aggregation in the solution. It is considered that monomers cause surface activity and not micelles, and any increase in the concentration of the surfactant only affects the micelling structure [73, 74] . At CMC, the unassociated monomers in the solution begin to move into the affinity phase to form micelles [75] . The monomers direct their hydrophobic tails outward to reduce their exposure to water [76] , while the hydrophilic parts are directed toward the water(inside) [77]



Figure(1-11) Micelle formation

The formation of a micelle implies a decrease in the entropy of the system. Such free energy will be used up in its formation. In an aqueous solution, the water molecules attract themselves by hydrogen bonds. Thus, in the dissolution of an ionic or polar substance, the necessary energy to break the hydrogen bonds is compensated by the hydration of dissolved species. However, aliphatic chains of surfactants are not appreciably hydrated. Then, Van der Waals forces act to their reciprocal attraction by decreasing the contact area among the surfactant molecules and water. Moreover, the hydrophilic head group of surfactants tends to solubilize in water. All these factors become the surfactant molecules agglomeration a spontaneous phenomenon. When the surfactant is dissolved in aqueous solution above its CMC, the hydrophobic group distorts the water liquid structure and this causes increase of free energy. This increase of free energy compensates for the necessary work to create a surface area permitting the micelle formation [78]

The most intensely studied and discussed type of microscopically ordered molecular aggregates are the micelles. Micelles are supramolecular structures of colloidal dimensions formed by surfactants molecules that aggregate in a spontaneous way in aqueous solution when critical micelle concentration (CMC) is attained. The CMC of a surfactant is based on several factors including its molecular structure, and experimental conditions such as temperature, ionic strength, counterions, etc. Below the CMC, the surfactant is usually in a nonassociated monomer form. However, when the CMC is reached, the formation process is favored. Micelles are not static structures. An important micelle characteristic is its dynamic equilibrium with the dissolved surfactant monomers, which remain at an approximately constant concentration after reaching the CMC. Micelles are thermodynamically stable and easily reproducible, but they can be destroyed by water dilution when the surfactant concentration becomes lower than its CMC[79, 80]

1.3.1 Micelles in Analytical Chemistry

In analytical chemistry the micellar media application can be described according to two aspects: the first one refers to the exploitation of micellar media properties such as water solubilization of hydrophobic substances, enhanced detection of spectroanalytical methods by changes of physical and chemical properties of the sample solution, transport and nebulization efficiency improvement, and reactions catalysis. The second aspect refers to the separation and pre-concentration by phase separation phenomenon in the cloud point [81].

In addition to the concentration of the surfactant, the temperature affects the propensity of the non-ionic surfactant in the micellar formation process and may also be affected by the presence of salts, alcohol, etc [82]. Temperature effect on aqueous solutions containing non-ionic surfactants are shown by dehydration of oxyethylene groups in the molecular, therefore, the micellar solution of converts from homogeneous into a cloudy [83]. The homogeneous system in micellar solutions is divided into two phases, one of which is rich in the micellar phase and the other contains a small amount of surfactant with a greater ratio of water [84]. It is also possible to return the surfactant solution to a homogeneous state, as the cloud point phenomenon is a reversible process [85].

Several authors suggested mechanisms depending on the phase separation phenomenon:

1. Proposed that above the T_c (the respective cloud point temperature) the temperature causes the outer portions of the micelles formed from the non-ionic surfactant to dry. This effect is mostly due to the dielectric constant of water, which decreases with increasing temperature. Thus, the hydrophilic portion of the surfactant is less able to interact with water molecules. This implies the breakdown of the hydrogen bonds between the water molecules and the surfactant molecules, then the capability of the micelles to be hydrophobic and thus the possibility of phase

separation increases. Figure (1-11) shows the effect of temperature on the phase separation process in a micellar solution [86].

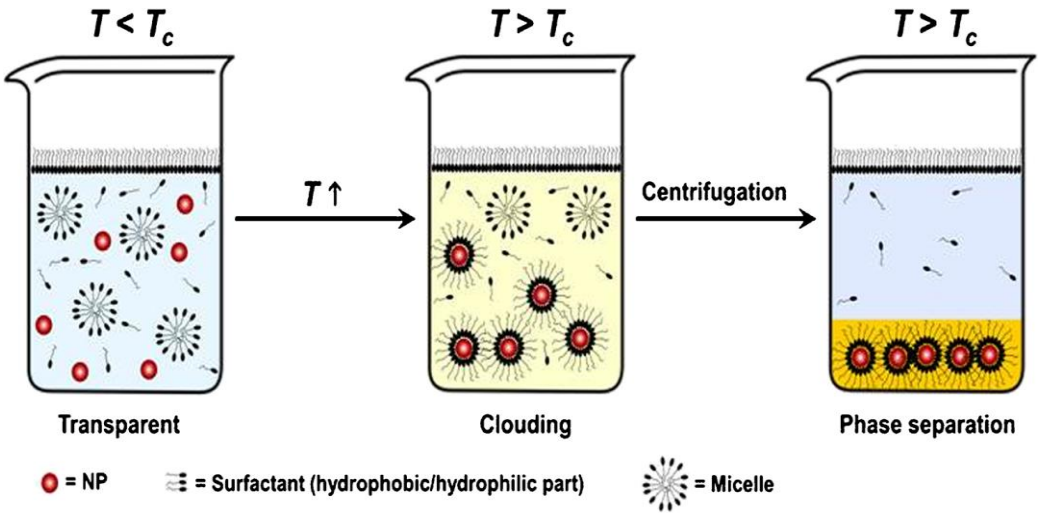


Figure (1-12): Schematic representation of the surfactant-mediated phase separation process.

2. The phase separation process over CPT may be due to the micellar attraction, which is weak mainly before it reaches the CPT due to the effect of the repulsive force between the particles (suggested Lindman and Wennerstrom 1991) [87].

3. Due to the opposition between the two free energies, the entropy that enhances the miscibility and the enthalpy that enhances the separation the phase separation process occurs. This indicates that phase separation and dimming are two processes that are reversible when controlling the two free energies (suggested Liu and et al. 1996)[88] .

1.3.2 Types of micelles

Surfactants tend to form different types of micelles in aqueous solutions depending on specific conditions, each with different properties and various applications. They are known as normal micelles, reverse micelles, and mixed micelle [89]

1. Normal Micelle

When the concentration is above than CMC of the surfactant in the water (the polar solvent) the monomers combine to form a spherical structure (micelle) as shown in (Figure.1-15) ,the center of the sphere is represented by the hydrophobic hydrocarbon parts and its radius is approximately equal to the length hydrocarbon chain [68]

2. Reverse Micelle

This type of micellar forms in aqueous systems that use a non polar solvent to dissolve the surfactant [90] . The structure of this species is arranged according to the direction of the polar head group to form a regular structure with a closed polar core surrounding the water molecules, as shown in Figure (1-15) [91,92] , while the tail group is oriented towards a non-polar organic solvent [44]. This type has many applications in the food, pharmaceutical, cosmetic, and petroleum refining industries [93]

3 .Mixed Micelle

Mixed micelles are formed when two different types of surfactant are used in an aqueous system, mixed surfactant based systems are of wide use in many applications due to their having distinct properties compared to single surfactant based systems. Due to their high surface activity, high ability to reduce surface tension and low micelle concentration (CMC) [94] . These mixtures have practical applications in industrial production such as detergents, pesticides, medicines and cosmetics [96] , applications in agriculture, soil treatment and food [95] . Figure (1-12) schematic for this type [97] .

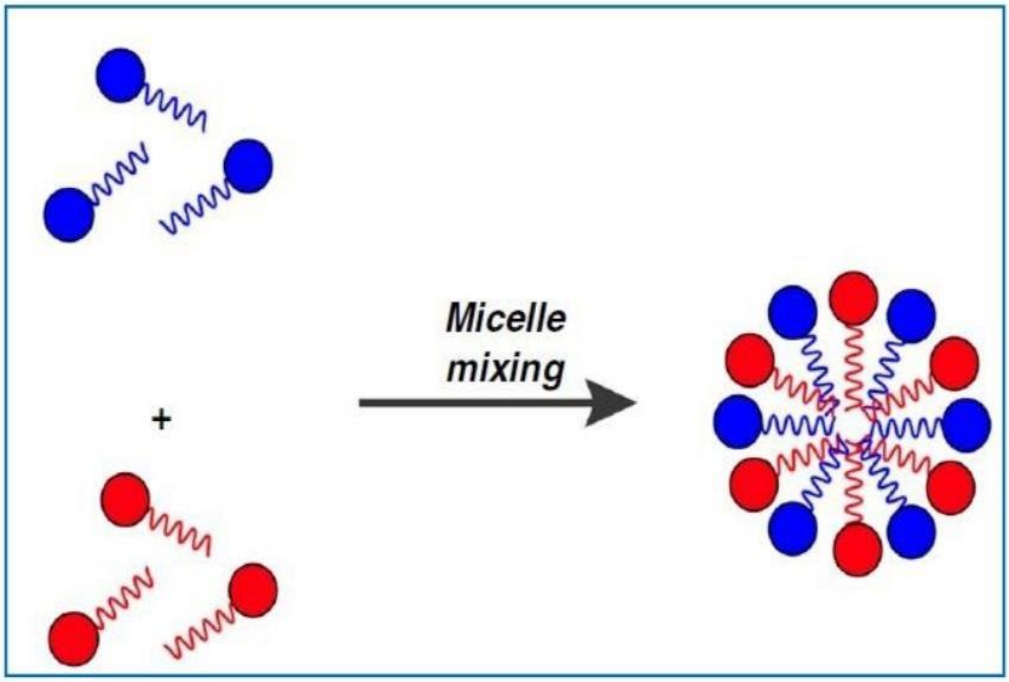


Figure (1-13): Schematic representation of formation of mixed micelle.

1.4 Factors Affecting on CPE

The CPE technique has received great attention by researchers for its simplicity, speed and ease of improving the extraction efficiency in it(by improving selectivity, sensitivity, detection limits, and some analytical properties prior to spectrophotometry) by improving the experimental conditions that affect the CPE process, such as salt addition, electrolyte type, temperature, pH values, type and concentration of the ionic surfactant and the time of extraction [98]. Table (1-2)summarizes some of the parameters affecting CPE systems

Table (1-2): Parameters optimized in CPE systems [99]

Type of surfac	Salt	pH	T. °C	Surfactant System
Triton X-1	none	pH 5.	40 °C	1.25% (v/v) Triton X-114
PEG600M	Na_2SO_4	none	45 °C	2 wt% PEG600M
$C_{13}E_{10}$	NaCl	none	70 °C	1wt% of $C_{13}E_{10}$
PONPE 5.	NaCl	pH 5.	70 °C	50% (v/v) of PONP

1.4.1 Type of surfactant

Since CPE technique depends on a surfactant [100] , so choosing the type of surfactant affects the extraction process, the preconcentration and the accuracy of the analytical results due to the different chemical properties of each type. The development of the CPE technique enabled it to use the amphoteric or anionic or cationic surfactants to eliminate some of the problems that the technique may happen when using a non-ionic surfactant and that choice depends on the conditions of the experiment or the type of analytical material if it is inorganic metal ions, biological materials, medicines and organic compounds or the type of Instrumental analysis used. For example, an anionic, cationic or amphoteric surfactant is used as an extract in the CPE technique to overcome the effect of the ultraviolet and visible absorption interference associated with the use of non-ionic surfactant. On this basis, interest has emerged in the charged surfactant in the extraction processes [101] . For different compounds of the same type, the effect also differs according to the compound, as one study showed that the extraction efficient using CPE is influenced by the kind of non-ionic surfactant applied in the extraction process, as the compounds used were: SDS, Triton X -114 and Triton X-100, which are all non-ionic surfactants [102] , while the non-ionic surfactant Triton X-114 is chosen when performing the separation and pre-

concentration of metal ions due to its high density in the rich surface phase and low CPT which facilitates phase separation through centrifugation [103]

1.4.2 Effect of the pH

The pH is one of the most important factors in the CPE process and is the first factor that needs improvement when performing the extraction process before moving to the next stage where pH is an important factor during analyzes involving the creation of metal-ligand complexes. Besides, the results of the extraction depend on determining the pH at which the compound is formed, as the possession of organic reagents with acidic or basic groups changes pH of their ionization form and solubility, and thus affects the ionization state for binding and the provision of binding sites with metal ions therefore, the importance of Introduction this parameter lies in the composition and chemical stability of complex and thus affects the sensitivity of the extraction [104, 105] , also pH affects some characteristics of surfactant [106] .

1.4.3 add salt

Cloud point extraction method extracts the analytical elements after forming the micelles, adding the salt increases the extraction efficiency by improving the conditions of internal reactions, this effect is known as the salting phenomenon, which results in a decrease in the solubility of non-electrolytic materials, as this addition increases the hydrophobic interactions between the micelles, which increases the turbidity of the solution, which facilitates the separation process [107] . Addition of appropriate salt can alter the cloud point (CP) of the non-ionic surfactant [108] .The CP is known as a physical parameter and one of the most important properties of non-ionic surfactants, so the addition of inorganic electrolytes affects the surfactant present in the solution [81] . The type of salt and its concentration also formation of the micelles required for an extraction process in CPE. For example, NaCl, KCl, K2SO4 and Na2SO4 (saline compounds), the latter showed a greater effect on extraction efficiency by increasing the turbidity of the solution, perhaps because it has a higher

ionic strength. The addition of salts sometimes negatively affects the extraction process when it reaches higher concentrations than required. The high concentration of the added salt makes it difficult to dilute the phase rich in organic solvents because the micelles do not tend to be soluble in these solvents [109] .

1.4.4 Effect of temperature and incubation time

These two parameters have a role no less important than the other parameters, in their impact on CPE technique to achieve the best results [110] . Crand and colleagues show that each surfactant has a specific cloud point temperature to reach the cloud state, cloud point temperature parameter changes depending on the type of surfactant and its chemical structure and may range from 30 °C to 160 °C The temperature may adversely affect the extraction efficiency and the effect includes a decrease in the surfactant concentration and an effect on its performance also high temperatures also affect surfactant precipitation [111] .

Incubation time has an important influence on the CPE process, and the incubation time may be inversely proportional to the temperature. The suitable incubation time and temperature lead to the completion of the separation of the two phases, which in turn affects the efficiency of extraction [112] .

1.4.5 Effects of diluents

In the last step of the CPE procedure, the sediment adheres to the inner walls of the test tube due to the centrifugation process, and the analyst must prepare this viscous mass for the analysis step by removing it from the walls and dissolved it completely [113]. This process is done using dilution agents, as the dilution agents reduce the high viscosity of the surfactant-rich phase and are often necessary for a spectrophotometric method to obtain a homogeneous solution with a compatible viscosity [114]

1.5 Applications of Cloud Point Extraction for the selected metals

The release of metal ions into the environment is a threat to humans and animals and leads to environmental pollution [115], as industrial activities and human gatherings are a source of emission of toxic elements to the environment, and among these elements are lead and copper ions, which have become a threat to the health of the population, so the use of efficient and selective analytical methods to determine the trace of copper and lead in real matrices is a very important topic [116, 117].

1.6 Extraction of Inorganic ions in CPE

The process of determining the concentrations of trace minerals in different matrices is not an easy task, due to the low concentrations of these metals, which require the provision of efficient and developed instrumental techniques [118]. CPE is one of the most widely used techniques for pre-concentration of trace minerals and it is a simple and easy procedure as its principle mainly depends on the formation and separation of two phases with different intensities when the system is heated to a higher degree than CPT, it is also environmentally friendly for dependence it on the use of surfactants non-ionic low toxicity [120].

The chelating reagents play a role in the extraction process; they are extracted from the hydrophobic part of the micellar solution by forming hydrogen bonds between the functional groups of the non-ionic surfactant or with the terminal OH group [121]. Generally, The CPE technique for quantifying trace metals is clear and fairly easy. It begins by adding small quantities of the surfactant solution to the aqueous sample, and a solution containing the reagent or chelate is added directly to the aqueous solution of the sample or after it is dissolved with a water-miscible organic solvent to forming covalent bonds between reagents and metals ions in the aqueous solution to separate them into the organic phase later, then the solution is induced to phase separation by heating to above CPT for the used non-ionic surfactant, then, the two phases are separated by centrifugation [122].

Figure (1-16) the schematic representation for cloud point extraction of metal ions [123].

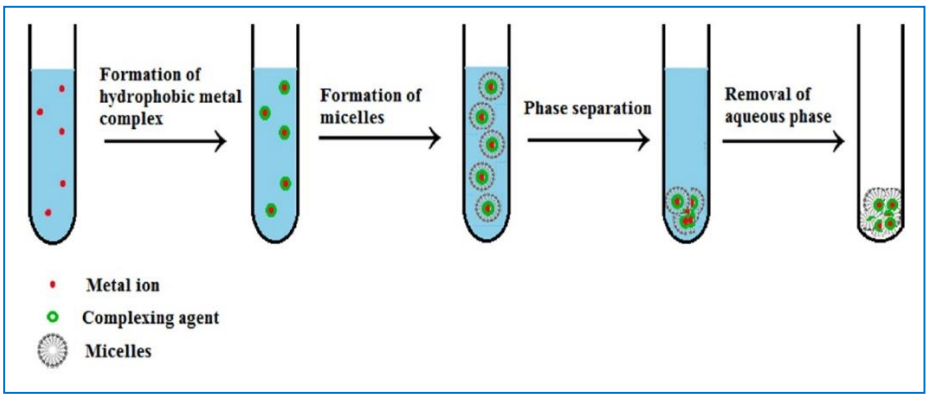


Figure (1-14): Cloud point extraction of metal ions

There are some different analytical techniques that are combined with CPE to analyze elements in different samples such as atomic absorption spectrometry (AAS) [124], atomic fluorescence spectrometry (AFS) [125], hydride generation atomic absorption spectrometry (HGAAS), graphite furnace atomic absorption spectrometry (GFAAS), inductively coupled plasma-mass spectrometry (ICP-MS), cold vapor atomic fluorescence spectrometry (CVAFS) [126], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [120],and spectrophotometer (UV-Vis) [127] .

UV-Vis spectrophotometer with CPE is a fairly common and effective solution for mineral analyzes compared to other spectroscopic methods, as it is characterized by low cost, simplicity, and compatibility with principles of green chemistry [128, 129].

1.7 Metal-Dithizone Complexes

The analytical reagent dithizone has now been used in analytical chemistry for just over 50 years, and forms the subject of an extensive literature comprising well over 2200 papers. Research work on this reagent was first carried out by Hellmuth Fischer, originally as a purely personal interest — virtually a hobby. However, the Siemens organization at Erlangen, Germany, was fortunate indeed to be able to employ his expertise when they were faced with the problems of determining small amounts of metallic impurities in certain highly purified metals.[130,131] In this field, its great sensitivity and considerable selectivity made an immediate impact. The intense

color of the reagent itself — and that of each of its metal complexes, their sparing solubility in water, but considerable solubility in organic solvents — led Hellmuth Fischer, and later many others, to elaborate liquid-liquid extraction procedures for concentrating and separating desired metals and to devise a whole variety of absorptiometric finishes. Selectivity was enhanced by a careful control of the pH employed in extractions, and by the use of masking agents.[132]

1.8 CPE for lead (II)

Water is an important and necessary resource for humans and their development [114]. Rapid industrial development has resulted in water pollution and deterioration of its quality [133], Industrial pollutants can cause severe damage to overall health and ecosystems [134]. Among the many industrial pollutants, lead is one of the most toxic and dangerous to human health [135].

The danger of lead is that it is highly toxic even in very low concentrations, in addition to its high stability, not biodegradable and the possibility of accumulation in living organisms during the food chain [136, 137]. According to the World Health Organization (WHO), acceptable concentrations of lead in drinking water ar 10 $\mu g.L^{-1}$, therefore, the exposure to lead causes many diseases: including cancer, anemia, mental retardation, kidney disease, nervous and reproductive system diseases [138, 139]. Due to its harm to humans and the environment, it was essential to use effective techniques to determine the lead content in aqueous samples.

1.9 CPE for cobalt(II)

Cobalt is an essential trace element in human body. Being a component of vitamin B12 (cyanocobalamin), it plays an important role in the production of the blood red cells and the prevention of pernicious anemia [140]. It is known that the toxicity of Cobalt is quite low, but high exposure to this element can cause diseases such as asthma and skin irritation. therefore, to determine trace amounts of cobalt becomes sig-nicantly important in the fields of environmental analysis, process control, and medicine [141, 142]

To design novel drugs, medicinal chemistry has benefited from the properties of metal ions. Hence, this has caused to have clinical application of chemotherapeutic agents for cancer treatment, such as cisplatin [143]. Some of these works were mentioned as follows. In 1952, the first biological activity of cobalt compounds was acquainted where cobalt(III) compounds of bidentate mustard move as if it were hypoxia selective agents [144].

Cobalt has many applications in a wide range of areas. A solution of cobalt (II)chloride can be utilized to measure moisture in the air. When the weather is humidity, paper is pink in color. Also, it may be used as an invisible ink [145]

The chemistry of cobalt complexes has attracted a lot of attention in recent years on account of their applications, among others, in biological systems such as antimicrobial agents and antibacterial agents (DNA studies and cytotoxicity studies) [146]

1.10 CPE for Cd (II)

Cadmium is one of the most toxic elements and accumulates in humans mainly in the kidneys and liver. A prolonged intake of cadmium leads to calcium regulation in biological systems, which induces cell injury and death. It also inhibits the action of zinc enzymes by substitution. Cd is also a teratogenic and carcinogenic agent [147]. Cd enters the organism primarily via the alimentary and/or respiratory tract. The sources of this metal are food, drinking water and air [148]. Due to that, trace and ultra-trace determinations of Cd in environmental and biological samples have become of increasing interest [149]. The exposure is obviously dependent upon the emission of that element and might be particularly serious in factories; 50% of inhaled Cd, for example, is absorbed and most of it is concentrated in the liver and kidneys [150]

Cadmium is considered as a toxic metal and is hazardous to both human and wild life. It acts as a mitogen and promotes cancer in a number of tissues. It also stimulates cell proliferation, inhibit DNA repair and inhibit apoptosis. On the one hand it induces

the cell death which leads to tissue damage in kidney. In cell culture systems, cadmium at low concentration cause apoptosis and with increase in concentration necrosis become evident. Cadmium also affects the renal function when exposed to the environment. [151] ,When the cadmium acetate is administered to the rat in varying concentration, there is interaction between the Cd^{2+} and the enzyme molecule which inhibits the activity of superoxide dismutase (SOD) to increase the lipid peroxidation in liver and kidney. It is indicated that Cd-induced elevation in lipid peroxidation is not only due to the inhibition of the activity of the superoxide dismutase (SOD) but also due to the direct action of Cd^{2+} on the peroxidation reaction. [152]

1.11 Aims of the Study

1. Spectral scanning of the reagent and its complexes with lead (II) ions and cobalt (II)ions and cadmium(II) ions according to the cloud point extraction technique in UV- visible region of the spectrum, in order to determine the values at the maximum wavelength of the complexes.

2. An application procedure of the proposed method for quantifying the selected ions in real samples from some water sources.

3. Verify Precision of cloud point extraction method by comparing it with the direct method for measuring metal ions using flame atomic absorption spectroscopy (FAAS).

4. Simple and effective analytical methods use in identifying lead (II) ions and cobalt (II)ions and cadmium(II) ions in aqueous samples, which will provide a great development in analytical chemistry as an alternative to the traditional and expensive methods in this field.

5. Determine the optimal experimental conditions when determining the effect of lead (II) ions and cobalt (II)ions and cadmium(II) ions in aqueous samples such as pH, reagent concentration (ligand), surfactant concentration (TritonX-114), equilibrium temperature, incubation time, salt effect, and Centrifugation rate and time.

6. Study contributes to the increasing popularity of using spectroscopic methods for determining trace elements, such as the use of UV-visible spectrophotometer, which are available and easy to use, and less polluting than the other methods used in this field.

chapter two experimental part

2.1 Apparatus used for this work.

1. Shimadzu (UV-1650) double beam UV- Visible spectrophotometer.
2. pH meter apparatus model pH200 l0vibond pH Meter
3. Water bath ,Hamburg – 90, model ,England.
4. Centrifuge apparatus , Medifuge model.
5. Heater and magnetic motor J Iab ,model (LMS-100)
6. Balance BP 3015,Sartorius ,Germany.
7. Atomic absorption spectrometry (AAS). 6300(Shimadzu, Japan)

2.2 Chemical Materials:

The chemicals used in this study were included in Table (1-2) with some important information about each of them.

