

Spectrofluorimetry - Cloud point extraction for the determination of cadmium and lead using Furosemide as a complexing agent

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Keywords: cloud point extraction, Spectrofluorimetry, TritonX-114, Preconcentration ,Furosemide , Benesi-Hildebrand .

ABSTRACT

In this paper , describes a separation and Preconcentration method for the determination of