Table 2-1: Chemicals used in this work

Substance	Company	Chemical Formula	Molecular weight (g/mol)	Purity or conc.
Triton X-114 CAS#: 9002-93-1	**AcrosOrga-nics,New Jersey,USA**	**$(C_2H_4O)_n$ $C_{14}H_{22}O$, n=7 or 8**	**558.75**	**100.0%**
Cobalt Nitrate hydrate	**Merck**	**$Co(NO_3)_2.3H_2O$**	**241.5**	**Analar**
Lead Nitrate	**Merck**	**$Pb(NO_3)_2$**	**331.2**	**Analar**
Absolute Ethanol	**GCC**	**C_2H_5OH**	**46.07**	**99%**
Sodium Nitrate	**Merck**	**$NaNO_3$**	**84.9947**	**Analar**
Magnesium Sulfate hydrate	**BDH**	**$MgSO_4.7H_2O$**	**120.415**	**99.9%**
Ammonium nitrate	**Fluka**	**NH_4NO_3**	**80.052**	**97.3%**
Sodium Chloride	**Fluka**	**NaCl**	**58.44**	**98%**
Ascorbic acid	**BDH**	**$C_6H_8O_6$**	**176.12**	**99%**

Citric acid	BDH	$C_6H_8O_7$	192.124	99.5%
Tartaric acid	Fluka	$C_4H_6O_6$	150.087	99.0%
Sodium fluoride	Fluka	Na F	41.98	99.0%
Acetic acid	BDH	$C_2H_4O_2$	60.05	99.7%
Ammonium Acetate	BDH	CH_3COONH_4	77.08	97.3%
Ammonia	BDH	30% NH_3	17.031	Analar

2.3 Cloud-Point Extraction for pb (II)

2.3.1 Preparation of Standard Solutions

Distilled water was used for dilution when preparing most of the chemical solutions required throughout the experimental work.

2.3.1.1 The organic reagent 3-anilino-1-phenylimino-thiourea 1 $\times 10^{-3}$ mol. L^{-1}

Reagent solution was prepared by dissolving 0.0128g of the organic reagent in 5mL NaOH (1M) and diluted with distilled water to 50.0 mLin volumetric flask

2.3.1.2 pb (II) Stock Solution (100) µg.mL^{-1}

This solution was prepared by dissolving 0.01598g of pb $(NO_3)_2$ in distilled water in a 100.0 mL volumetric flask and then the volume was completed to the mark. From this standard solution, the other standard solutions were prepared by successive dilution using distilled water.

2.3.1.3 5% (v/v) Triton X-114

This solution was prepared by adding 2.5 ml of Triton X-114 in a 50.0 mL volumetric flask and diluted with distilled water to the required volume.

2.3.1.4 Ionic salt Agents 0.1 mol.L^{-1}

The ionic salting agent solutions were prepared at a concentration of 0.1 mol.L^{-1} for each of the substances listed in Table (2-2) using a 100.0 mL volumetric flask with dilution with distilled water to the mark.

Table 2-2: Preparation of salting agents

Salt out	Weight (g)
$NaNO_3$	0.8501
$MgSO_4.{}_7H_2O$	1.2041
NH_4NO_3	0.8001
NaCl	0.585

2.3.1.5 Preparation of acid and base Solutions

solutions were prepare by dissolving 0.4g sodium hydroxide in distilled water and then complete volume to 100 mL by distilled water

were prepare hydrochloric acid by dissolving 0.9mL and then complete volume to 100 mL by distilled water and measurement PH by different addition from acid and base.

2.3.2 Spectrophotometric Investigation

The complex formed by adding the pb(II) to the complexing agent was revealed by UV-visible spectroscopy as shown in Figure (3-1). Using the reagent (R) , the spectrophotometric scanning of the visible and ultraviolet regions of the spectrum was performed with a Shimadzu UV-Visible Spectrophotometer-1650 (Tokyo) equipped with quartz cells. Absorption spectrum of complex showed λ_{max} at 560 nm, this wavelength was used to determine the pb(II) in all experiments.

2.3.3 General Procedure for CPE for Determination of Pb (II)[153]

In a 5.0 mL volumetric flask, added 3µg .mL^{-1} of pb(II) standard solution and 0.8 mL 1 x 10^{-3} mol.L^{-1} of (R) as a complex agent with presence of 0.6 mL (5% (v/v)) of Triton. X-114 as a micellar medium and diluted the mixture with distilled water to the desired volume. The solution is transferred to a test tube to be heated in a water bath at 50 °C for 15 min and then the solution is centrifuged using a centrifuge at 4000 rpm for 20 min to complete the phase separation process. The aqueous phase can be easily poured and then 3.0 mL of ethanol absolute is added to the rich phase of the surfactant to treat the viscosity and complete the required volume in cuvette for analysis. The analytical signal of the sample is recorded at λ_{max} (560) nm to determine the pb (II) content.

2.3.4 Optimization parameters for CPE for determination of pb (II)

There are some parameters that affect CPE and can improve its efficiency by increasing the sensitivity and reducing the detection limit of pb (II) as pH, reagent concentration (R), Triton concentration, equilibrium temperature, incubation time, salting, effect of foreign ions and centrifugation (rate and time).

2.3.4.1 Effect of pH

Similar solutions were prepared with 5.0 mL volumetric flasks, containing 3 µg.mL^{-1} of pb (II) standard solution and 0.8 mL1×10^{-3} mol.L^{-1} of (R) in the presence of 0.6 mL of 5% (v/v) of Triton. X-114 within pH (4.2) which were adjusted with different NaOH and HCl solutions. After completing the required volume with NaOH and HCl solutions and applying the general procedure for CPE, the absorption was recorded at 560 nm

2.3.4.2 Effect of (R) Concentration

Similar solutions were prepared with 5.0 mL volumetric flasks, containing of 3.0 $\mu g.mL^{-1}$ of pb (II) standard solution and varying volumes (0.2-1.2) mL, 1×10^{-3} $mol.L^{-1}$ of (R) in the presence of 0.6 mL 5% (v/v) of Triton X-114 at optimum pH (4.2). After completing the required volume with distilled water and applying the general procedure for CPE, the absorption was recorded at 560 nm.

2.3.4.3 Effect of Triton X-114 Concentration

Using various volumes (0.2-1) mL, 5% (v/v) of Triton X-114 with keeping previous optimum conditions, such as pH and concentration of (R), CPE procedure was applied to the test solutions and measured the absorption at 560 nm.

2.3.4.4 Effect of Equilibrium Temperature

While keeping the optimum experimental conditions constant such as pH, concentration of (R), concentration of Triton X-114 in the presence of 3.0 $\mu g.mL^{-1}$ of pb(II) standard solution, the temperature effect was tested on the extraction process in the ranges of (30-60)°C ; after completing the CPE steps, the absorbance measurement of all test solutions were measured at 560 nm.

2.3.4.5 Effect of Heating Time

Effect of the incubation time on the extraction process of lead (II) was carried out by using different times of 5-30 min as the incubation time of samples in the water bath after applying all the optimal experimental conditions.

2.3.4.6 Effect of Centrifugation (Time and Rate)

Effect of the centrifugation time on the pb (II) extraction process in the CPE was tested by using different times (5 - 25) min for applying the centrifugation processOn

the other hand, the effect of centrifugation rate was tested by using different rates ranging from 1000 to 5000 rpm for a period of 20 min

2.3.4.7 Effect of Salt out

Effect of salts on CPE were studied by adding 0.5 mL, 0.1 $mol.L^{-1}$ of NaCl, NH_4NO_3, $MgSO_4.7H_2O$ and $NaNO_3$ to volumetric flasks containing of 3.0 $\mu g.mL^{-1}$ of pb(II) standard solution, 0.8 mL (1×10^{-3}) $mol.L^{-1}$of (R) and 0.6 mL (5% (v/v)) of Triton X-114 and with distilled water to the required volume. The test solutions were exposed to 50 °C for 15 min (as an optimal incubation time). With all CPE steps completed, absorbance was measured for all samples at 560 nm.

2.4 Stoichiometric Complex Determination of [pb(II)-R]

The Job's method was used to find the stoichiometric ratio between pb (II) and (R). Using a series of solutions were prepared containing distinct volumes of pb(II) standard solution (0.5−4.0) mL and the reagent (4.0−0.5) mL at the same concentration level ($1x10^{-3}$ $mol.L^{-1}$). after measuring the absorbance of the solutions, the relationship between the molar fraction and the absorbance was drawn as explain in Figure (3-17).

On the other hand, the molar ratio method was used for the same purpose, where the absorbance was measured for a series of solutions containing constant volumes (1.0) mL of pb (II) and various volumes (0.5-3.5)mL of (R) at the same concentration level (1×10^{-3}) $mol.L^{-1}$, then diluted to the required volume. By using the same analytical metho

2.5 Preparation of Calibration Curve

Calibration Curve was obtained by preparing seven various solutions from pb(II) standard solution (0.06- 3.0) $\mu g.mL^{-1}$ using 5.0 mL volumetric flasks in the presence of 0.8 mL (1×10^{-3}) $mol.L^{-1}$ of (R), pH 4.2 and 0.6 mL(5% (v/v)) of Triton X-114 with dilution with distilled water to the required volume. The CPE procedure was applied while keeping all the optimal experimental conditions obtained from previous experiments constant and measuring the absorbance at λ_{max} 560 nm for [pb(II)-R] complex.

2.6 Cloud-Point Extraction for Cobalt (II)

2.6.1 Preparation of Standard Solutions

Distilled water was used for dilution when preparing most of the chemical solutions required throughout the experimental work.

2.6.1.1 Co(II) Stock Solution (100 µg. mL^{-1})

This solution was prepared by dissolving 0.0310 g of $Co(NO_3)_2$ with distilled water in a 100.0 mL volumetric flask and then the volume was completed to the mark. From this standard solution, the other standard solutions were prepared by successive dilution using distilled water.

2.6.1.2 (5% v/v) of Triton X-114

This solution was prepared by adding 2.5 ml of Triton X-114 in a 50.0 mL volumetric flask and diluted with distilled water to the required volume.

2.6.2 Spectrophotometric Investigation

The complex formed by adding the Co(II) to the complexing agent was revealed by UV-visible spectroscopy as represented in Figure(3-20). Using the reagent (R), the spectrophotometric scanning of the visible and ultraviolet regions of the spectrum was carried out with a Shimadzu UV-Visible Spectrophotometer-1650 equipped with two quartz cell.

Absorption spectrum of complex showed λ_{max} at 550 nm, this wavelength was used to determine the cobalt (II) in all experiments

2.6.3 General Procedure for CPE for Determination of Co(II)

In a 5.0 mL volumetric flask, added 6 μg.mL^{-1} of Co (II) standard solution and 0.6 mL (1 x 10^{-3}) mol.L^{-1} of (R) as a complex agent in the presence of 0.8 mL (5% (v/v)) of Triton. X-114 as a micellar The solution is transferred to a test tube to be heated in a water bath at 45 °C for 15 min and then the solution is centrifuged using a centrifuge at 4000 rpm for 20 min to complete the phase separation process. The aqueous phase can be easily poured and then 3.0 mL of ethanol absolute is added to the rich phase of the surfactant to treat of the viscosity and complete the required volume in cuvette for analysis.

To measure the absorbance of the sample, the analytical signal is recorded at λ_{max} (550) nm to determine the cobalt(II) content.

2.6.4 Optimization Parameters for CPE of Co(II)

There are some parameters that affect CPE and can improve its efficiency by increasing the sensitivity and reducing the detection limit of cobalt(II) as pH, reagent concentration (R), Triton concentration, equilibrium temperature, incubation time, salting, effect of foreign ions and masking agent and centrifugation (time and rate).

2.6.4.1 Effect of pH

Similar solutions were prepared with 5.0 mL volumetric flasks, containing of 6 μg.mL^{-1} of cobalt (II) standard solution and 0.6 ml (1 × 10^{-3}) mol.L^{-1} of (R) with presence of 0.8 mL of 5% (v/v) of Triton X-114 within pH ranges (3-6.5) which were adjusted with different NaOH and HCl solutions. After completing the required volume with NaOH and HCl solutions and applying the general procedure for CPE, The absorption was recorded at 550 nm.

2.6.4.2 Effect of (R) Concentration.

Similar solutions were prepared with 5.0 mL volumetric flasks, containing of 6 $\mu g.mL^{-1}$ of cobalt (II) standard solution and varying volumes (0.2-1) mL (1×10^{-3}) $mol.L^{-1}$ of (R) in the presence of 0.8 mL (5% (v/v)) of Triton X-114 at optimum pH (4.9) . After completing the required volume with distilled water and applying the general procedure for CPE, The absorption were recorded at 550 nm.

2.6.4.3 Effect of Triton X-114 Concentration

Using various volumes (0.2-1) mL (5% (v/v)) of Triton X-114 in with keeping optimum conditions constant , such as pH and concentration of (R), the CPE procedure were applied on the test solutions and measured the absorbance at 550 nm.

2.6.4.4 Effect of Equilibrium Temperature

While keeping the optimum experimental conditions constant such as pH, concentration of (R), concentration of Triton X-114 in presence of 6 $\mu g.mL^{-1}$ of cobalt(II) standard solution, the temperature effect was tested on the extraction process in the ranges of (35-55)°C. After completing the CPE steps, the absorbance measurement of all test solutions were measured at 550 nm.

2.6.4.5 Effect of Heating Time

Effect of the incubation time on the extraction process of cobalt (II) was carried out by using different times (5-30) min as the incubation time of samples in the water bath after applying all the optimal experimental conditions.

2.6.4.6 Effect of Centrifugation Time and Rate

Effect of the centrifugation time on the cobalt (II) extraction process in the CPE was tested by using different times (5-30) min for the centrifugation process. results were presented in Figures (3-31) and (3-32). On the other hand, effect of centrifugation rate was tested by using different rates ranging from 1000 to 5000 rpm for a period of 20 min as ideal time

2.6.4.7 Effect of Salt out

Effect of salts on CPE was studied by adding 0.5 mL of 0.1 $mol.L^{-1}$ of NaCl, NH_4NO_3, $MgSO_4.7H_2O$ and $NaNO_3$ to 5.0 mL volumetric flasks containing of 6 $\mu g.mL^{-1}$ of cobalt (II) standard solution, 0.6 mL (1×10^{-3}) $mol.L^{-1}$ of (R) and 0.8 mL (5% (v/v)) of Triton X-114 and with distilled water to the required volume. The test solutions were exposed to 45 ° C for 15 min as an optimal incubation time. With all CPE steps completed

2.7 Stoichiometric Complex Determination of [Co(II)-R]

Using a series of solutions were prepared containing distinct volumes of cobalt(II) standard solution (0.5-4.5) mL and the reagent (4.5-0.5) mL at the same concentration level ($1x10^{-3}$ $mol.L^{-1}$). After measuring the absorbance of the solutions, the relationship between the molar fraction and the absorbance

On the other hand, the molar ratio method was used for the same purpose, where the absorbance was measured for a series of solutions containing of constant volumes 1.0 mL of Co(II) and various volumes (0.5-4.0) mL of (R) at the same concentration level (1×10^{-3} $mol.L^{-1}$), then diluted to the required volume. By using the same analytical method

2.8 Preparation of Calibration Curve

Calibration curve was obtained by preparing seven various solutions from the standard solution (0.3-6.0) $\mu g.mL^{-1}$ using 5.0 mL volumetric flasks in the presence of 0.6 ml (1×10^{-3}) $mol.L^{-1}$ of (R), pH 4.9 and 0.8 mL (5% (v/v)) of Triton X-114 and dilution with distilled water to the required volume. The CPE procedure was applied while keeping all the optimal experimental conditions obtained from previous

experiments constant and measuring the absorbance at λ_{max} 550 nm for [Co(II)-R] complex.

2.9 Cloud-Point Extraction for Cadmium (II)

2.9.1 Preparation of Standard Solutions

Distilled water was used for dilution when preparing most of the chemical solutions required throughout the experimental work.

2.9.1.1 Cd(II) Stock Solution (100 µg. mL^{-1})

This solution was prepared by dissolving 0.0210g of $Cd(NO_3)_2$ with distilled water in a 100.0 mL volumetric flask and then the volume was completed to the mark. From this standard solution, the other standard solutions were prepared by successive dilution using distilled water.

2.9.1.2 (5% v/v) of Triton X-114

This solution was prepared by adding 2.5 mL of Triton X-114 in a 50.0 mL volumetric flask and diluted with distilled water to the required volume.

2.9.2 Spectrophotometric Investigation

The complex formed by adding the Cd(II) to the complexing agent was revealed by UV-visible spectroscopy as shown in Figure(3-39). Using the (R) as a blank, the spectrophotometric scanning of the visible and ultraviolet regions of the spectrum was carried out with a Shimadzu UV-Visible Spectrophotometer-1650 equipped with two quartz cell.

Absorption spectrum of complex showed λ_{max} at 525 nm, this wavelength was used to determine the cadmium(II) in all experiments

2.9.3 General Procedure for CPE for Determination of Cd(II)

In a 5.0 mL volumetric flask, added 3 μg.mL^{-1} of Cd (II) standard solution and 1 mL (1 x 10^{-3}) mol.L^{-1} of (R) as a complex agent in the presence of 0.4 mL (5% (v/v)) of Triton. X-114 as a micellar The solution is transferred to a test tube to be heated in a water bath at 55 °C for 20 min and then the solution is centrifuged using a centrifuge at 4000 rpm for 15 min to complete the phase separation process. The aqueous phase can be easily poured and then 3.0 mL of ethanol absolute is added to the rich phase of the surfactant to treat of the viscosity and complete the required volume in cuvette for analysis.

To measure the absorbance of the sample, the analytical signal is recorded at λ_{max} (525) nm to determine the cadmium(II) content.

2.9.4 Optimization Parameters for CPE of Cd(II)

There are some parameters that affect CPE and can improve its efficiency by increasing the sensitivity and reducing the detection limit of cadmium(II) as pH, reagent concentration (R), Triton concentration, equilibrium temperature, incubation time, salting, effect of foreign ions and masking agent and centrifugation (time and rate).

2.9.4.1 Effect of pH

Similar solutions were prepared with 5.0 mL volumetric flasks, containing of 3 μg.mLl^{-1} of cadmium(II) standard solution and 1mL (1 × 10^{-3}) mol.L^{-1} of (R) with presence of 0.4 mL of 5% (v/v) of Triton X-114 within pH ranges (3.3-6.1) which were adjusted with different buffer solutions. After completing the required volume with buffer solutions and applying the general procedure for CPE, The absorption was recorded at 525nm.

2.٩.4.2 Effect of (R) Concentration.

Similar solutions were prepared with 5.0 mL volumetric flasks, containing of 3 μg.mL^{-1} of cadmium (II) standard solution and varying volumes (0.2-1) mL (1×10^{-3}) mol.L^{-1} of (R) in the presence of 0.4 mL5% (v/v)) of Triton X-114 at optimum pH (4.7) . After completing the required volume with distilled water and applying the general procedure for CPE

2.9.4.3 Effect of Triton X-114 Concentration

Using various volumes (0.2-1.2) mL (5% (v/v)) of Triton X-114 in with keeping optimum conditions constant , such as pH and concentration of (R), the CPE procedure were applied on the test solutions and measured the absorbance at 525 nm.

2.9.4.4 Effect of Equilibrium Temperature

While keeping the optimum experimental conditions constant such as pH, concentration of (R), concentration of Triton X-114 in presence of 3 μg.mL^{-1} of cadmium(II) standard solution, the temperature effect was tested on the extraction process in the ranges of (30-60)°C. After completing the CPE steps, the absorbance measurement of all test solutions were measured at 525 nm.

2.9.4.5 Effect of Heating Time

Effect of the incubation time on the extraction process of cadmium(II) was carried out by using different times (5-25) min as the incubation time of samples in the water bath after applying all the optimal experimental conditions.

2.9.4.6 Effect of Centrifugation Time and Rate

Effect of the centrifugation time on the cadmium (II) extraction process in the CPE was tested by using different times (5-25) min for the centrifugation process. On

the other hand, effect of centrifugation rate was tested by using different rates ranging from 1000 to 5000 rpm for a period of 15 min as ideal time

2.9.4.7 Effect of Salt out

Effect of salts on CPE was studied by adding 0.5 mL of 0.1 mol.L^{-1} of NaCl, NH_4NO_3, $MgSO_4.7H_2O$ and $NaNO_3$ to volumetric flasks containing of 3μg.mL^{-1} of cadmium (II) standard solution,1mL (1 x 10^{-3}) mol.L^{-1} of (R) and 0.4 mL (5% (v/v)) of Triton X-114 and with distilled water to the required volume. The test solutions were exposed to 55 ° C for 20 min as an optimal incubation time. With all CPE steps completed, absorbance were measured for all samples at 525 nm

2.10 Stoichiometric Complex Determination of [Cd(II)-R]

Using a series of solutions were prepared containing distinct volumes of cadmium(II) standard solution (0.5-4.5) mL and the reagent (4.5-0.5) mL at the same concentration level (1x10^{-3} mol.L^{-1}). After measuring the absorbance of the solutions, the relationship between the molar fraction and the absorbance

On the other hand, the molar ratio method was used for the same purpose, where the absorbance was measured for a series of solutions containing of constant volumes 1.0 mL of Cd (II) and various volumes (0.5-4.0) mL of (R) at the same concentration level (1×10^{-3} mol.L^{-1}), then diluted to the required volume. By using the same analytical method

2.11 Preparation of Calibration Curve

Calibration curve was obtained by preparing seven various solutions from the standard solution (0.012-3.0) μg.mL^{-1} using 1ml (1 x 10^{-3}) mol.L^{-1} of (R), pH 4.7 and 0.4 mL (5% (v/v)) of Triton X-114 and dilution with distilled water to the required volume. The CPE procedure was applied while keeping all the optimal experimental

conditions obtained from previous experiments constant and measuring the absorbance at λ_{max} 525 nm for [Cd(II)-R] complex.

2.12. Preparation of water samples

Water samples were sampled from different water sources for applying the proposed method to real samples in the process of determining the metals selected in this study. The impurities and suspended particles in the samples drawn by the filtration process were eliminated using appropriate filter papers, then the lead and cobalt and cadmium ions of each sample were determined using the standard method(FAAS) and the proposed method (CPE) using the standard additives method.

Results and Discussion

3.1 Cloud-Point Extraction for [lead (II) -(R)] complex

3.1.1 Spectrophotometric Investigation

The complex formed by adding the pb(II) to the complexing agent was revealed by UV-visible spectroscopy. Figure (3-1) shows the absorption spectrum of [pb (II)-R] complex and λ_{max} at 560 nm. By comparing the absorption spectrum of the complex with the absorption spectrum of (R) and pb(II), the analytical signal of [pb (II)-R] complex can be considered as evidence of its formation. Accordingly, this wavelength was used to determine the lead content in all experiments.

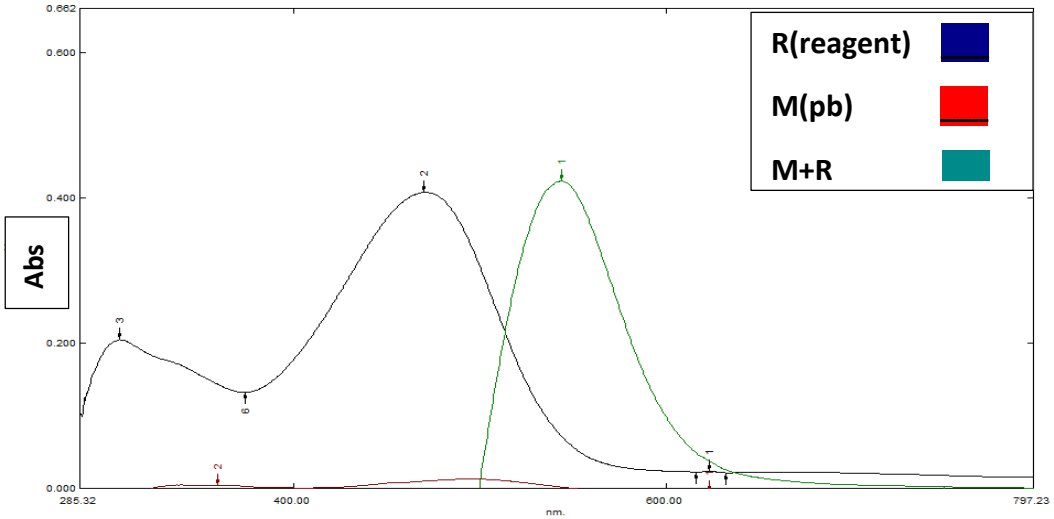


Figure (3-1): Absorption spectrums of lead (II), reagent(R), and [pb (II)-R] complex.

3.1.2 pH effect

The pH is the main and most important parameter in CPE, as the formation process of complex and its stability are two factors that are directly affected by pH [154]. On this view, a series of experiments were carried out to evaluate the effect of pH on the extraction process by preparing similar solutions containing of 3 $\mu g.mL^{-1}$ lead (II) standard solution, 0.8 mL $(1x10^{-3})$ $mol.L^{-1}$ of (R) in the presence of 0.6 mL (5% (v/v)) of Triton X-114 within pH ranges (3.5-5.8) which were adjusted with different buffer solutions. Figures (3-2) and (3-3) are represented the experimental results of this test after the absorbance was recorded for all samples at 560 nm.

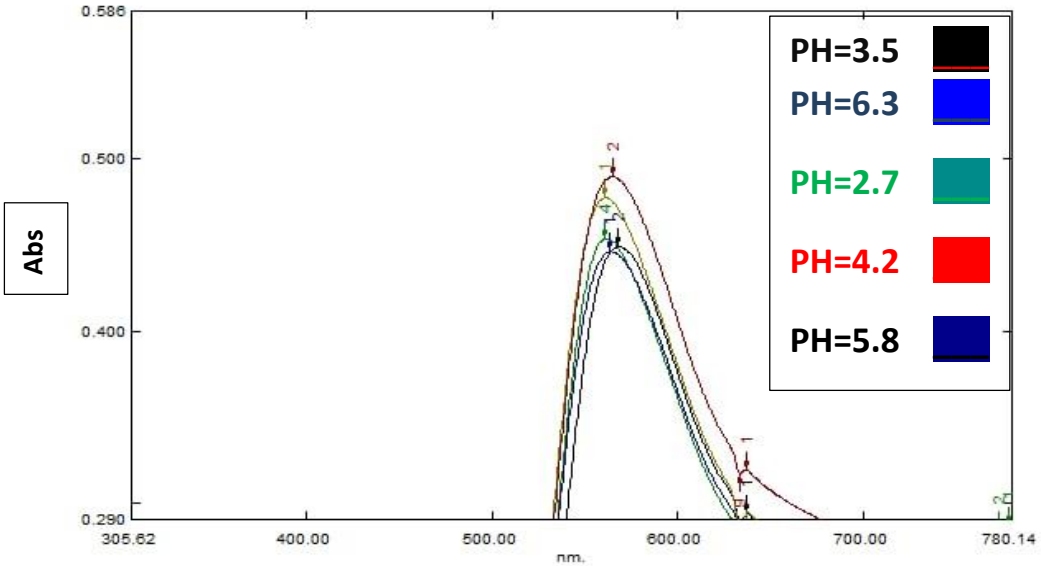


Figure (3-2): Absorption spectra of [pb(II)-R] complex under effecting of pH.

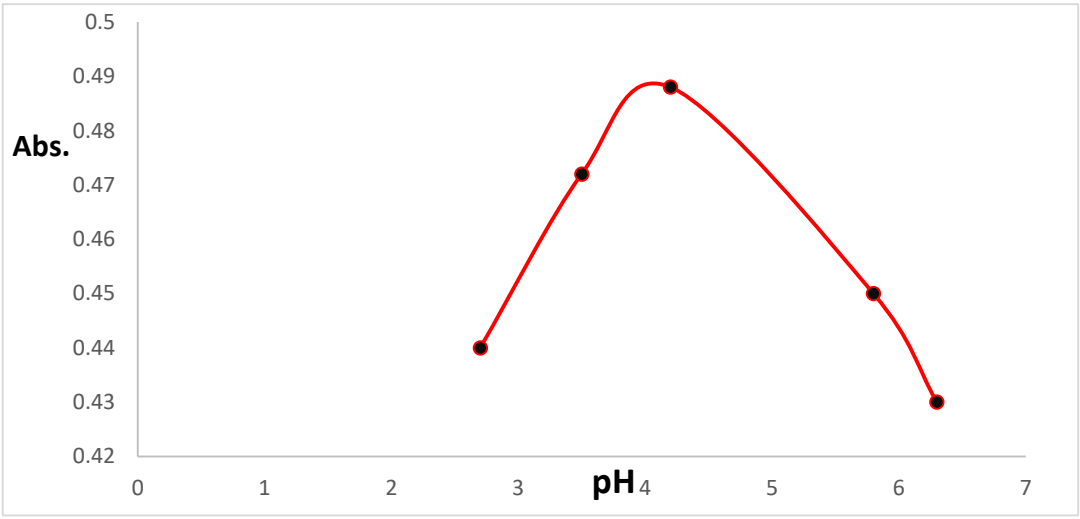


Figure (3-3): Effect of pH on formation of [pb(II)-R] complex. Conditions: 3 µg mL^{-1} of pb(II) ;0.8mL (1×10^{-3}) $mol.L^{-1}$ of (R); 0.6mL of (5% (v/v)) of Triton X- 114; Equilibration temperature 50 °C and heating time at 15 min)

The results in Figure (3-3) explain that the absorbance of the complex increases gradually with increasing pH up to pH of 4.2 and then the absorption value decrease with the increase pH of the solution. This contrast is due to the effect of the pH on the absorbance of the metal in the solution and the ionization state of the functional groups in the organic reagent that provide the binding sites [155].

3.1.3 Effect of (R) Concentration

To achieve maximum efficiency in extraction process of the complex, it was necessary to know the optimal concentration of the reagent at the optimum pH and in the presence of the surfactant. Therefore, the effect of reagent concentration on the extraction process in CPE was tested by preparing similar solutions containing different reagent volumes (0.2-1.2) mL at 1 x 10^{-3} mol.L^{-1} and adding them to 3 µg.mL^{-1} of lead (II) standard solution in the presence of 0.6 mL (5% (v/v)) of TritonX-114. Figure (3-4) represents the absorption spectrum of complex formed by the effect of reagent concentration and to illustrate the behavior of this effect, the relationship between absorption of the compound and the volume of (R) was drawn in Figure. (3-5).

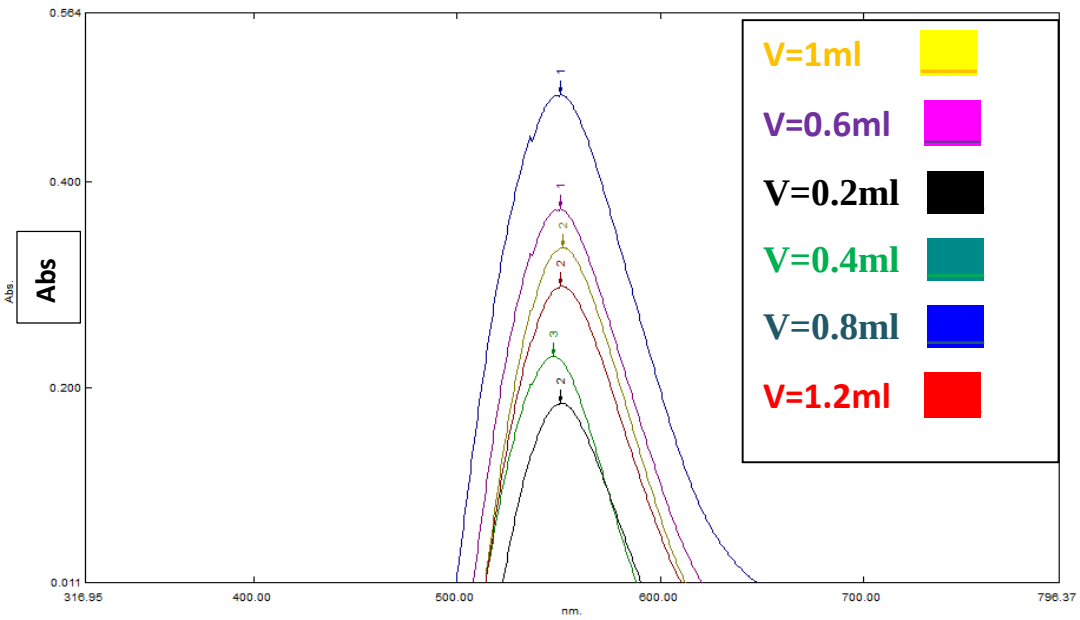


Figure (3-4): Absorption spectra of [pb (II) -R] complex by effect the concentration of reagent.

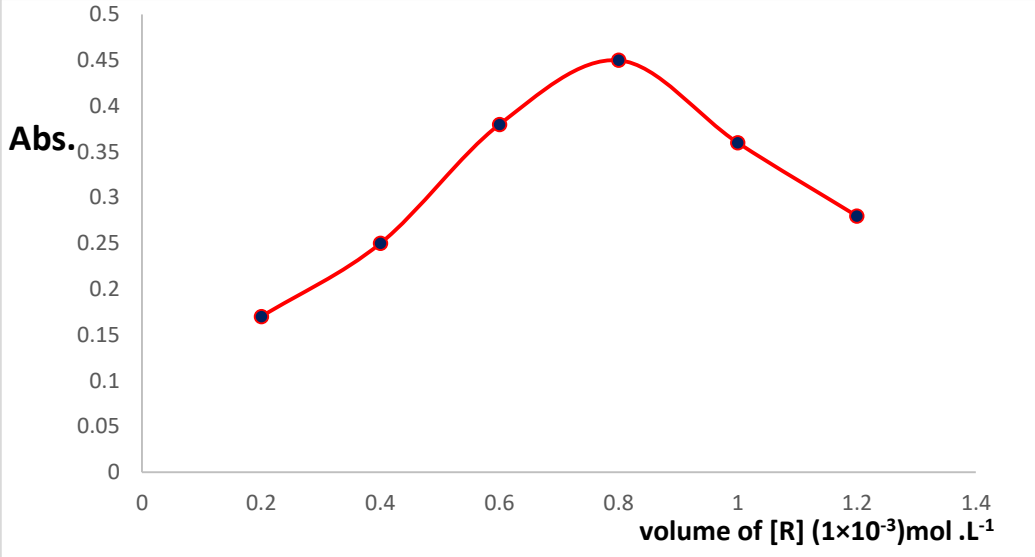


Figure (3-5): Effect of concentration of reagent on the determination of pb(II). Conditions: pH 4.2 ,3µg.mL^{-1} of pb (II); 0.6 mL (5% (v/v)) of Triton X-114; Equilibration temperature 50 °C and heating time 15 min).

The results in Figure (3-5)represented that the analytical signals increased with increasing reagent volume until reached 0.8 mL as maximum, this is due to the incomplete complexity of the metal at low concentrations of (R) in solution [156]. On the other hand, a gradual decrease in the absorbance value is observed with the continuous increase in concentration of complexing agent, pointing out that the increase for reagent was not necessary and lead to competing of free (R) with the complexes and thus low sensitivity [157]. Therefore, 0.8 mL was chosen to be the optimal volume of (R) in all experiments.

3.1.4 Effect of Triton X-114 Concentration

The amount of surfactant plays an important role in the efficiency of the extraction process in CPE [158]. According to this view, the effect of surfactant concentration on [pb(II)-R] extraction was studied by using varying volumes within ranges (0.2-1) mL (5% (v/v)) of Triton X-114 while keeping other parameters at optimum condition. By applying the general CPE procedure, the results were obtained in Figures (3-6) and (3-7).

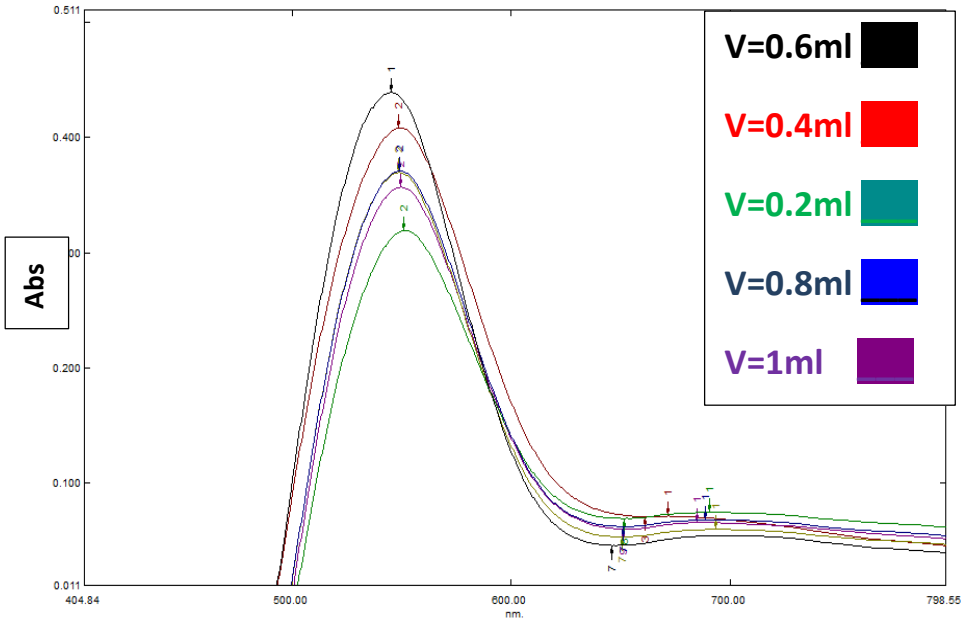


Figure (3-6):): Absorption spectra of [pb(II)-R] complex under effecting of vlume of Triton X-114

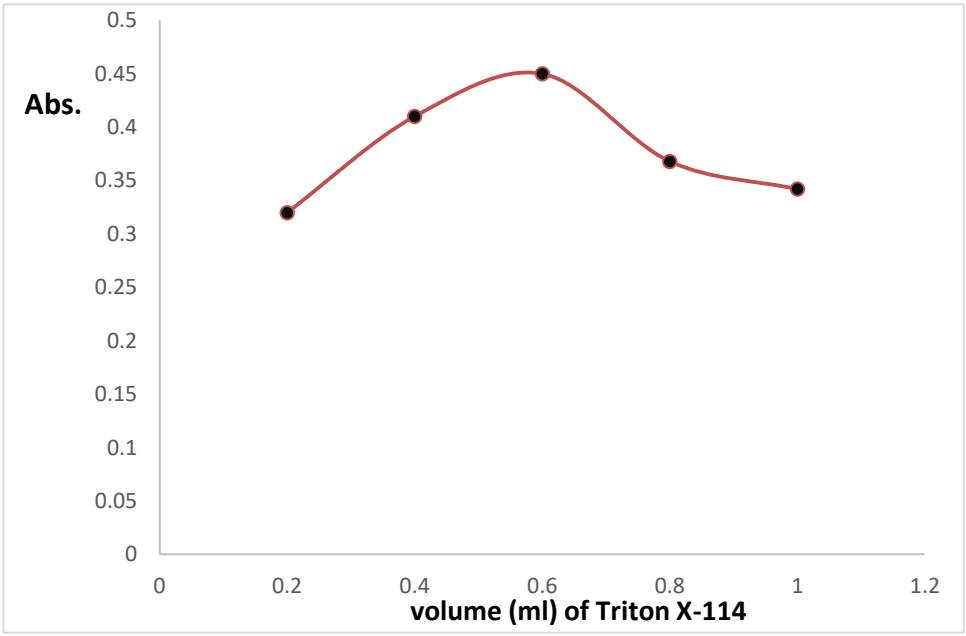


Figure (3-7): Effect of Triton X- 114 concentration on the determination of pb(II). Conditions: pH 4.2, 3 µg.mL^{-1} of pb (II); 0.8 mL (1.0 x 10^{-3}) mol.L^{-1} of (R.); Equilibration temperature 50°C and heating time 15 min).

The results in Figure (3-7) show that the analysis signals continue to increase until 0.6 ml of Triton X-114, the reason may be due to insufficient amount of Triton X-114 to capture the largest possible amount of the complex in the solution. On the other hand,

the continuous increase in amount of Triton X-114 reduces the absorption value gradually due to the increase in the viscosity of the surfactant-rich phase, which reduces the extraction efficiency [157]. Therefore, 0.6 mL (5% (v/v)) of TritonX-114 was selected as the optimal amount to be used in subsequent experiments.

3.1.5 Effect of Equilibration Temperature

Some of literature indicates that the equilibrium temperature has a major influence on the extraction efficiency through its effect on micelle formation and thus the complete quantitative extraction for analyte [159]. Effect of temperature on the extraction efficiency was tested by applying different temperatures to the test samples (30-60) °C at optimum conditions for the previous tests. A constant temperature showing excellent condition for the extraction process was obtained at 50 °C as shown in Figures (3-8) and (3-9).

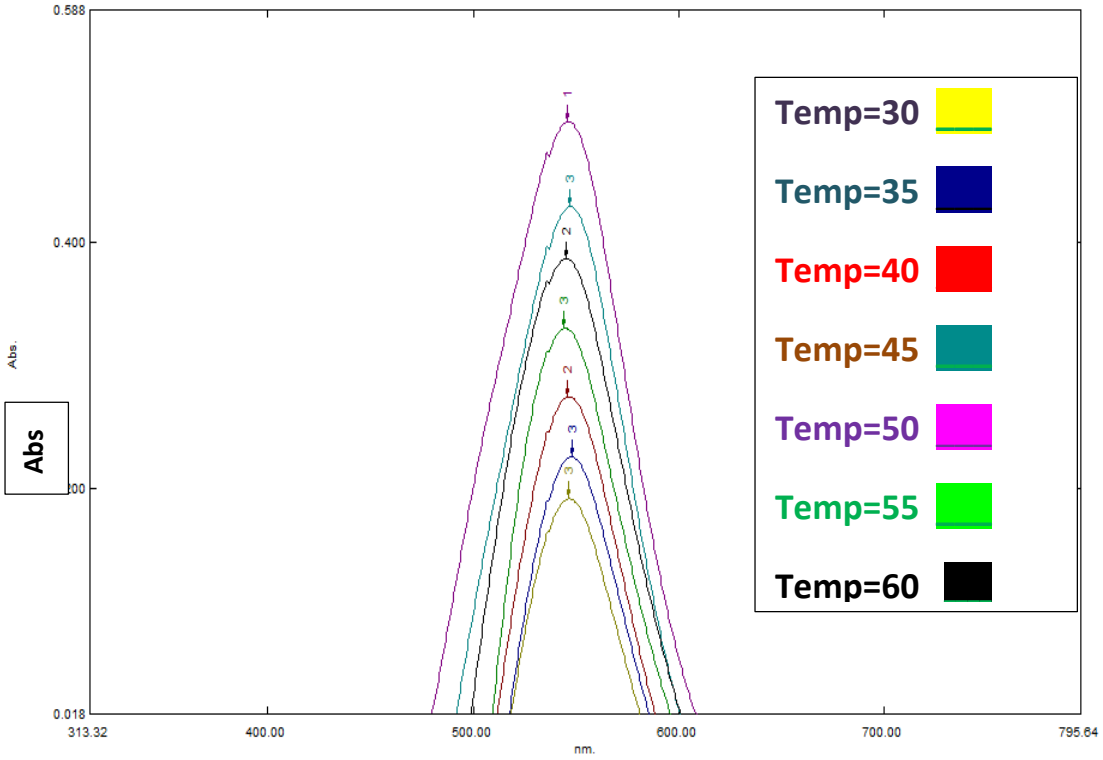


Figure (3-8): Absorption spectra of [pb(II)-R] complex under effecting of equilibration temperature.

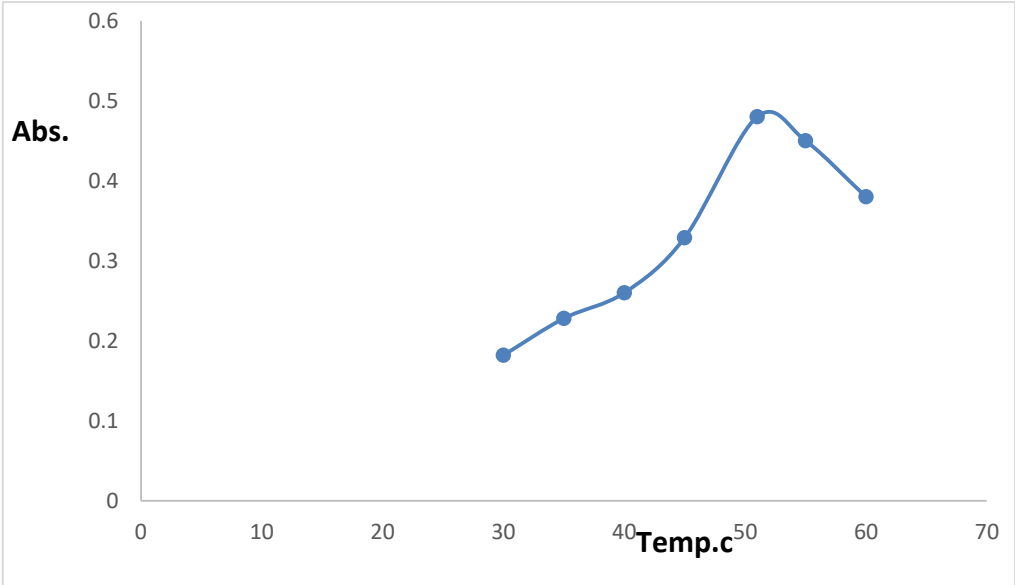


Figure (3-9): Effect of equilibration temperature on the determination of pb(II). Conditions: pH 4.2, 3µg.mL^{-1} of pb (II), 0.8 mL (1.0 x 10^{-3}) mol.L^{-1} of (R.); 0.6 mL (5% (v/v)) of Triton X – 114 and heating time 15 min).

Figure (3-9) shows that the extraction efficiency increases gradually with raising the temperature to 50 °C. This can be explained result the effect of the temperature on the volume of the SRP due to the disturbance of the hydrogen bonds, which leads to a decrease in the water content and thus an increase in the extraction sensitivity [155].On the other hand, a gradual decrease in the absorption values of the complex was observed while continuing to increase the temperature, as higher temperatures may lead to dissociation of the complex [156], and thus a decrease in the analytical signals at the point (55,60)°C. Therefore, selected were 50 °C for all subsequent experiments**.**

3.1.6 Effect of Incubation Time

The effect of the incubation time starts from the moment the solution reaches the CPT, so this parameter greatly influences the extraction efficiency in the CPE process [160]. To evaluate the effect of the incubation time, repeated tests were done for different times (5-25) minutes after fixing the equilibrium temperature and other parameters at the optimum conditions obtained through previous experiments. Figures (3-10) and (3-11) represented the results of the incubation time test.

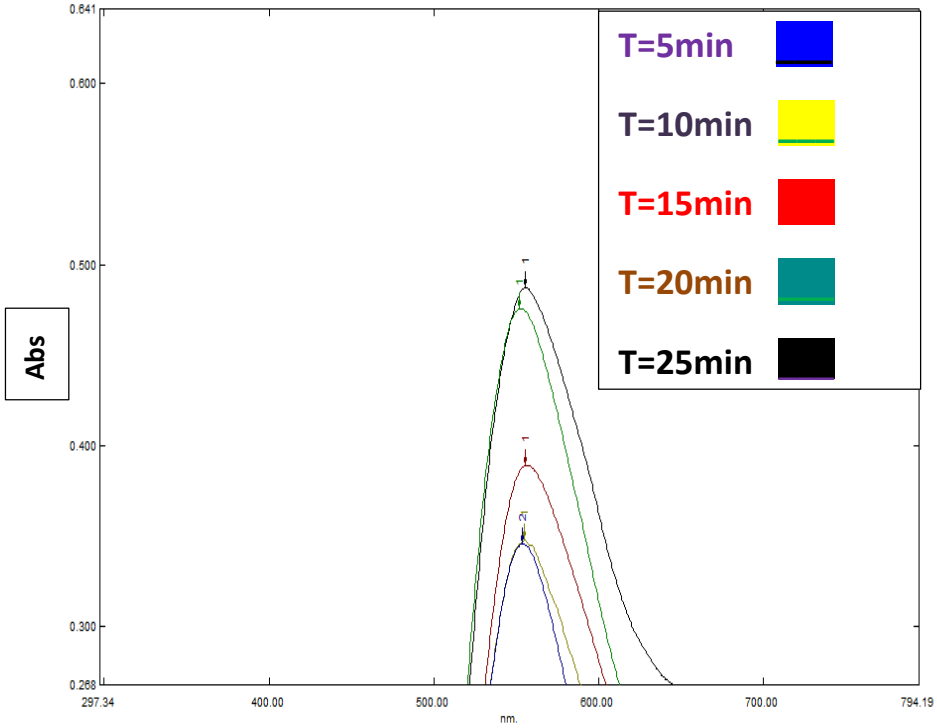


Figure (3-10): Effect of Incubation time on absorption of [pb(II)-R] complex

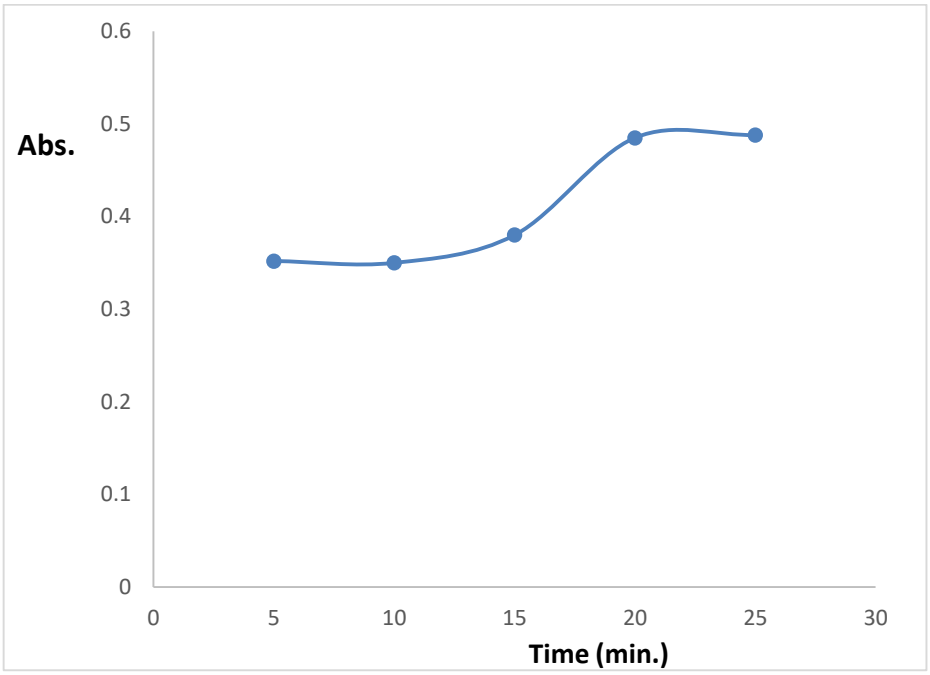


Figure (3-11): Effect of equilibration time on the determination of pb(II). Conditions: pH 4.2, 3µg.mL^{-1} of pb(II); 0.8 mL (1.0 x 10^{-3}) mol.L^{-1} of (R.); 0.6 mL (5%(v/v)) of Triton X-114 and equilibration temperature 50 °C).

Through the results in Figure (3-11) the analytical signal increases until it reaches the point (15) minutes and then stabilizes relatively with the increasing time. This time can be considered sufficient to achieve a good separation of the solution as preferable that the extraction process takes place in a shorter time and with higher efficiency. Therefore, chosen was (15) min as the best incubation time.

3.1.7 Effect of centrifugation Time and Rate

The time factor is just as important as the other parameters and desirable that the extraction process be more efficient and less time-consuming. Therefore, the effect of centrifugation time on the extraction efficiency of lead (II) ions in CPE was studied using different times (5-25) min to evaluate the effect of time on the centrifugation process. Through the results explain in Figures (3-12) and (3-13 The time (20) min was chosen as a best time for higher extraction.

While keeping time constant at (20 min), the Centrifuging rate was tested within a range of 1000 to 5000 round per minute (rpm). according to the obtained data in Figure (3-14) and (3-15), it represented that at 4000 rpm available for complete phase separation, so it was chosen as its perfect rate.

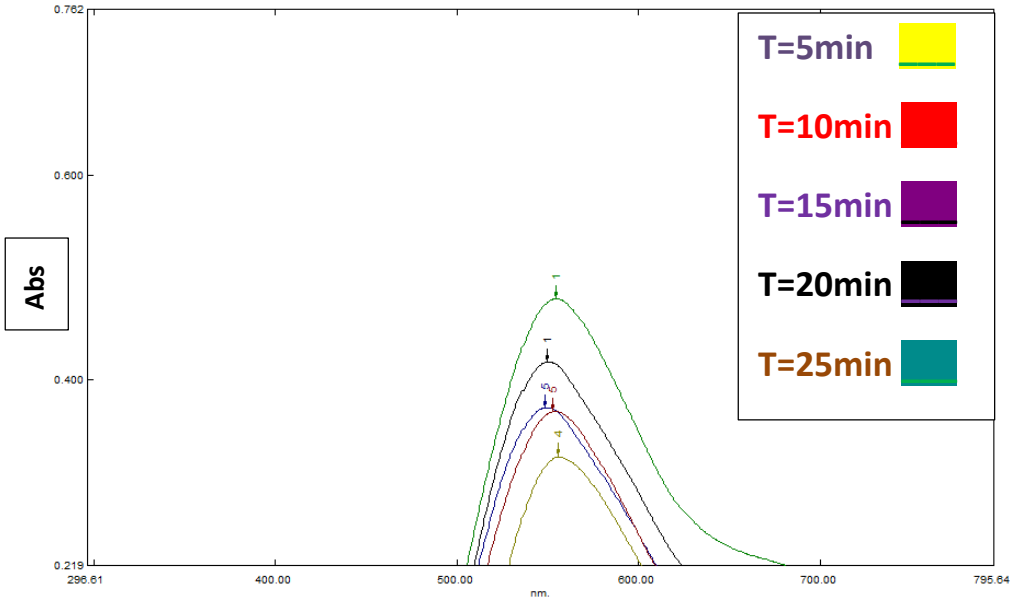


Figure (3-12): Effect centrifugation time on absorption of [pb(II)-R] complex

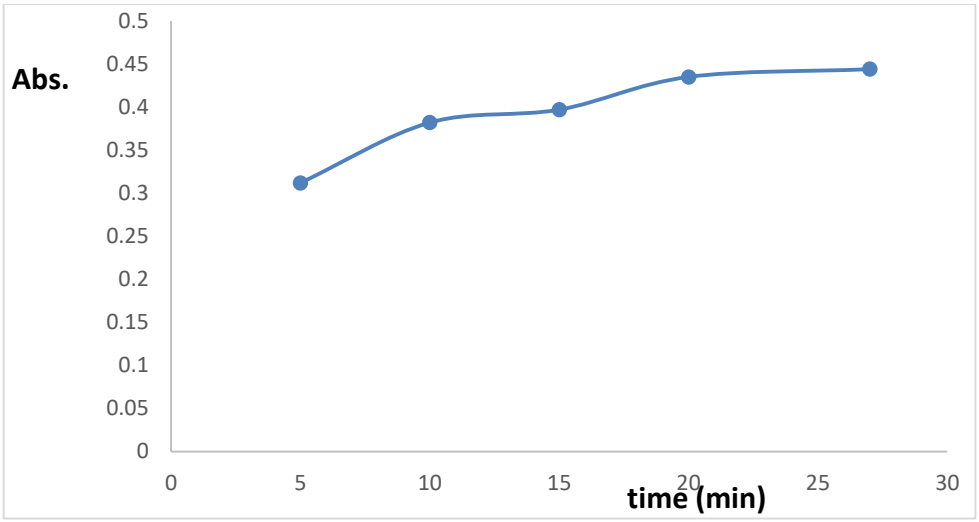


Figure (3-13): Effect of centrifuge time on the determination of pb(II). Conditions: pH 4.2, 3µg.mL^{-1} of pb(II); 0.8 mL (1.0 x 10^{-3} mol.L^{-1}) of (R.); 0.6mL (5%(v/v)) of Triton X– 114; Equilibration temperature 50 °C and heating time 15 min)

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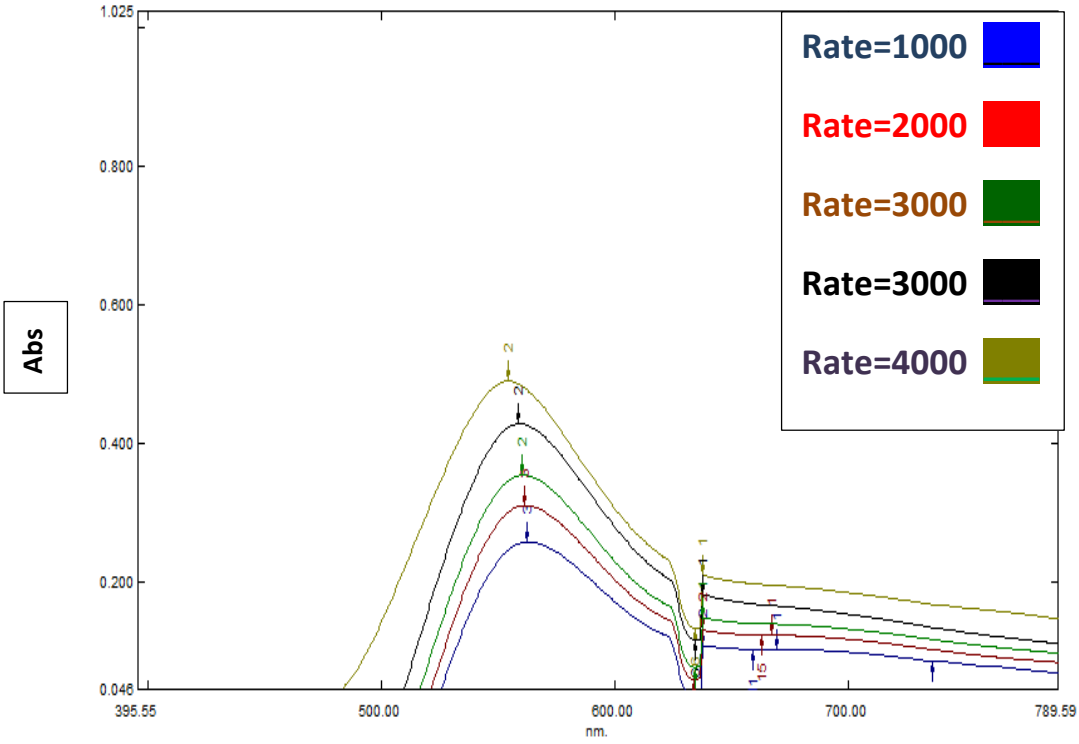


Figure (3-14): Effect centrifuge rate on absorption of [pb(II)-R] complex

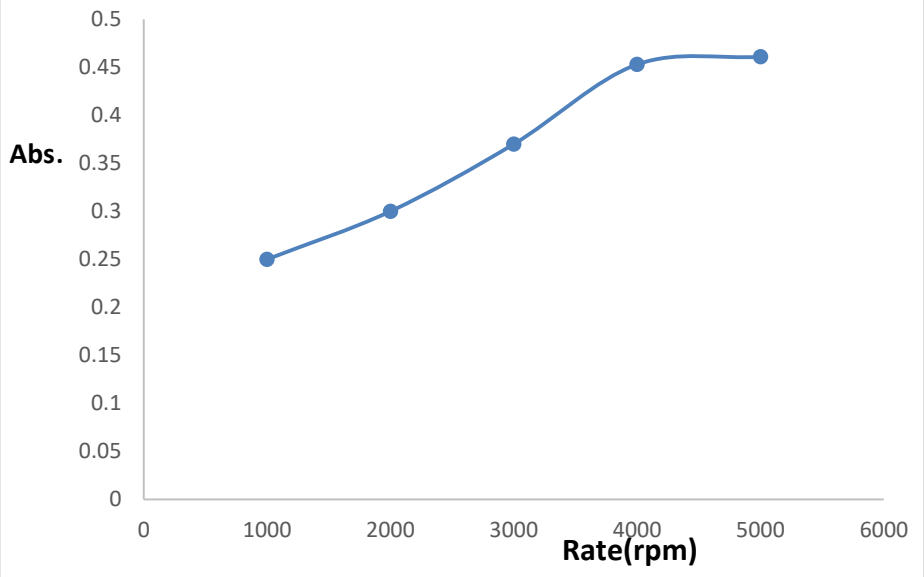


Figure (3-15): Effect of centrifuge Rate on the determination of pb(II). Conditions: pH 4.2, 3µg.mL^{-1} of pb(II); 0.8mL (1.0 x 10^{-3}) mol.L^{-1} of (R.); 0.6mL (5%(v/v)) of Triton X– 114; Equilibration temperature 50 °C and heating time 15 min).

3.1.8 Effect of Salt out

The salts are added to improve internal reaction conditions and increase extraction efficiency. The presence of the salts affects the increase of hydrophobic interactions between the micelles, which increases the turbidity of the solution and thus increases the efficiency of the extraction. The effect of salts on CPE was tested by adding 1ml (0.1) mol L^{-1} of the salts listed in Table (3-1). After completion of the required steps of CPE, the absorbance of the test solutions was measured. From the results shown in Figure (3-16), it is noticed that the salts used in this experiment have no effect on the absorption of the complex.

Table 3-1: Absorbance values under the influence of salt out

Salt out	Abs
NaCl	0.483
$MgSO_4.7H_2O$	0.411
$NaNO_3$	0.409
NH_4NO_3	0.488

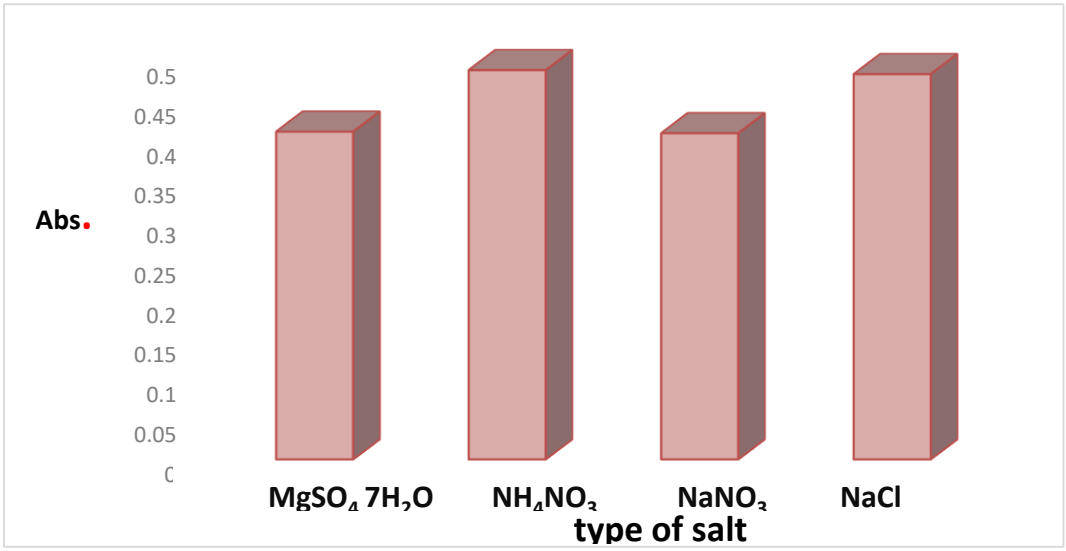


Figure (3-16): Effect of salt out on the determination of pb(II). Conditions: pH 4.2; 3 µg.mL^{-1} of pb(II); 0.8 mL (1.0 x 10^{-3}) mol.L^{-1} of (R.); 0.6mL (5%(v/v)) of Triton X– 114; Equilibration temperature 50 °C and heating time 15 min.

3.2 Stoichiometric Complex Determination

The Job's method was used to find the stoichiometric ratio between lead (II) and (R) by measuring the absorption of the complex formed using a Uv-visible spectrophotometer at (560) nm for a series of prepared solutions by adding distinct volumes for each of them and at the same concentration level (1×10^{-3}mol.L^{-1}). The results in Figure (3-17) represented that the stoichiometric ratio between pb (II) and (R) was 1: 2.

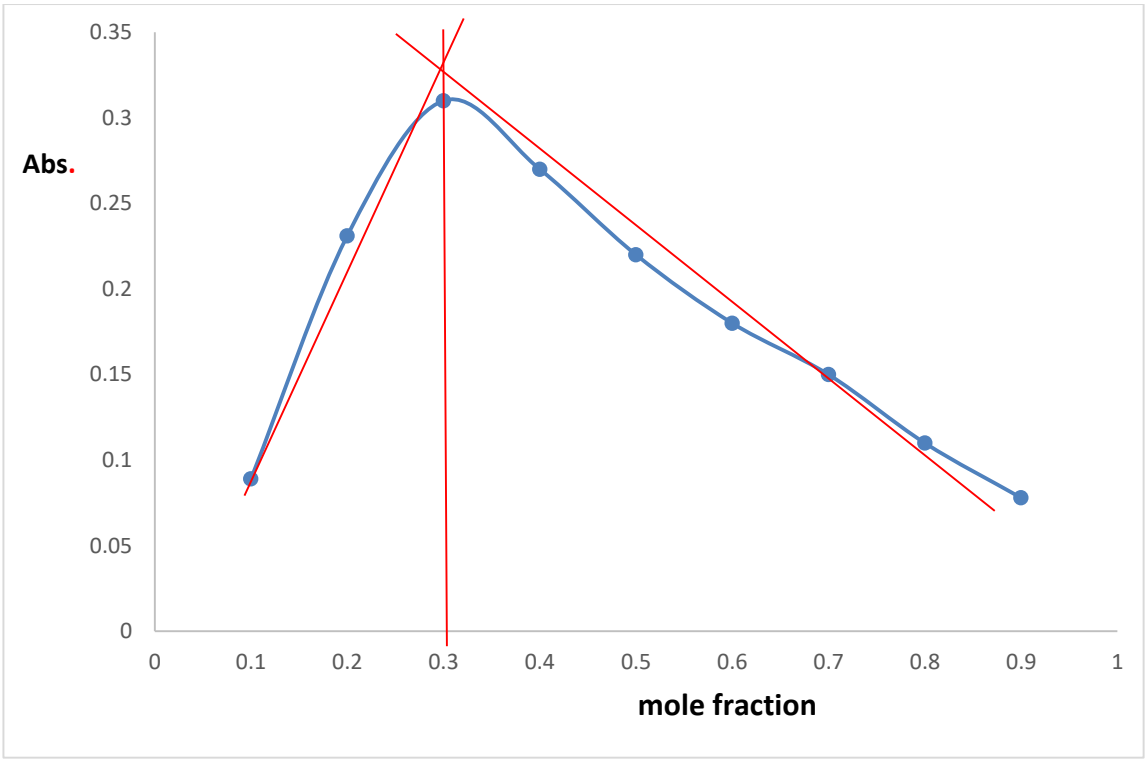
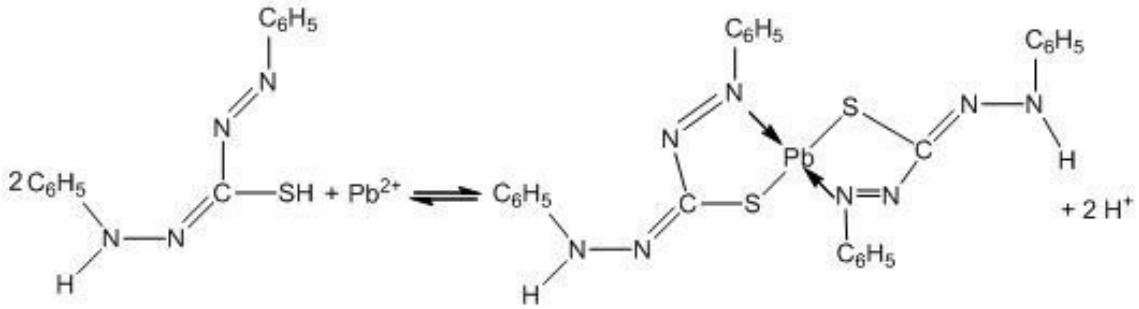


Figure (3-17): Continuous variables method

In molar ratio method, the absorbance was measured for a series of solutions containing constant volumes of pb(II) and various volumes of (R) at the same concentration level (1×10^{-3} mol.L^{-1}), then diluted to the required volume. By using the same analytical method, the stoichiometric ratio was determined as shown in Figure (3-18) to be 1: 2.



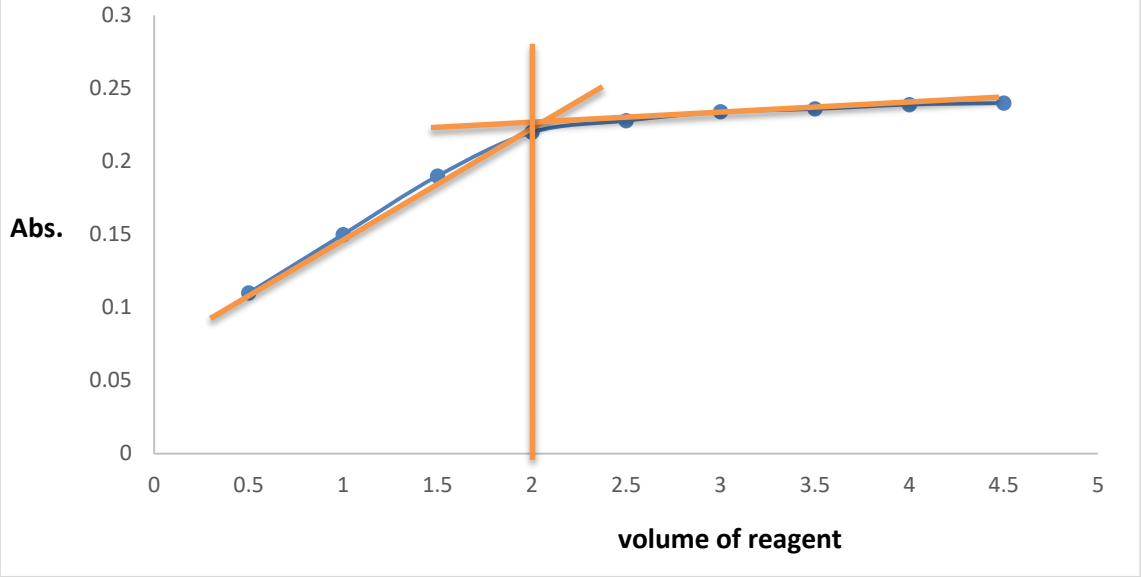


Figure (3-18): Mole-ratio method

3-3 Calibration Curve for lead(II)

The linear calibration curve was obtained with CPE by preparing solutions containing standard concentrations of copper within the range (0.06 - 3) $\mu g.mL^{-1}$, applying the CPE procedure at optimal conditions obtained from previous experiments and represented in Table (3-2). Absorbance was measured at λ_{max} (560) nm, and a linear calibration curve was established between the absorbance values versus the lead(II) concentration as in Figure (3-19). Information for the calibration carve and other analytical parameters for this work is summarized in Table (3-3). The standard deviation of the response and the slope of the calibration curve were obtained using the following equations; Limit of detection (LOD) = 0.018 σ_B /s; Limit of quantification (LOQ) = 0.08 σ_B/s, where (σ_B) is the standard deviation and (s) its slope.

Table 3-2: Optimum conditions for CPE to determine pb (II)

Conditions	Value
pH	4.2
Conc. of Triton X- 114 and volume	5 % (v/v) of 0.6mL
Centrifugation time (min.)	20
Centrifugation rate (rpm)	4000
Temperature. (°C)	50
Conc. of complexing agent and volume used	1×10^{-3} (mol.L $^{-1}$) by 0.8 mL
Incubation time (min.)	15

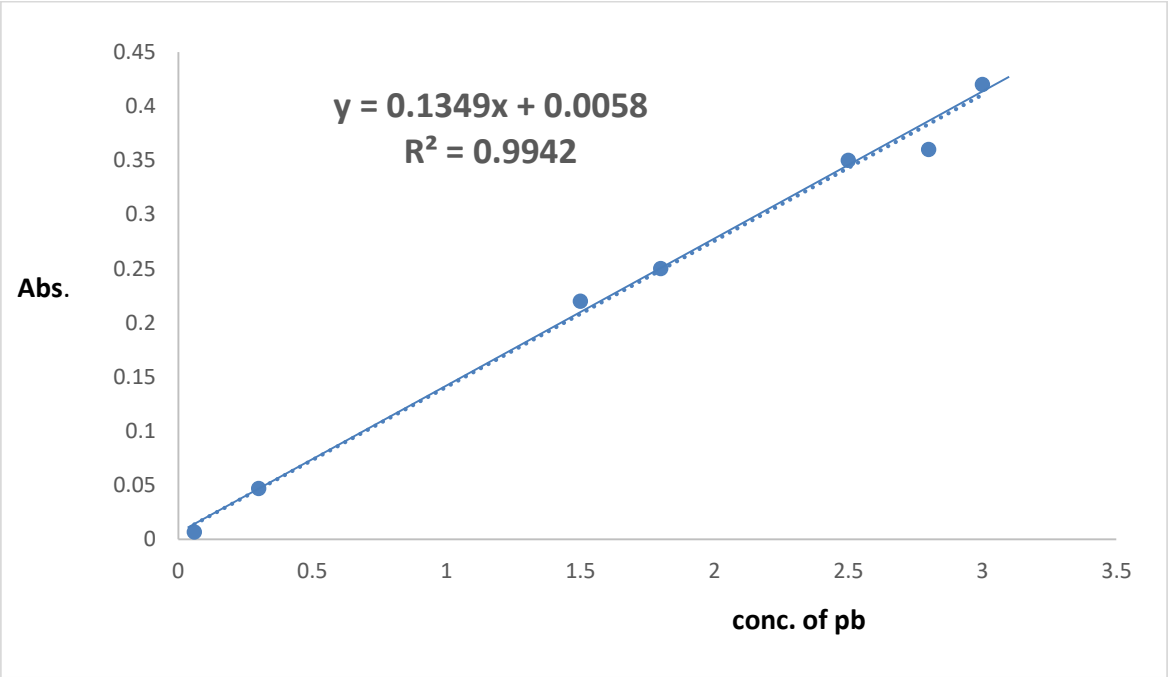


Figure (3-19): Calibration curve for [pb(II)-R] complex using CPE method

Table 3-3: Analytical properties of CPE in determining pb(II)at optimum conditions

Parameters	Value
λ_{max} (nm)	560
Regression equation	y = 0.1349x + 0.0058
Correlation coefficient, R^2	0.9942
Linear calibration range μg.mL^{-1}	0.06-3
Relative standard deviation	1.9
Limit of quantification μg.mL^{-1}	0.018
Molar absorption coefficient L.mole^{-2}. cm^{-2}	27951.28
Enrichment factor	17
M:L	1:2

3.4 Comparison CPE Method with another Analytical Method

After determining the optimal experimental conditions for the determination of lead (II) by CPE and shown in Table (3-2), the method was applied to real samples from different water samples The impurities and suspended particles were removed using a 0.45 μm membrane filter. After applying all the experimental conditions and removing the effect of probable interferences by adding ascorbic acid at an appropriate concentration and using the standard addition method, the lead content was measured in all samples and the results of the proposed method were compared with the standard method (method (FAAS)). Table (3-5) shows the results of the two methods.

Statistical analysis was applied to the results of the two methods in determining lead (II) and listed in Table (3-4) to evaluate the trueness and precision of the proposed method and to reveal whether there is a difference between the two methods or not, as it was noted that the value of t and F were (1.65) and (1.08) respectively, which are less than the critical values for each of (T) and (F) Critical two-tailed at a 95% confidence level and as shown in Table (3-4), which means that the two methods do not differ

significantly at 2.681 a 95% confidence level and that the proposed method has the precision and reliability and can be applied in determining lead (II) in aqueous samples.

Table 3-4: Results of statistical analysis

Statistical Analysis (*t* and *F* tests)			
Observation	15	Mean FAAS	0.2166
Degree freedom (df)	14	Mean CPE	0.2058
Pearson Correlation		0.9942	
***t* Stat**	1.65	*F* calculated	1.08
T Critical two-tailed	2.12	*F* Critical two-tailed	2.681

Table 3-5: Results of (CPE) method compared to (FAAS) method for determining Pb (II) in aqueous samples

Seq	Pb(II)	Pb(II) atomic	
١	٠,٠١١٢	٠,٠١٧٨	**Shatt al-Hilla (Al-Farsi sewage station)**
٢	٠,٠٢٠٥	٠,٠٢١٤	**Shatt al-Hilla (The drain point of treatment unit)**
٣	٠,٠٠٥٩	٠,٠٠٧٥	**Shatt al-Hilla –Batah bridge**
٤	٠,٠١٩٠	٠,٠٢٠١	**AL-Jerboa River**
٥	٠,٠٢٧١	٠,٠٢٩١	**AL-kifl river**
٦	٠,٠٣٩٨	٠,٠٤٠٣	**AL-Yahodia River**
٧	٠,٠٢٣٩	٠,٠٢٧١	**Abu al-Agyar**
٨	٠,٠١٩٦	٠,٠٢١٠	**The big well for Prophet Job**
٩	٠,٠٢٠٠	٠,٠٢٣١	**The small well for Prophet Job (1)**
١٠	٠,٠١٤٤	٠,٠١٥٧	**The small well for Prophet Job (2)**
١١	٠,٠٠٩٢	٠,٠١٠٢	**Bakerly**
١٢	٠,٠١٠٤	٠,٠١٩٤	**Nader /2**
١٣	٠,٠٣٩٠	٠,٠٤١٢	**The new treatment unit for wastewater**
١٤	٠,٠٢٦٠	٠,٠٢٨٥	**Old sewage treatment unit for wastewater**
١٥	٠,٠٣٥٢	٠,٠٣٧٨	**Standard solution of pb(II)**
١٦	٠,٠٢٤٢	٠,٠٢٦٣	**Shatt al-Hilla (Al-Farsi sewage station)**
١٧	٠,٠٢٠٨	٠,٠٢٢٤	**Shatt al-Hilla (The drain point of treatment unit)**
١٨	٠,٠٢٣٧	٠,٠٢٦٩	**Shatt al-Hilla –Batah bridge**
١٩	٠,٠٢٤٢	٠,٠٢٨٧	**AL-Jerboa River**
٢٠	٠,٠٣٣٥	٠,٠٣٤٥	**AL-kifl river**
٢١	٠,٠٣٨٠	٠,٠٤٠٢	**AL-Yahodia River**
٢٢	٠,٠٣٨٢	٠,٠٣٩١	**Abu al-Agyar**

3.5 Cloud-Point Extraction for [Cobalt (II) -(R)] complex

3.5.1 Spectrophotometric Investigation

The complex formed by adding the Co(II) to the complexing agent was revealed by UV-visible spectroscopy. Figure (3-20) shows the absorption spectrum of [Co (II)-R] complex and λ_{max} at 550 nm. By comparing the absorption spectrum of the complex with the absorption spectrum of (R) and Co(II), the analytical signal of [Co (II)-R] complex can be considered as evidence of its formation. Accordingly, this wavelength was used to determine the cobalt(II) ions content in all experiments.

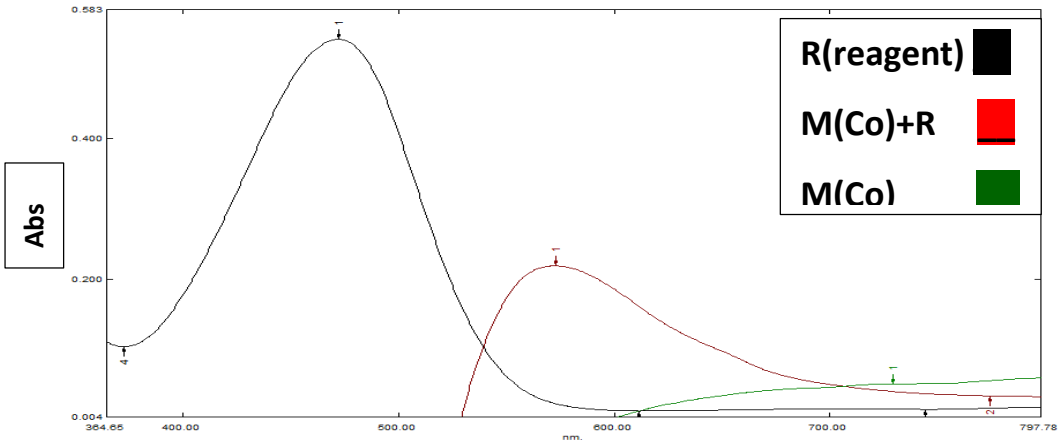


Figure (3-20): Absorption spectrums of: cobalt (II) ions, complexing agent (R), and [Co (II)-R] complex.

3.5.2 pH effect

A pH plays a paramount role, in the CPE procedure and it is the first factor that needs to be adjusted when applying an extraction process before moving on to the next step ,as well as pH is an important factor during the embedding process for analyzes of generating [metal-ligand] complexes ,so the results of the extraction depend on determining the degree of pH at which the complex is formed [161]. On this view, a series of experiments were carried out to evaluate the effect of pH on the extraction process by preparing similar solutions containing 6 $\mu g.mL^{-1}$ of cobalt (II) standard solution and 0.6 mL $1x10^{-3}$ $mol.L^{-1}$ of (R) in presence of 0.8 mL (5% (v/v)) of TritonX-114 within pH ranges (3-6.5) which were adjusted with

different NaOH and HCl solutions. Figures (3-21) and (3-22) show the experimental results of this test after the absorbance was recorded for all samples at 550 nm

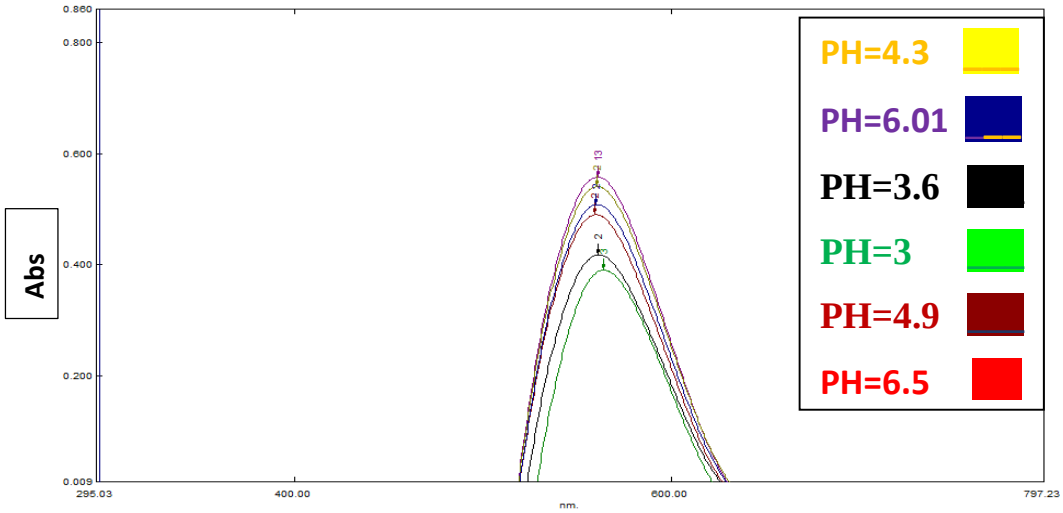


Figure (3-21): Absorption spectra of [Co(II) -R] complex under effect of pH

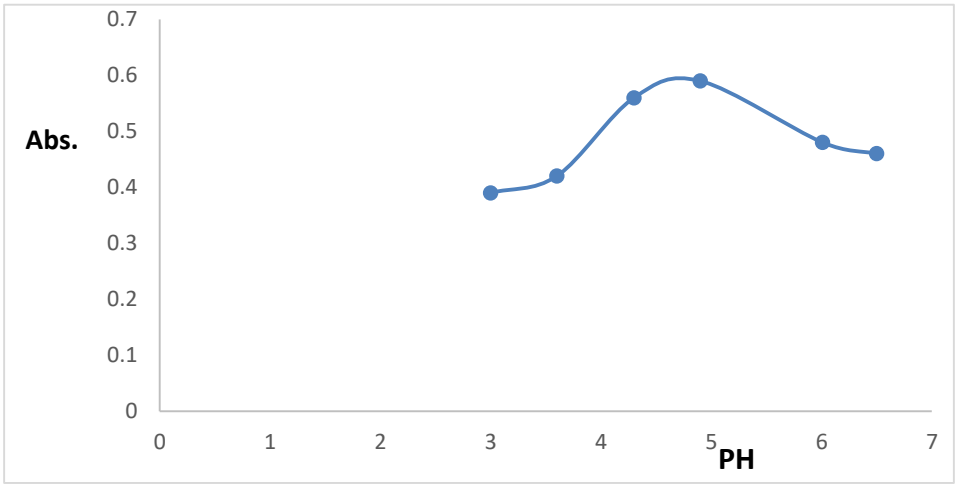


Figure (3-22): Influence of pH of the medium on extraction efficiency of Co (II). Conditions:6 µg mL^{-1} of Co (II); 0.6 mL (1x10^{-3}) mol.L^{-1} of (R); 0.8 mL (5% (v/v)) of Triton X– 114; Equilibration temperature 45 °C and heating time 20 min).

On the other hand, absorption values a decrease with the direction of the solution towards alkalinity, may be due to the instability of the complex at basic medium that results in dissociation of the complex. Accordingly, a pH of 4.9 was chosen as the best point for forming the complex and which do not require another NaOH and HCl solution.

3.5.3 Effect of (R) Concentration

Concentration of reagent is one of the factors that affect the CPE procedure because it is the transport medium for metal ions to the rich phase of the surfactant [162]. Therefore, it is necessary to know the appropriate amount of reagent to transfer the maximum possible amount of cobalt ions to SRP and extract it. In this work, the effect of reagent concentration on the extraction process in CPE was tested by preparing similar solutions containing different reagent volumes (0.2-1) mL (1x 10^{-3})mol.L^{-1} with 6 µg.mL^{-1} of cobalt (II) standard solution in the presence of 0.8 mL 5% (v/v) of TritonX-114 at pH 4.9. After applying the CPE procedure, the absorption spectra of the complex were obtained at different levels as in Figure (3-23), also Figure (3-24) explain the behavior of this effect.

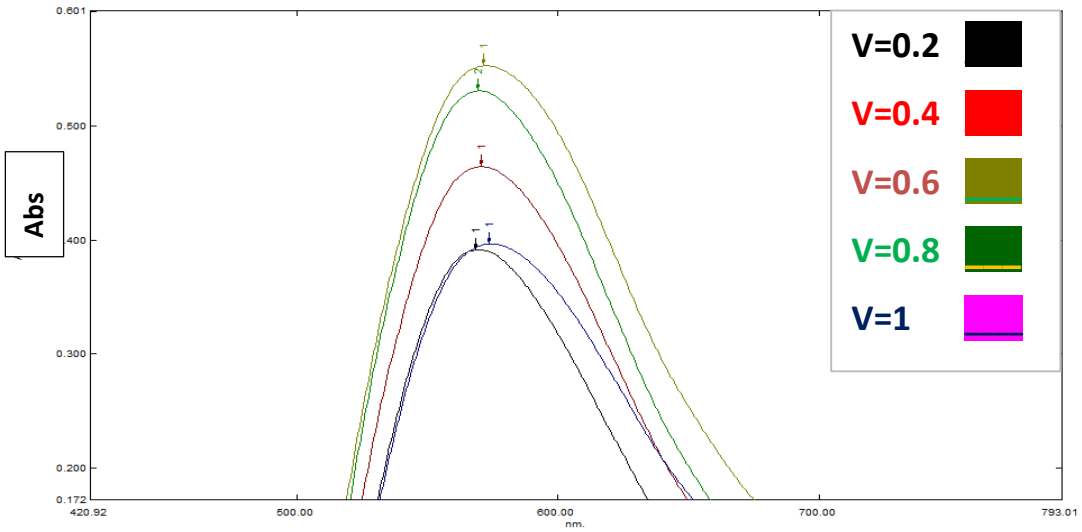


Figure (3-23): Absorption spectra of [Co (II) -R] complex under effect of concentration of reagent (R).

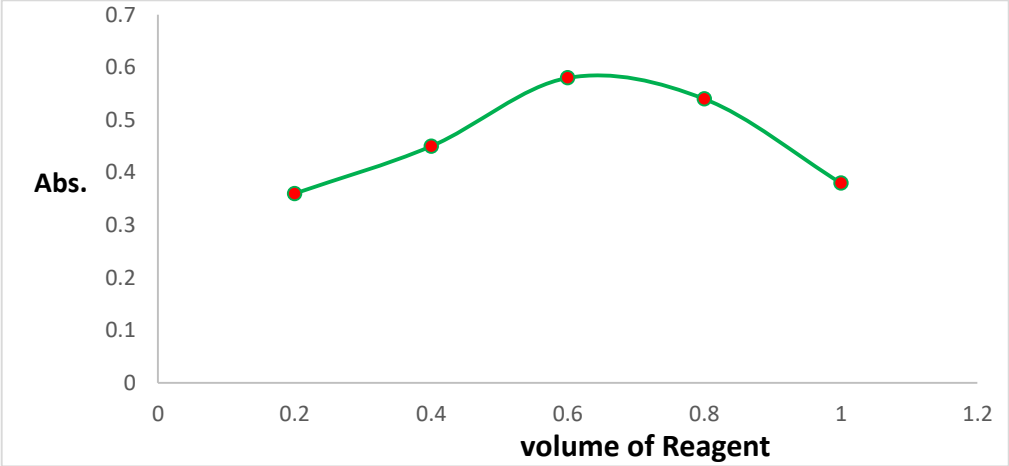


Figure (3-24): Test results for effect of reagent concentration on extraction efficiency of Co(II). Conditions: pH 4.9; 6µg.mL^{-1} of Co(II), 0.8 mL 5% (v/v) of Triton X– 114, Equilibration temperature 45 °C and heating time 20 min).

From Figure (3-24), It is possible to choose 0.8 mL of the reagent because it provides less consumption in the amount of reagent with maximum extraction, also, that high concentrations of (R) are not necessary and have resulted in a significant decrease in the absorbance values.

3.5.4 Effect of TritonX-114 Concentration

Concentration of surfactant is one of the factors affecting the action of CPE, since surfactant is an extraction medium by which the pre-concentration agent can be optimized to reach maximum extraction efficiency by reducing the volume ratio of the surfactant-rich phase to the aqueous phase [(163)]. Therefore, effect of surfactant concentration on [Co(II)-R] extraction was studied by using varying volumes within ranges (0.2-1) mL of 5% (v/v) Triton X-114. After applying the general CPE procedure, the results were obtained in Figures (3-25) and (3-26)

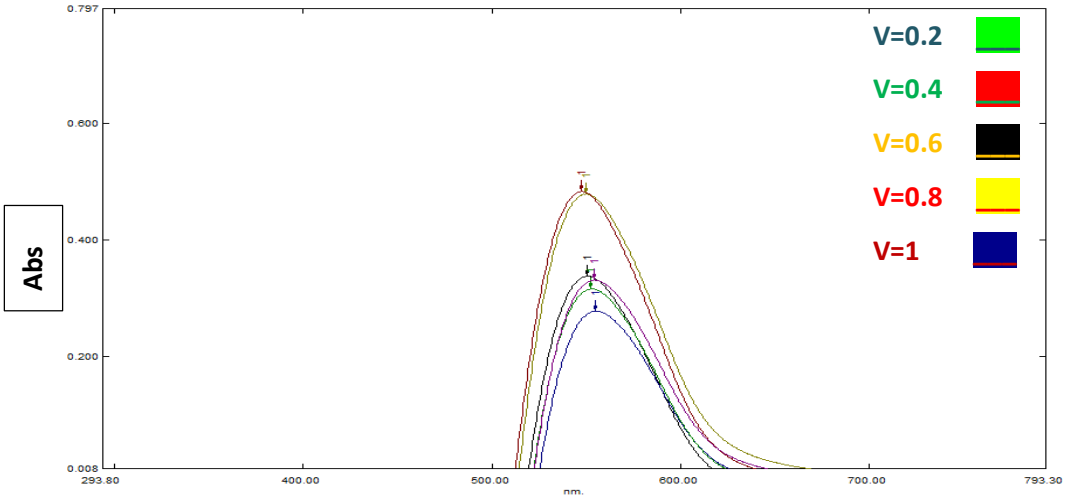


Figure (3-25): Absorption spectra of [Co (II) -R] complex under effect of concentration of Triton X-114.

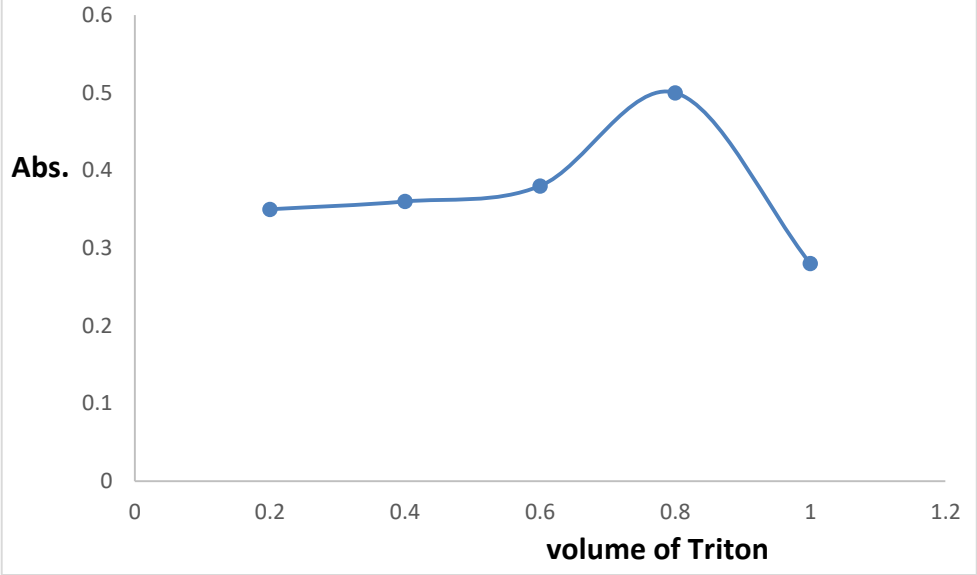


Figure (3-26): Test results for the effect of Triton X- 114 concentration on extraction efficiency of Co(II). Conditions: pH 4.9; 6 µg.mL^{-1} of Co (II); 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); Equilibration temperature 45°C and heating time 20 min).

It is shown in Figure (3-26) that the cobalt(II) ions extraction rate is increased to the maximum possible at 0.8 mL of Triton X-114 and it appears that the concentration of Triton X-114 at this point was able to capture the greatest possible amount of complex in SRP. On the other hand, the gradual decrease in the absorption values is associated with the presence of high concentrations of the surfactant due to an increase in the viscosity of the rich surface phase, which leads to a decrease in the sensitivity [(164)]. Therefore, 0.8 mL, 5% (v/v) of Tritonx-114 was chosen as the ideal concentration and used in all subsequent experiments.

3.5.5 Effect of Equilibrium Temperature

Several published studies indicated that increasing the temperature above the cloud point reduces the hydrophilic surfactant property of non-ionic surfactants during the application of the CPE procedure and helps to induce phase separation phenomenon [165]. In addition, the temperature is a critical factor in the formation of the micelle and it may affect the stability of the complex [162]. To reach the maximum efficiency in the extraction process, we performed a temperature effect test by applying different temperatures to the test samples (35-55)°C under the optimum conditions for the previous tests. A constant temperature was obtained for the extraction process showing an excellent condition at 45°C, as represented in Figures (3-27) and (3-28).

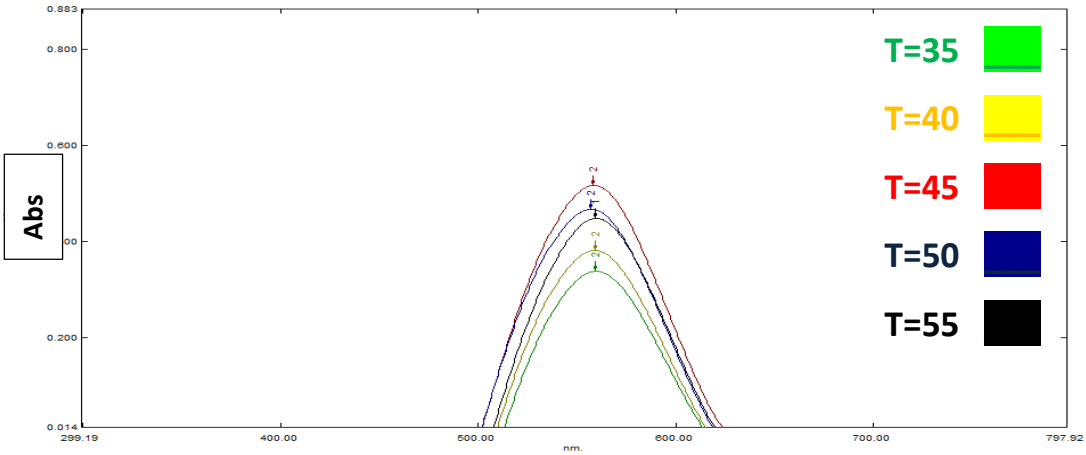


Figure (3-27): Absorption spectra of [Co (II) -R] complex undr effect of equilibration temperature

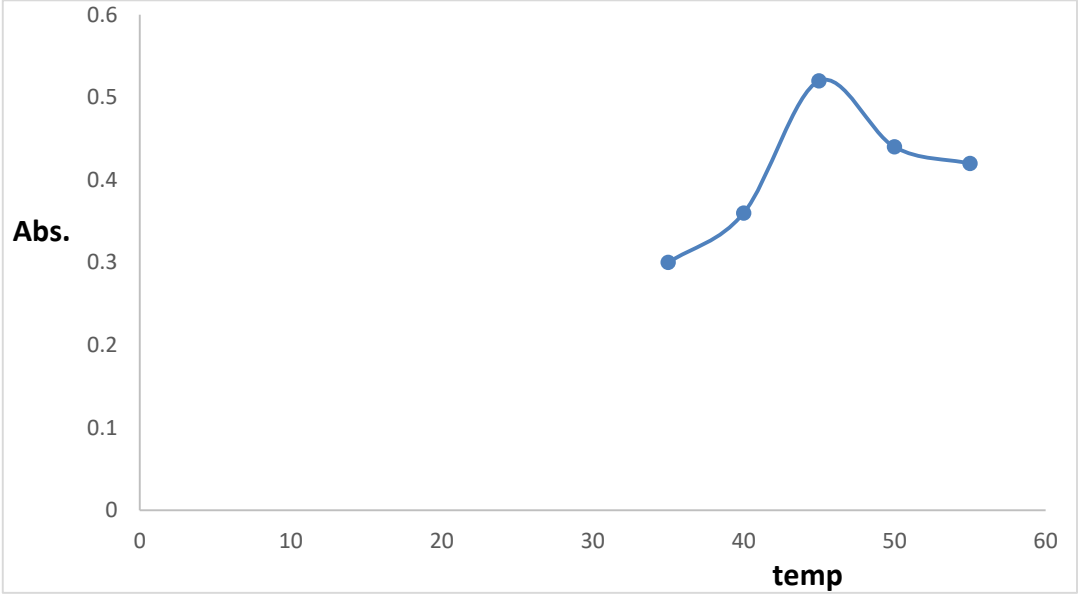


Figure (3-28): Test results for the effect of equilibration temperature on extraction efficiency of Co(II). Conditions: pH 4.9, 6µg.mL^{-1} of Co (II); 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); 0.8 mL 5% (v/v) of Triton X– 114 and heating time 20 min).

From the test results in Figure (3-28), it was observed that the absorption values of the extracted complex increase gradually with the rise in temperature to 45 °C .This can be explained results the decrease in the volume of the rich phase of the surfactant because of decrease in the water content which leads to an increase in sensitivity [157]. On the other hand, it is observed that the absorption values of the complex decrease when the temperature rises, maybe the reason that is due to the instability of the complex at high temperatures and thus causes this decrease.

3.5.6 Effect of Incubation Time

Incubation time has great influence on CPE process, as the appropriate incubation time and temperature lead to the completion of the separation of the two phases and thus affect the extraction efficiency [166]. To evaluate the effect of incubation time, repeated tests were carried out for different times (5-30) min when placing the samples in the water bath, while keeping other parameters at the optimal conditions obtained through previous experiments. Figures (3-29) and (3-30) explain the results of the incubation time test.

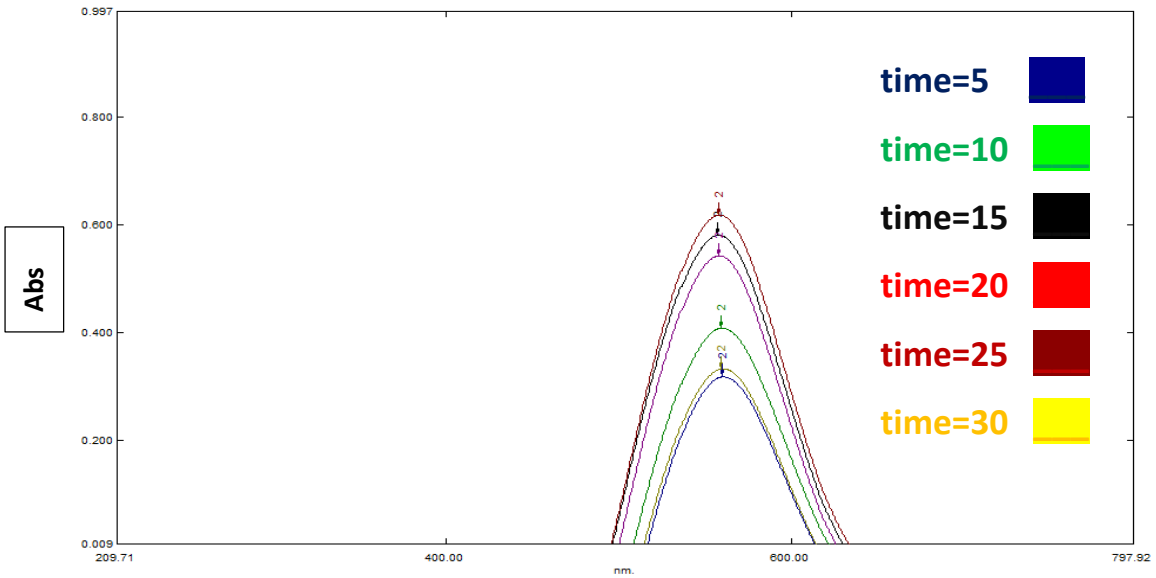


Figure (3-29): Absorption spectra of [Co (II) -R] complex under effect of Incubation time

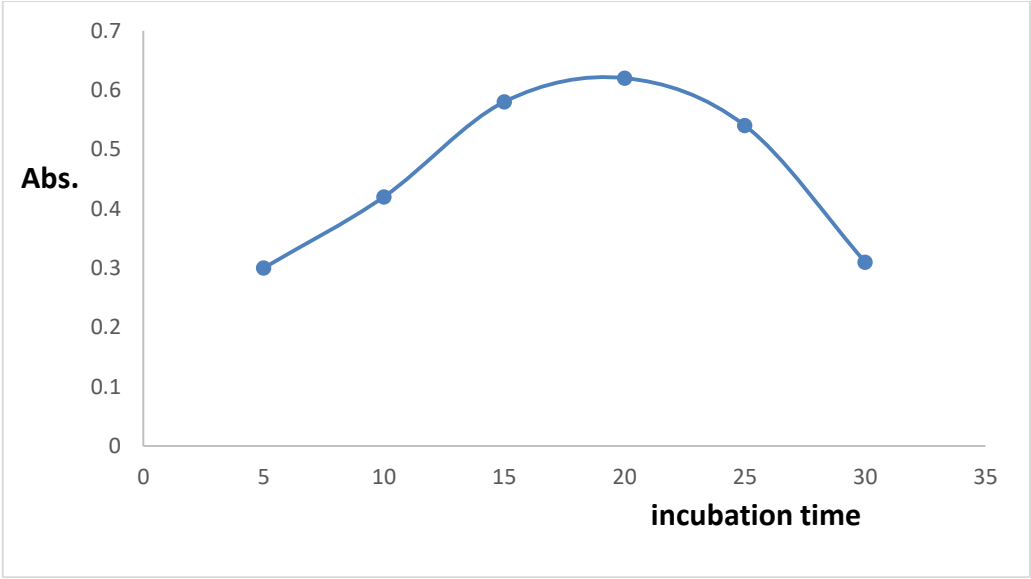


Figure (3-30): Test results for the effect of equilibration time on extraction efficiency of Co (II). Conditions: pH 4.9; 6µg.mL^{-1} of Co(II); (R.), 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); 0.8 mL 5% (v/v) of Triton X– 114 and equilibrium temperature 45 °C).

It was found that the analytical signal increases until it reaches the point (20) min and then stabilizes relatively with the increasing time. It can be considered that 20 min is sufficient time to achieve a good separation of the solution, as it is preferable that the extraction process takes place in a shorter time and with higher efficiency. Therefore, this point was chosen as the best incubation time and applied in all subsequent experiments.

3.6.7 Effect of Centrifugation Time and Rate

The effect of centrifugation time on the extraction efficiency of cobalt (II) ions in CPE was studied using different times (5-30) minutes to assess the effect of time on the centrifugation process. Through the results shown in Figures (3-31) and (3-32), The time (20) min was chosen as a short time for higher extraction

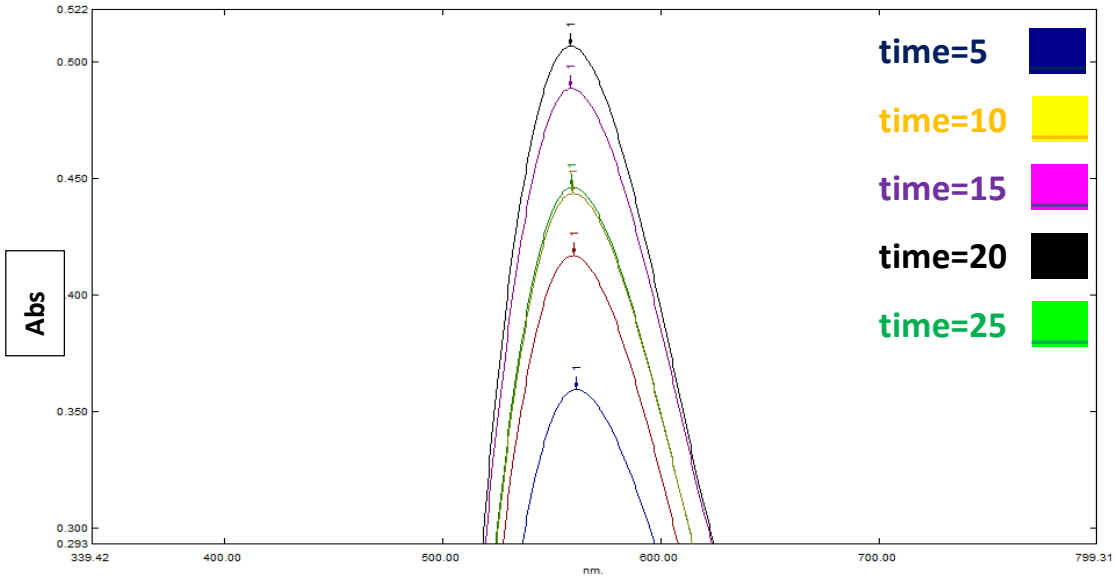


Figure (3-31): Absorption spectra of [Co(II) -R] complex under effect of centrifugation time

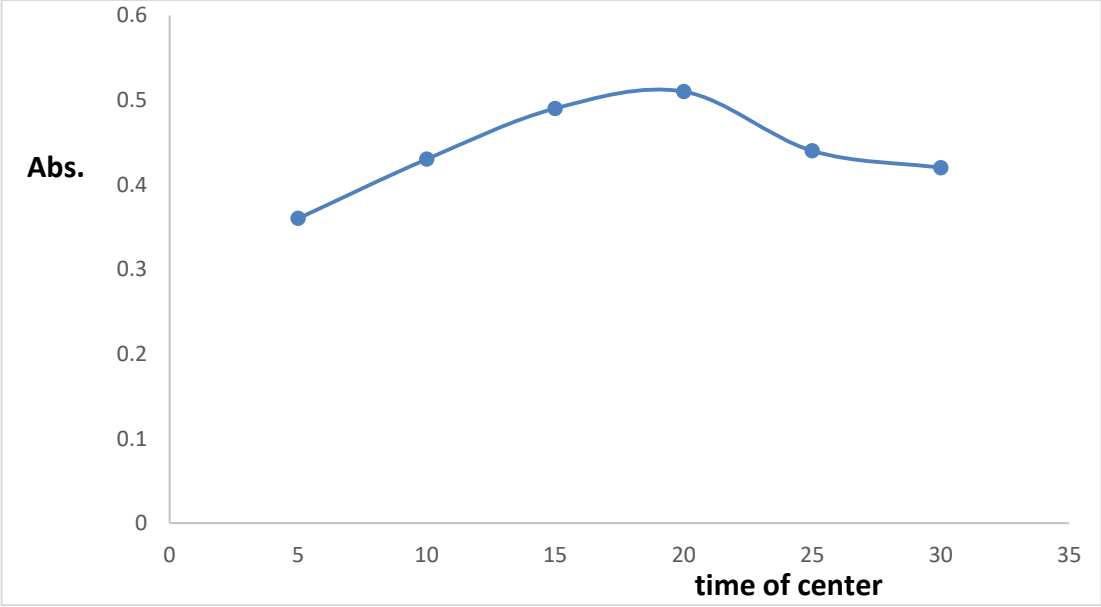


Figure (3-32): Test results for the effect of of centrifuge time on extraction efficiency of Co (II). Conditions: pH 4.9; 6µg.mL^{-1} of Co (II); 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); 0.8 mL 5% (v/v) of Triton X– 114; Equilibration temperature 45 °C and heating time 20 min).

Keeping time constant at (20 min), the centrifugation rate was tested in the range of 1,000 to 5,000 rpm. According to the data obtained in Figure (3-33) and (3-34), it is indicated that at 4000 rpm the complete phase separation is available with the organic phase pooling at the bottom of the test tube and sticking to it. This allows for easy pouring of the aqueous phase without losing any part of the analyte, so 4000 rpm was chosen as the ideal rate.

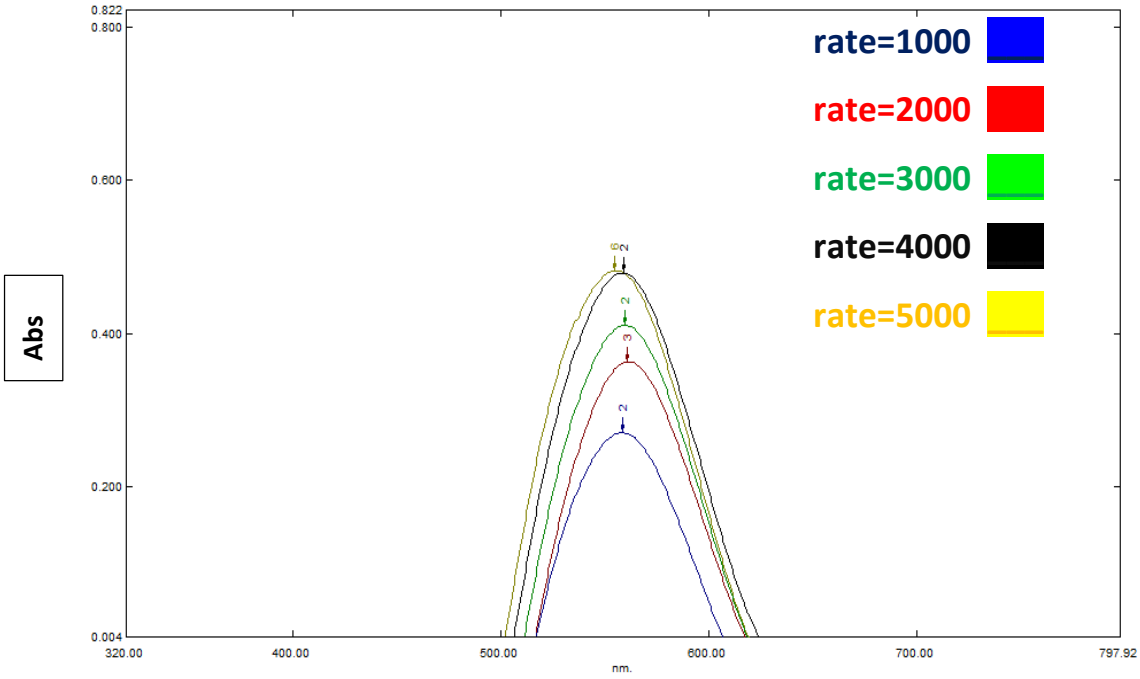


Figure (3-33): Absorption spectra of [Co (II) -R] complex under effect of centrifuge rate

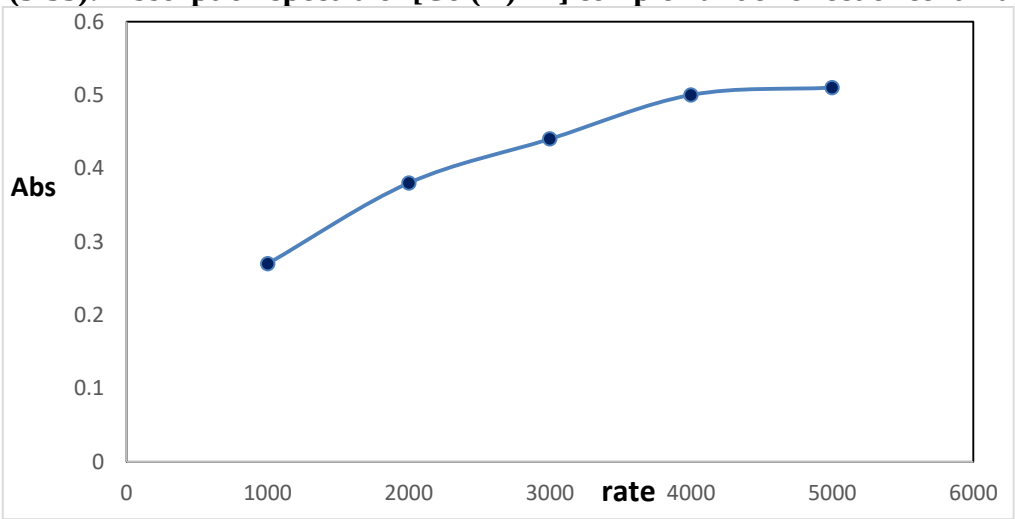


Figure (3-34): Test results for the effect of of centrifuge Rate on extraction efficiency of Co(II). Conditions: pH 4.9; 6 µg.mL^{-1} of Co(II); 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); 0.8 mL 5% (v/v) ofTriton X– 114; Equilibration Temperature 45°C and heating time 20 min).

3.5.8 Effect of salt out

The literature indicates that salting may improve the extraction process to achieve more efficient extraction by enhancing the hydrophobic character in the accumulation of dissolved organic matter [167]. The effect of salts on CPE were tested by adding 1 mL of 0.1 mol.L^{-1} of NaCl, $MgSO_4.{}_7H_2O$, $NaNO_3$, NH_4NO_3 to the solution. After applying the steps required for CPE, the absorbance of the test solutions was measured as shown in Table (3-6). Figure (3-35) represented that the salts used in this experiment had no effect on the absorption of the complex.

Table (3-6): Absorbance of [Co(II)–(R.)] complex under effect of salt out

Salt out	Abs
NaCl	0.592
$MgSO_4.7H_2O$	0.557
$NaNO_3$	0.523
NH_4NO_3	0.492

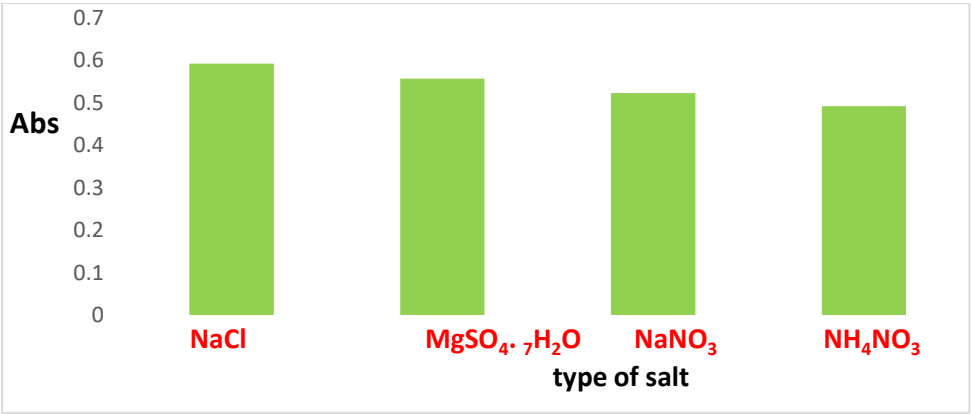


Figure (3-35): Test results for the effect of salt out on extraction efficiency of Co(II). Conditions: pH 4.9; 6µg.mL^{-1} of Co(II); 0.6 mL (1×10^{-3}) mol.L^{-1} of (R); 0.8 mL 5% (v/v) of Triton X– 114; Equilibration temperature 45 °C and Equilibration time 20 min.

3.6 Stoichiometric Complex Determination

Job's method was used in order to find the stoichiometric ratio between Co (II) and (R). A series of solutions were prepared by adding distinct volumes of standard cobalt (II) solution and reagent solution at the same concentration level $1x10^{-3}$ $mol.L^{-1}$. The results in Figure (3-36) showed that the stoichiometric ratio between Co (II) and (R.) was 1:2.

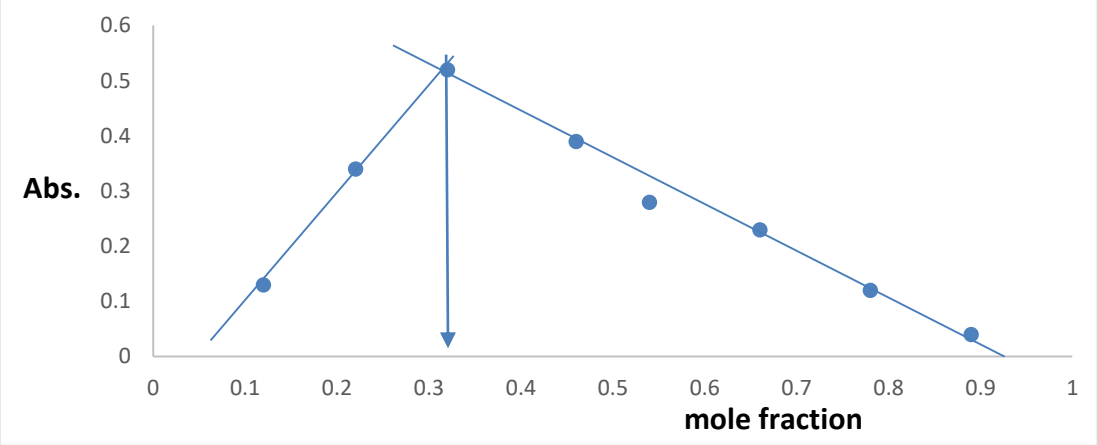


Figure (3-36): Continuous variables method

In the molar ratio method, the absorbance was measured for a series of solutions containing constant volumes of Co(II) and various volumes of (R) at the same concentration level (1×10^{-3} $mol.L^{-1}$) then diluted to the required volume. By using the same analytical method, the stoichiometric ratio was determined as shown in Figure (3-37) to be 1:2.

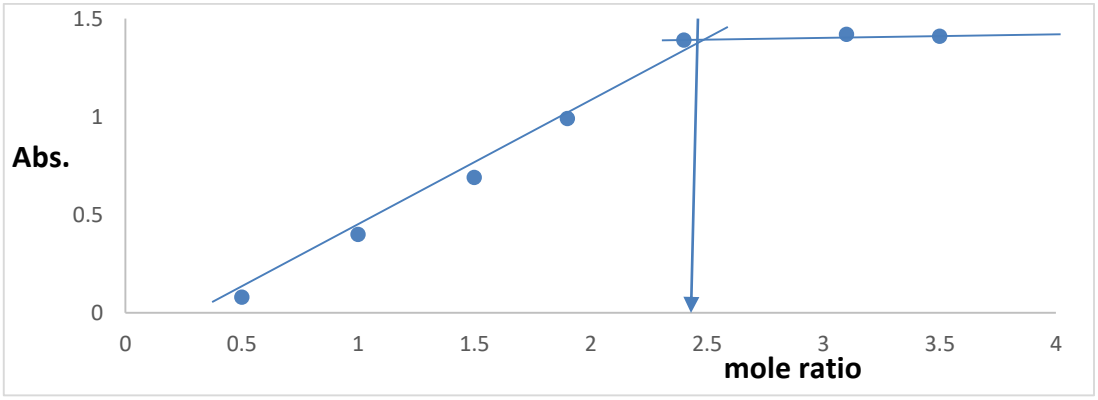


Figure (3-37): Mole-ratio method

3.7 Calibration Curve for Co(II)

The linear calibration curve was obtained with CPE by preparing solutions containing standard concentrations of cobalt(II) ions within the ranges (0.3-6.0) $\mu g.mL^{-1}$ after applying CPE procedure at optimal conditions obtained from previous experiments and shown in Table (3-7), where absorbance was measured at λ_{max} (550) nm, and a linear calibration curve was established between the absorbance values versus the cobalt (II) concentration as in the Figure (3-38).

Information for the calibration curve and other analytical parameters for this work are summarized in Table (3-8).

Table 3-7: Optimum conditions for CPE to determine Co (II).

Conditions	Value
pH	**4.9**
Concentration of (R).	**(1×10^{-3}) $(mol.L^{-1})$ by 0.8 mL**
Concentration of surfactant	**0.6 mL (5% (v/v)) of TritonX-114**
Equilibrium temperature(°C)	**45**
Equilibration time (min.)	**20**
Centrifugation rate (rpm)	**4000**
Centrifugation time (min.)	**20**

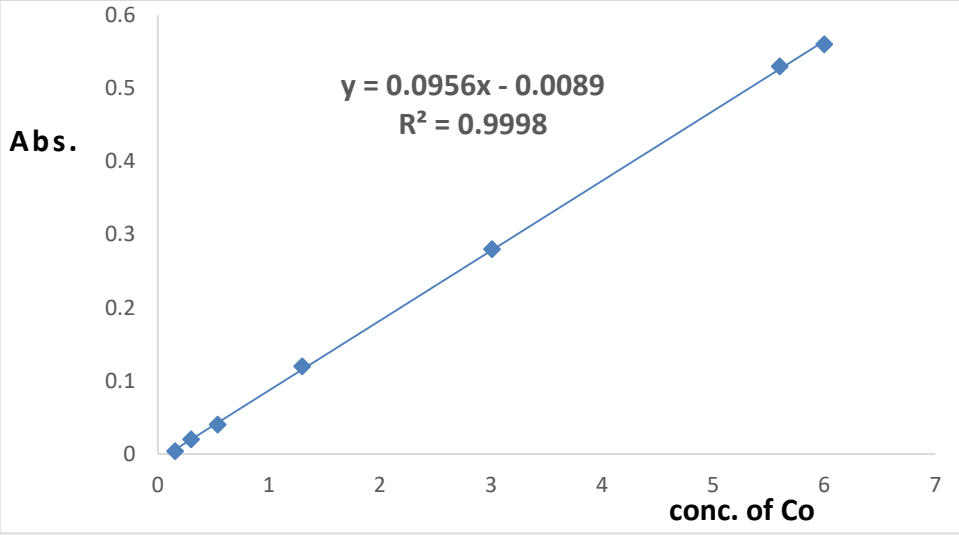


Figure (3-38): Calibration curve for [Co(II)-R] complex using CPE method

Table 3-8: Analytical properties of CPE in determining Co(II)

Parameters	Value
λ_{max} (nm)	550
Regression equation	0.0956x-0.0089
Correlation coefficient, R^2	0.9998
Linear calibration range μg.mL^{-1}	0.3 -6.0
Relative standard deviation	1.9
Limit of detection μg.mL^{-1}	0.17
Limit of quantification μg.mL^{-1}	0.49
Molar absorption coefficient L.mole^{-2}. m^{-2}	5630.84
Enrichment factor	23
M:L	1:2

3.8 Comparison CPE Method with another Analytical Method

After determining the optimal experimental conditions for the determination of cobalt (II) by CPE and shown in Table (3-7), the method was applied to real samples from different water samples. The impurities and suspended particles were removed using a 0.45 μm membrane filter. After applying all the experimental conditions and removing the effect of probable interferences by adding ascorbic acid at an appropriate concentration and using the standard additives method, the lead content was measured in all samples and the results of the proposed method were compared with the standard method (method (FAAS)). Table (3-10) shows the results of the two methods.

Statistical analysis was applied to the results of the two methods in determining cobalt(II) and listed in Table (3-9) to evaluate the trueness and precision of the proposed method and to reveal whether there is a difference between the two methods or not, as it was noted that the value of *t* and *F* were (1.66) and (0.98) respectively, which are less than the critical values for each of (*T*) and (*F*) Critical two-tailed a 2.12 at 95% confidence level and as shown in Table (3-9), which means that the two methods do not differ significantly at a 95% confidence level and that the proposed method has the precision and reliability and can be applied in determining cobalt (II) in aqueous samples.

Table (3-9): Results of statistical analysis

Statistical Analysis (*t* and *F* tests)			
Observation	15	Mean FAAS	0.5633
Degree freedom (df)	14	Mean CPE	0.5543
Pearson Correlation		0.9998	
t Stat	1.66	*F* calculated	0.98
t- crtical two- tailed	2.12	F-crtical two- tailed	2.681

Table 3-10: Results of (CPE) method compared to (FAAS) method for determining Co (II) in aqueous samples

Seq	Co(II)	Co(II) atomic	
١	٠,١٥٢١	٠,١٥٤٥	Shatt al-Hilla (Al-Farsi sewage station)
٢	٠,٠٧٤٧	٠,٠٨٠١	Shatt al-Hilla (The drain point of treatment unit)
٣	٠,٠٩٣٤	٠,٠٩٧٧	Shatt al-Hilla –Batah bridge
٤	٠,١٢٧٨	٠,١٣٠١	AL-Jerboa River
٥	٠,٠٨٧٣	٠,٠٩١٢	AL-kifl river
٦	٠,٠٩٦١	٠,١٠٠١	AL-Yahodia River
٧	٠,١٣٦٠	٠,١٣٨٥	Abu al-Agyar
٨	٠,١٣٧٢	٠,١٣٩٢	The big well for Prophet Job
٩	٠,٠٩٢٩	٠,١٠٠١	The small well for Prophet Job (1)
١٠	٠,١٢٦٤	٠,١٢٩٤	The small well for Prophet Job (2)
١١	٠,١٥٥٢	٠,١٥٢٨	Bakerly
١٢	٠,١٠٠٨	٠,١١٠٠	Nader /2
١٣	٠,٠٩٦٩	٠,١٠١٠	The new treatment unit for wastewater
١٤	٠,١٨٨٠	٠,١٩٠١	Old sewage treatment unit for wastewater
١٥	٠,٠٩٣٩	٠,١٠٠٩	Standard solution of pb(II)
١٦	٠,١٠٠٥	٠,١٠٩٨	Shatt al-Hilla (Al-Farsi sewage station)
١٧	٠,٠٩٥٧	٠,١٠٠٤	Shatt al-Hilla (The drain point of treatment unit)
١٨	٠,٠٨٨٢	٠,٠٩٢٣	Shatt al-Hilla –Batah bridge
١٩	٠,١١٠٠	٠,١١٢٧	AL-Jerboa River
٢٠	٠,١٤٢٠	٠,١٤٩٨	AL-kifl river
٢١	٠,٠١٢٢	٠,٠١٧٢	AL-Yahodia River
٢٢	٠,٠٨٩١	٠,٠٩٢٠	Abu al-Agyar

3.9 Cloud-Point Extraction for [cadmium (II) -(R)] complex

3.9.1 Spectrophotometric Investigation

The complex formed by adding the Cd(II) to the complexing agent was revealed by UV-visible spectroscopy. Figure (3-39) explain the absorption spectrum of [Cd (II)-R] complex and λ_{max} at 525 nm. By comparing the absorption spectrum of the complex with the absorption spectrum of (R) and Cd(II), the analytical signal of [Cd (II)-R] complex can be considered as evidence of its formation. Accordingly, this wavelength was used to determine the cadmium(II) ions content in all experiments

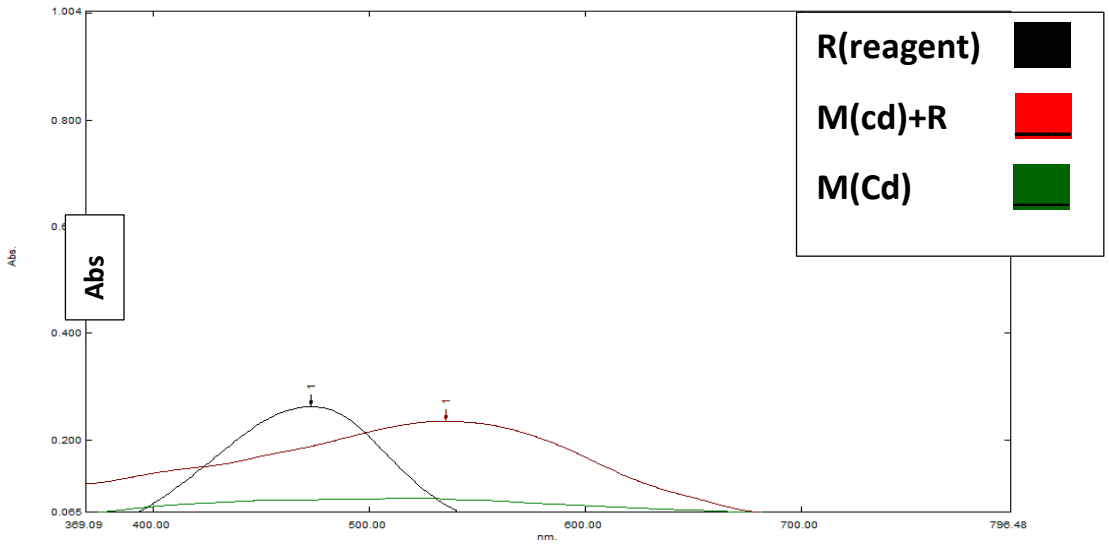


Figure (3-39): Absorption spectrums of: cadmium (II) ions, complexing agent (R), and [Cd (II)-R] complex.

3.9.2 pH effect

A pH plays a paramount role, in the CPE procedure and it is the first factor that needs to be adjusted when applying an extraction process before moving on to the next step ,as well as pH is an important factor during the embedding process for analyzes of generating [metal-ligand] complexes ,so the results of the extraction depend on determining the degree of pH at which the complex is formed [157]. On this view, a series of experiments were carried out to evaluate the effect of pH on the extraction process by

preparing similar solutions containing 3 µg.mL^{-1} of cadmium (II) standard solution and 1mL 1x10^{-3} mol.L^{-1} of (R) in presence of 0.4 mL (5% (v/v)) of TritonX-114 within pH ranges (3.3-6.1) which were adjusted with different buffer solutions. Figures (3-40) and (3-41) show the experimental results of this test after the absorbance was recorded for all samples at 525 nm

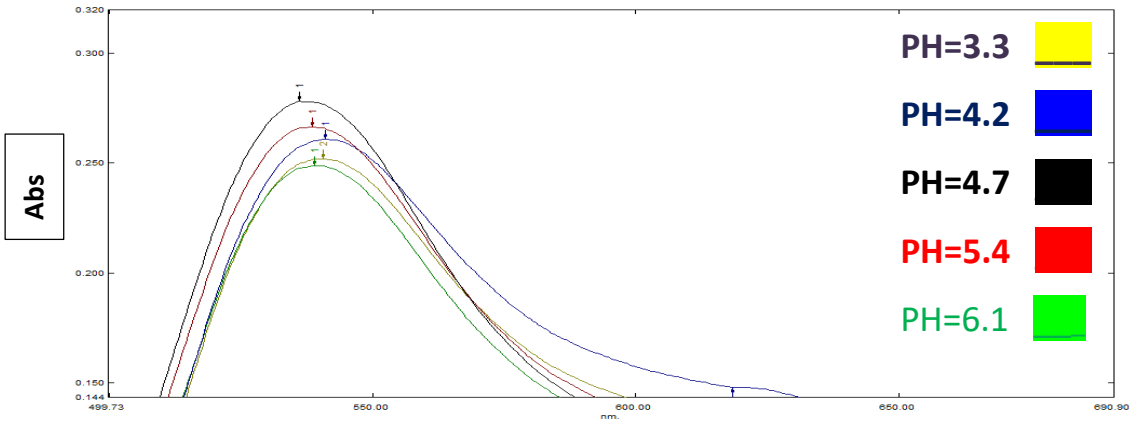


Figure (3-40): Absorption spectra of [Cd(II) -R] complex under effect of pH

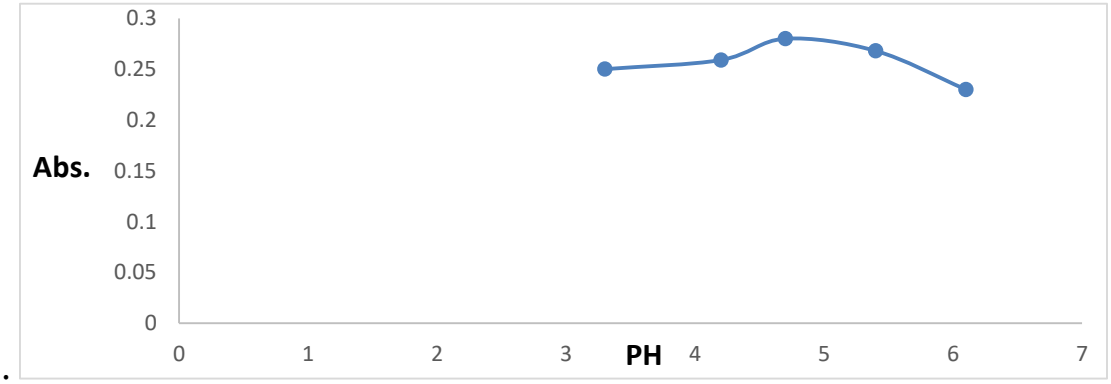


Figure (3-41): Influence of pH of the medium on extraction efficiency of Cd (II). Conditions:3 µg mL^{-1} of Cd (II); 1 mL (1x10^{-3}) mol.L^{-1} of (R); 0.4 mL (5% (v/v)) of Triton X– 114; Equilibration temperature 55 °C and heating time 20 min).

On the other hand, absorption values a decrease with the direction of the solution towards alkalinity, may be due to the instability of the complex at basic medium that results in dissociation of the complex. Accordingly, a pH of 4.7 was chosen as the best point for forming the complex and which do not require buffer solution.

3.9.3 Effect of (R) Concentration

Concentration of reagent is one of the factors that affect the CPE procedure because it is the transport medium for metal ions to the rich phase of the surfactant [158]. Therefore, it is necessary to know the appropriate amount of reagent to transfer the maximum possible amount of lead ions to SRP and extract it. In this work, the effect of reagent concentration on the extraction process in CPE was tested by preparing similar solutions containing different reagent volumes (0.2-1.2) mL 1x 10^{-3} $mol.L^{-1}$ with 3 $\mu g.mL^{-1}$ of cadmium (II) standard solution in the presence of 0.4 mL 5% (v/v) of TritonX-114 at pH 4.7. After applying the CPE procedure, the absorption spectra of the complex were obtained at different levels as in Figure (3-42), also Figure (3-43) represented the behavior of this effect.

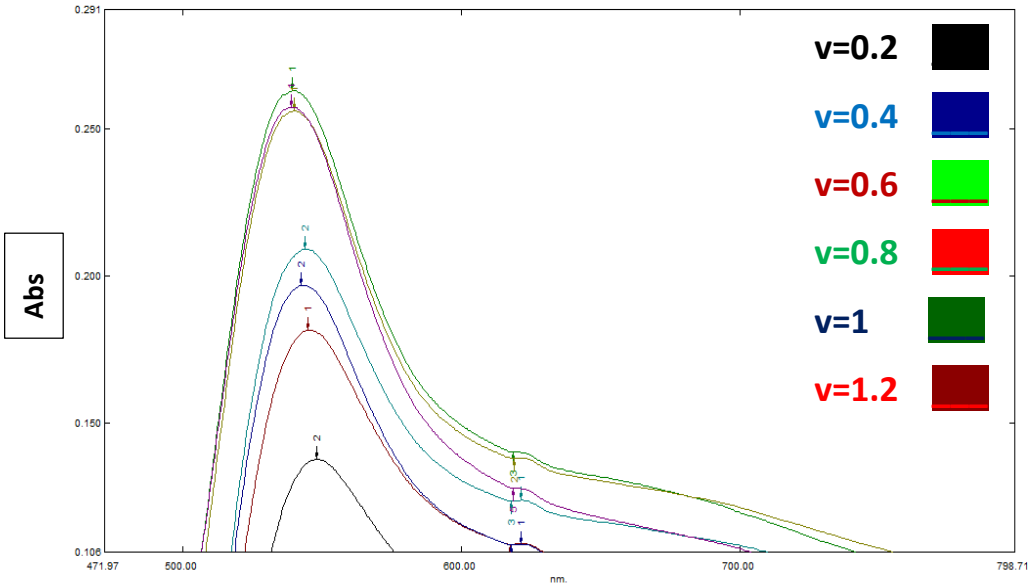


Figure (3-42): Absorption spectra of [Cd (II) -R] complex under effect of concentration of reagent (R).

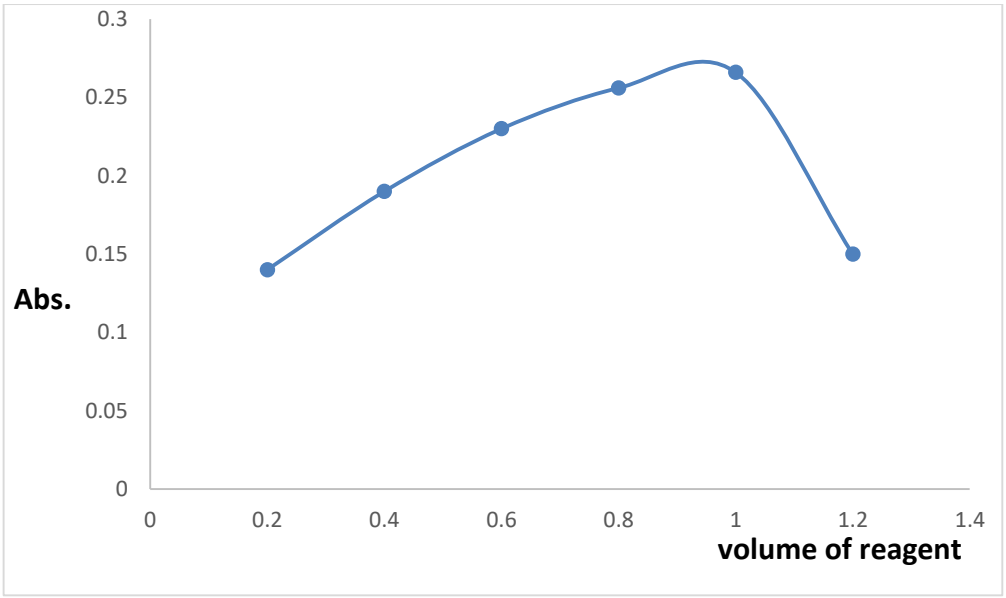


Figure (3-43): Test results for effect of reagent concentration on extraction efficiency of Cd(II). Conditions: pH 4.7; 3µg.mL^{-1} of Cd(II), 0.4 mL 5% (v/v) of Triton X– 114, Equilibration temperature 55 °C and heating time 20 min).

From Figure (3-43), It is possible to choose 1 mL of the reagent because it provides less consumption in the amount of reagent with maximum extraction, also, that high concentrations of (R) are not necessary and have resulted in a significant decrease in the absorbance values.

3.9.4 Effect of TritonX-114 Concentration

Concentration of surfactant is one of the factors affecting the action of CPE, since surfactant is an extraction medium by which the pre-concentration agent can be optimized to reach maximum extraction efficiency by reducing the volume ratio of the surfactant-rich phase to the aqueous phase [162]. Therefore, effect of surfactant concentration on [Cd(II)-R] extraction was studied by using varying volumes within ranges (0.2-1.2) mL of 5% (v/v) Triton X-114. After applying the general CPE procedure, the results were obtained in Figures (3-44) and (3-45)

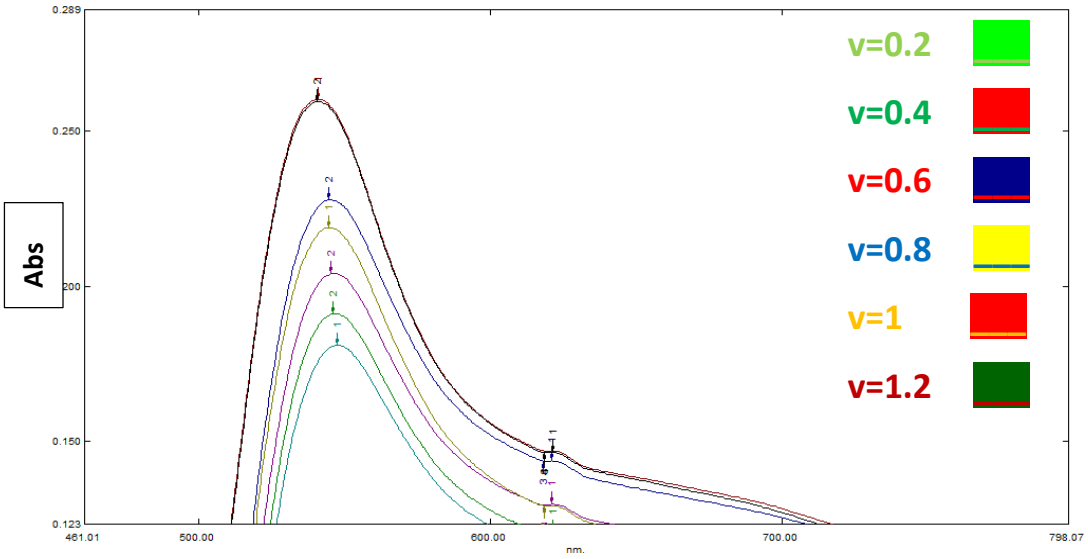


Figure (3-44): Absorption spectra of [Cd (II) -R] complex under effect of concentration of Triton X-114.

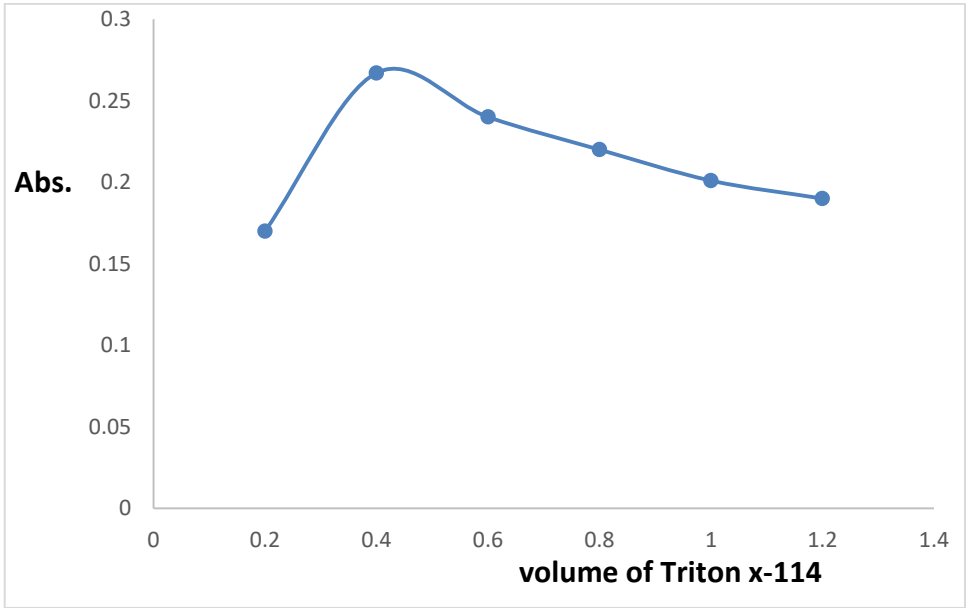


Figure (3-45): Test results for the effect of Triton X- 114 concentration on extraction efficiency of Cd(II). Conditions: pH 4.7; 3µg.mL^{-1} of Cd (II); 1 mL (1×10^{-3}) mol.L^{-1} of (R); Equilibration temperature 55°C and heating time 20 min).

It is shown in Figure (3-45) that the cadmium(II) ions extraction rate is increased to the maximum possible at 0.4 mL of Triton X-114 and it appears that the concentration of Triton X-114 at this point was able to capture the greatest possible amount of complex in SRP. On the other hand, the gradual decrease in the absorption values is associated with the presence of high concentrations of the surfactant due to an increase in the viscosity

of the rich surface phase, which leads to a decrease in the sensitivity [163]. Therefore, 0.4 mL, 5% (v/v) of Tritonx-114 was chosen as the ideal concentration and used in all subsequent experiments.

3.9.5 Effect of Equilibrium Temperature

To reach the maximum efficiency in the extraction process, we performed a temperature effect test by applying different temperatures to the test samples (30-60)°C under the optimum conditions for the previous tests. A constant temperature was obtained for the extraction process showing an excellent condition at 55°C, as explain in Figures (3-46) and (3-47).

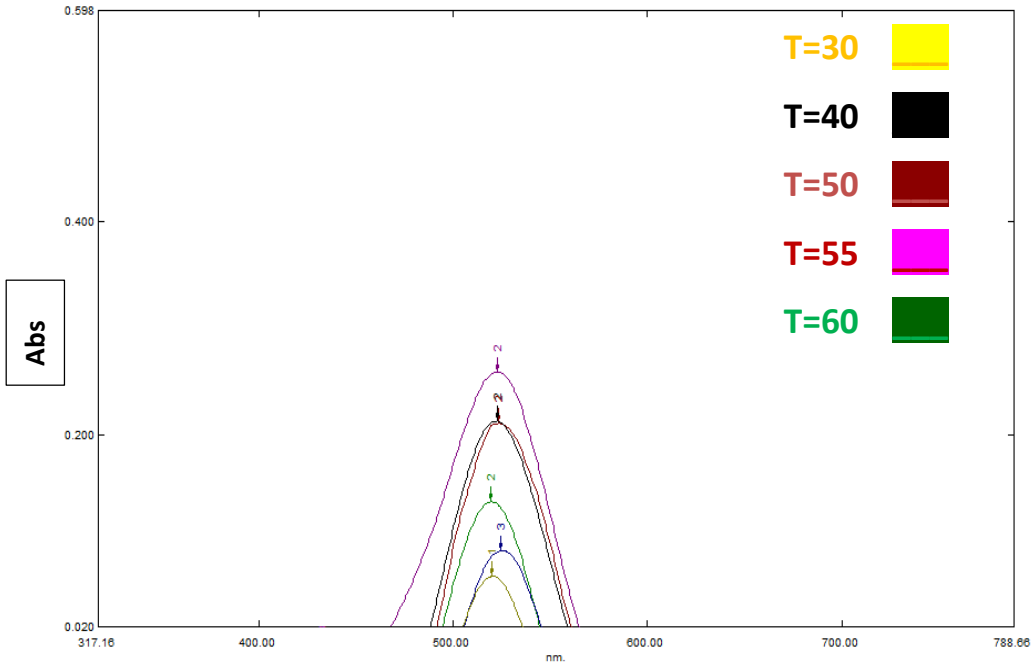


Figure (3-46): Absorption spectra of [Cd (II) -R] complex undr effect of equilibration temperature

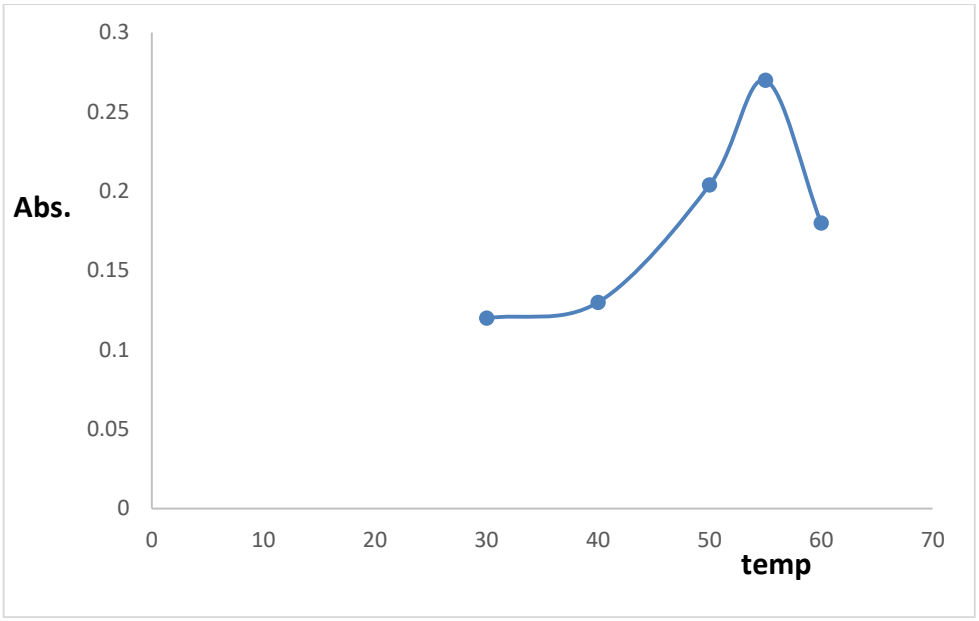


Figure (3-47): Test results for the effect of equilibration temperature on extraction efficiency of Cd(II). Conditions: pH 4.7, 3µg.mL^{-1} of Cd (II); 1 mL (1×10^{-3}) mol.L^{-1} of (R); 0.4 mL 5% (v/v) of Triton X– 114 and heating time 20 min).

From the test results in Figure (3-47), it was observed that the absorption values of the extracted complex increase gradually with the rise in temperature to 55 °C .This can be explained results the decrease in the volume of the rich phase of the surfactant because of decrease in the water content which leads to an increase in sensitivity.

3.9.6 Effect of Incubation Time

To evaluate the effect of incubation time, repeated tests were carried out for different times (5-25) min when placing the samples in the water bath, while keeping other parameters at the optimal conditions obtained through previous experiments. Figures (3-48) and (3-49) show the results of the incubation time test.

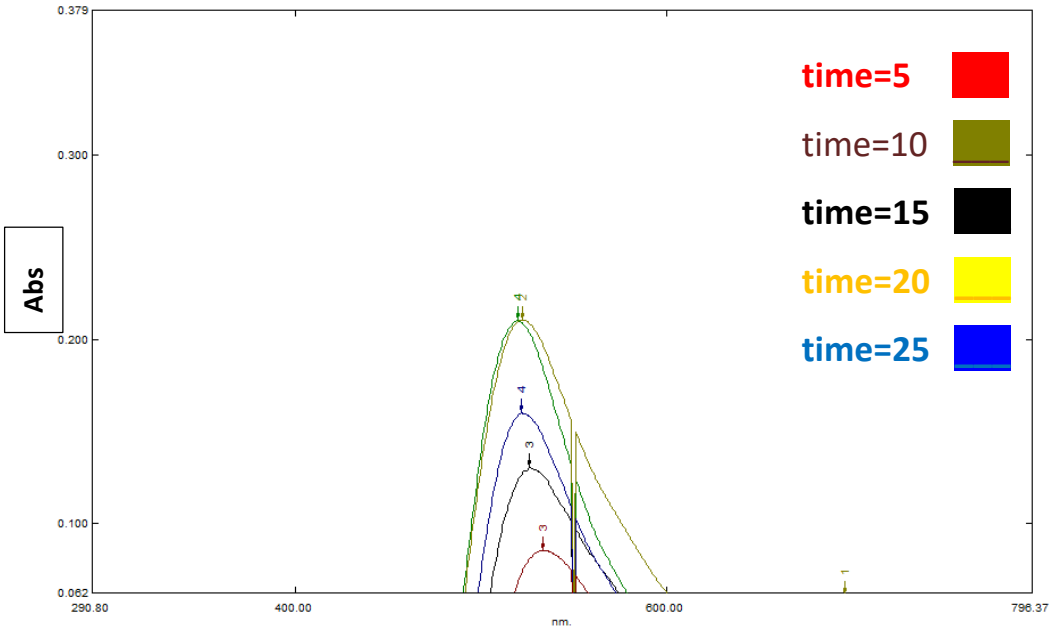


Figure (3-48): Absorption spectra of [Cd (II) -R] complex under effect of Incubation time

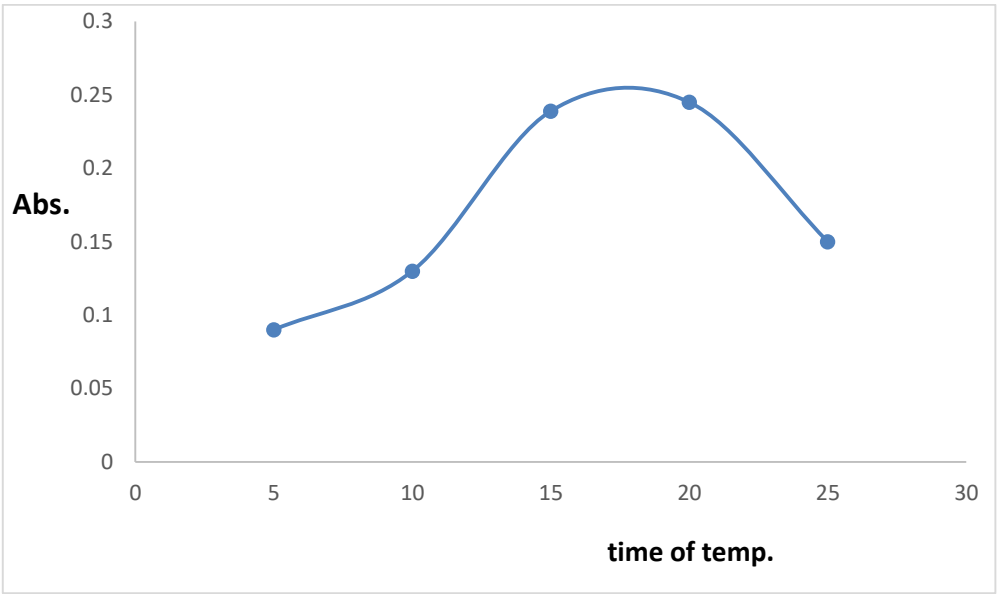


Figure (3-49): Test results for the effect of equilibration time on extraction efficiency of Cd (II). Conditions: pH 4.7; 3µg.mL^{-1} of Cd(II); (R.), 1 mL (1×10^{-3}) mol.L^{-1} of (R); 0.4 mL 5% (v/v) of Triton X– 114 and equilibrium temperature 55 °C)

It was found that the analytical signal increases until it reaches the point (20) min and then stabilizes relatively with the increasing time. It can be considered that 20 min is sufficient time to achieve a good separation of the solution, as it is preferable that the extraction process takes place in a shorter time and with higher efficiency. Therefore, this point was chosen as the best incubation time and applied in all subsequent experiments.

3.9.7 Effect of Centrifugation Time and Rate

The effect of centrifugation time on the extraction efficiency of cadmium (II) ions in CPE was studied using different times (5-25) minutes to assess the effect of time on the centrifugation process. Through the results shown in Figures (3-50) and (3-51), The time (20) min was chosen as a short time for higher extraction.

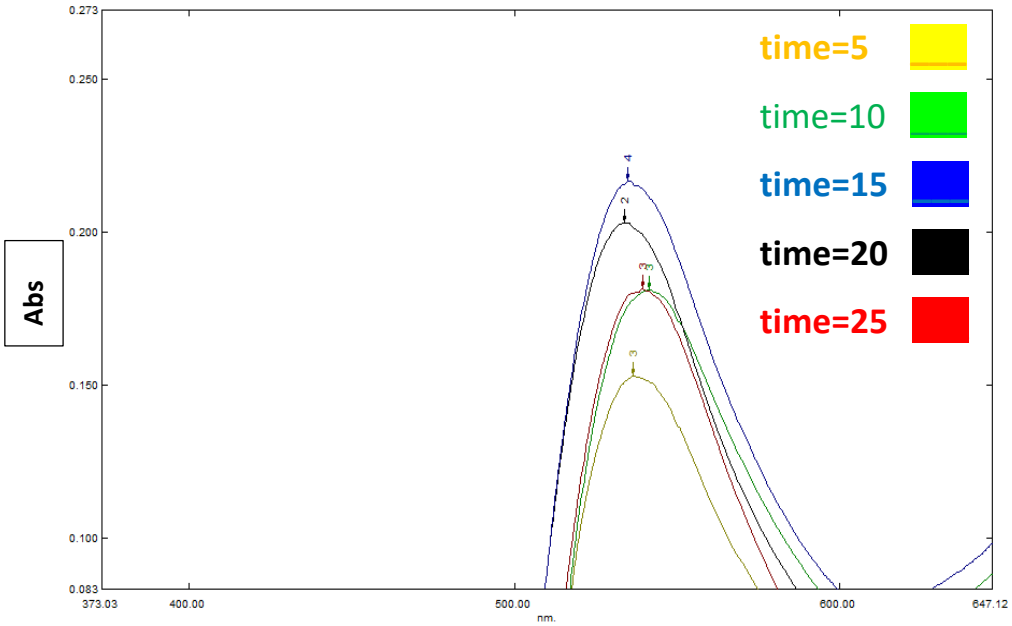


Figure (3-50): Absorption spectra of [Cd(II) -R] complex under effect of centrifugation time

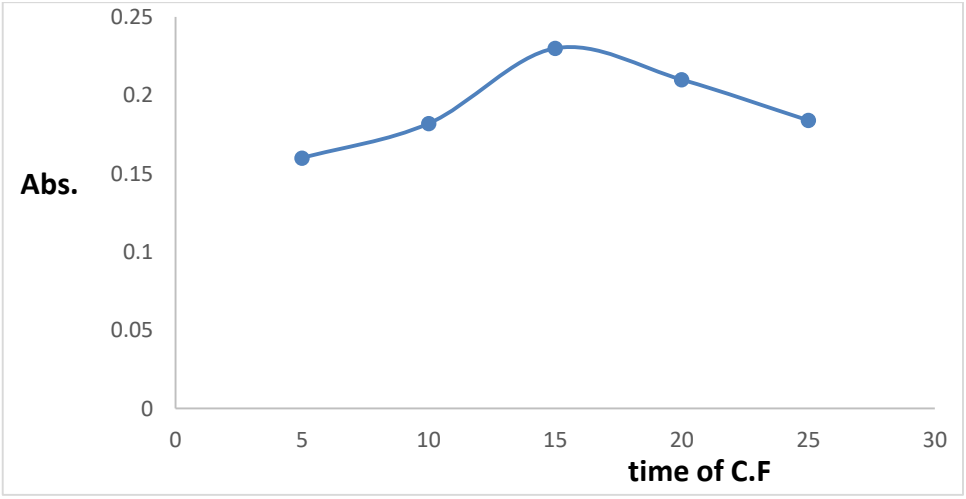


Figure (3-51): Test results for the effect of of centrifuge time on extraction efficiency of Cd (II). Conditions: pH 4.7; 3µg.mL^{-1} of Cd (II); 1 mL (1×10^{-3}) mol.L^{-1} of (R); 0.4mL 5% (v/v) of Triton X– 114; Equilibration temperature 55 °C and heating time 20 min).

Keeping time constant at (15 min), the centrifugation rate was tested in the range of 1,000 to 5,000 rpm. According to the data obtained in Figure (3-52) and (3-53), it is indicated that at 4000 rpm the complete phase separation is available with the organic phase pooling at the bottom of the test tube and sticking to it. This allows for easy pouring of the aqueous phase without losing any part of the analyte, so 4000 rpm was chosen as the ideal rate.

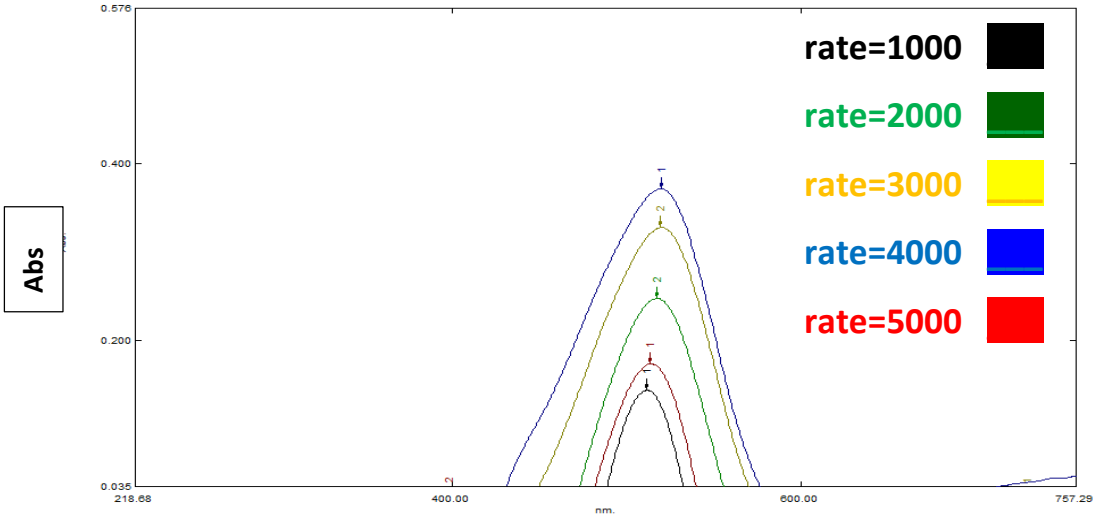


Figure (3-52): Absorption spectra of [Cd (II) -R] complex under effect of centrifuge rate

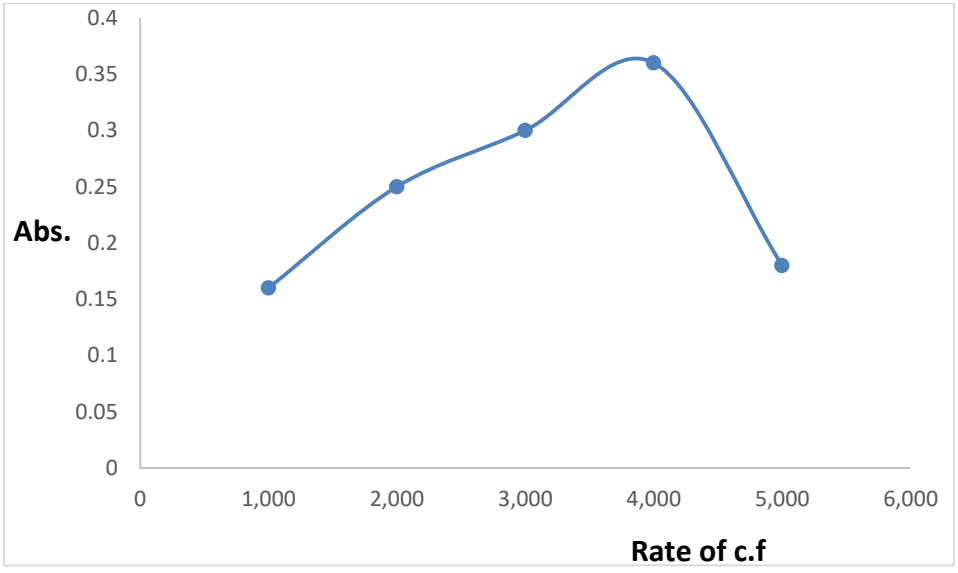


Figure (3-53): Test results for the effect of of centrifuge Rate on extraction efficiency of Cd(II). Conditions: pH 4.7; 3 µg.mL^{-1} of Cd(II); 1 mL (1×10^{-3}) mol.L^{-1} of (R); 0.4 mL 5% (v/v) of Triton X– 114; Equilibration Temperature 55°C and heating time 20 min).

3.9.8 Effect of salt out

The effect of salts on CPE were tested by adding 1 mL of 0.1 mol.L^{-1} of NaCl, $MgSO_4.7H_2O$, $NaNO_3$, NH_4NO_3 to the solution. After applying the steps required for CPE, the absorbance of the test solutions was measured as shown in Table (3-12). Figure (3-54) shows that the salts used in this experiment had no effect on the absorption of the complex. (168)

Table (3-11): Absorbance of [Cd(II)–(R.)] complex under effect of salt out

Salt out	Abs
NaCl	0.22
$MgSO_4.7H_2O$	0.27
$NaNO_3$	0.21
NH_4NO_3	0.29

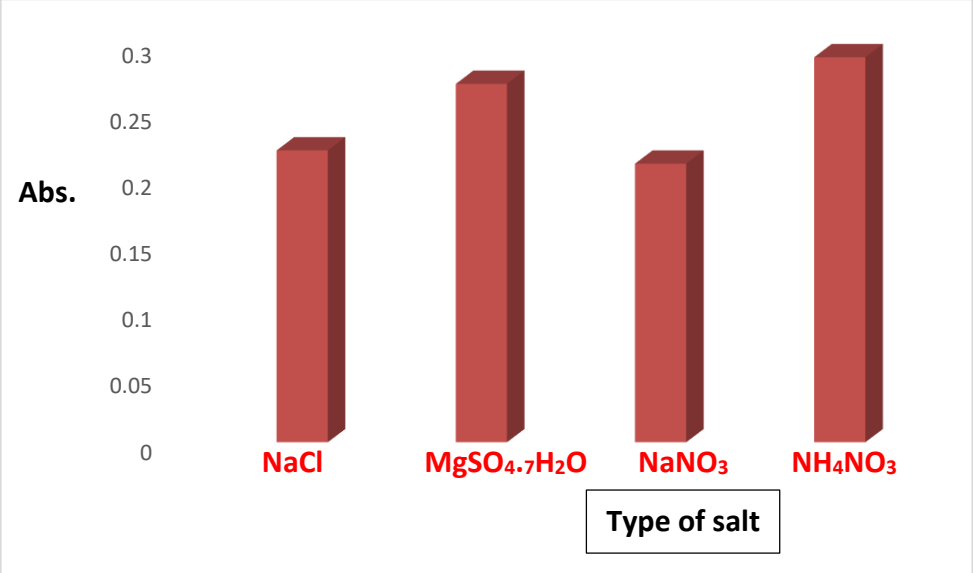


Figure (3-54): Test results for the effect of salt out on extraction efficiency of Cd(II). Conditions: pH 4.7; 3µg.mL^{-1} of Cd(II); 1 mL (1×10^{-3}) mol.L^{-1} of (R); 0.4 mL 5% (v/v) of Triton X– 114; Equilibration temperature 55 °C and Equilibration time 20 min.

3.10 Stoichiometric Complex Determination

Job's method was used in order to find the stoichiometric ratio between Cd (II) and (R). A series of solutions were prepared by adding distinct volumes of standard cadmium (II) solution and reagent solution at the same concentration level $1x10^{-3}$ $mol.L^{-1}$. The results in Figure (3-55) represented that the stoichiometric ratio between Cd (II) and (R.) was 1:2.

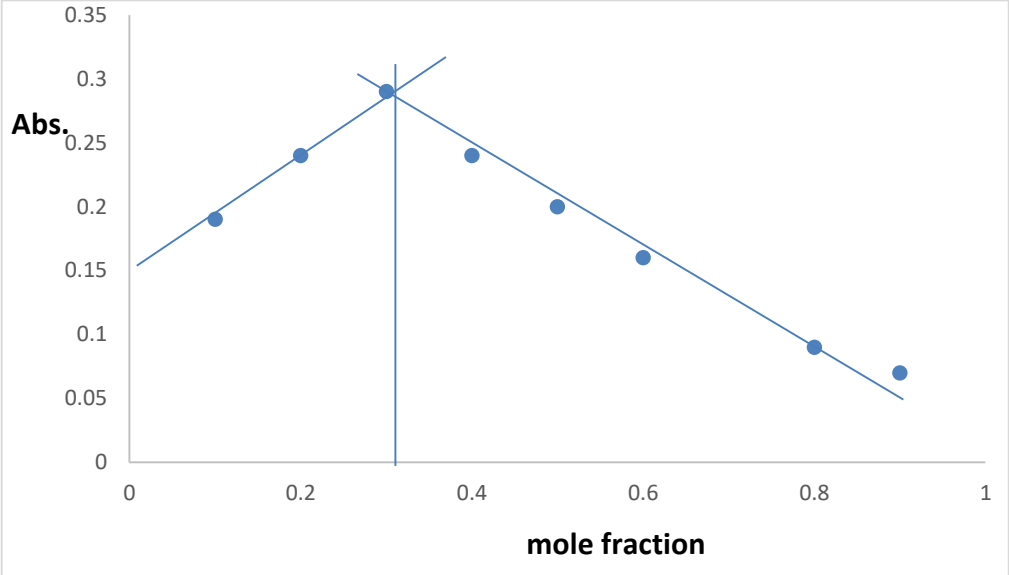


Figure (3-55): Continuous variables method

In the molar ratio method, the absorbance was measured for a series of solutions containing constant volumes of Cd(II) and various volumes of (R) at the same concentration level (1×10^{-3} $mol.L^{-1}$) then diluted to the required volume. By using the same analytical method, the stoichiometric ratio was determined as shown in Figure (3-56) to be 1:2.

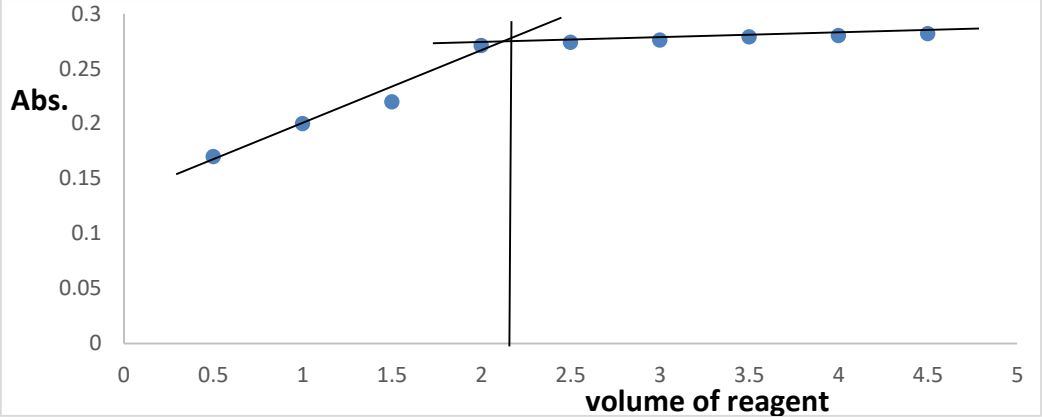


Figure (3-56): Mole-ratio method

3.11 Calibration Curve for Cd(II)

The linear calibration curve was obtained with CPE by preparing solutions containing standard concentrations of cadmium(II) ions within the ranges (0.012-3.0) μg.mL^{-1} after applying CPE procedure at optimal conditions obtained from previous experiments and explain in Table (3-15), where absorbance was measured at λ_{max} (525) nm, and a linear calibration curve was established between the absorbance values versus the cadmium (II) concentration as in the Figure (3-57).

Information for the calibration curve and other analytical parameters for this work are summarized in Table (3-13).

Table 3-12: Optimum conditions for CPE to determine Cd (II).

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

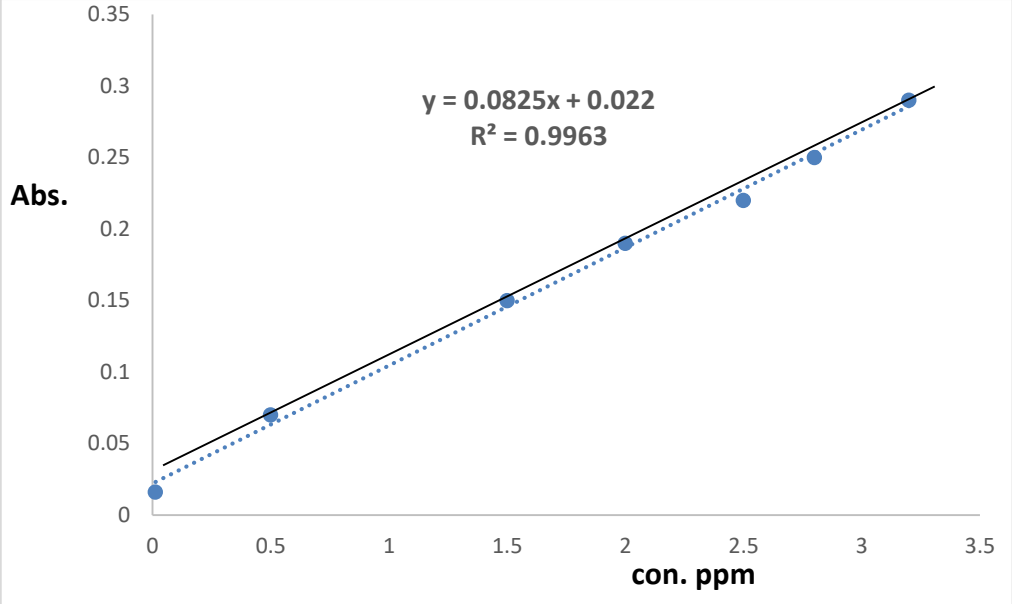


Figure (3-57):Calibration curve for [Cd(II)-R] complex using CPE method

Table 3-13: Analytical properties of CPE in determining Cd(II)

Parameters	Value
λ_{max} (nm)	525
Regression equation	0.0825X+0.022
Correlation coefficient, R^2	0.9963
Linear calibration range µg.mL^{-1}	0.012 -3.0
Relative standard deviation	1.9
Limit of detection µg.mL^{-1}	**0.0091**
Limit of quantification µg.mL^{-1}	**0.02**
Molar absorption coefficient L.mole^{-2}. cm^{-2}	**9273**
Enrichment factor	14
M:L	1:2

3.12 Conclusions

1. The CPE method is easy, inexpensive, and has high sensitivity compared to other conventional methods and conforms to the principles of green chemistry
2. Ease of controlling the experimental conditions of the method and increasing its sensitivity, which gave encouraging results in the process of extracting the trace elements selected in this work.
3. The use of a non-ionic surfactant, Triton X 114, reduced the time factor to possess (CPT) suitable for achieving the clouding phenomenon with less consumption of the surfactant to achieve high extraction efficiency.
4. The use of spectroscopy and correlation with CPE to measure the content of the extracted trace element complexes in the SRP is important due to their ease of use, cheapness, and high efficiency.
5. The proposed method is dependable for determining lead(II),cobalt (II) and cadmium (II) in aqueous solutions, with the possibility of obtaining accurate results when applied to real water samples.

3.13 Future Works

The method of extracting cloud points has proven its role in the development of analytical chemistry, especially in the science of separation, as it is able to separate metal ions and compounds in different matrices, so we can put some suggestions that we think will open new horizons for this technology in the field of chemical analyzes:

1. The development of the CPE method with the ability to separate and concentrate more than one ion in the sample in one step, which allows for faster extraction and less cost and effort.
2. Conducting the separation and concentration for many elements by the CPE method and determining the optimal conditions for each element will provide a great development in

contemporary analytical chemistry and open up broad paths towards easy, simple, and inexpensive routine analysis instead of using expensive tools in this field.

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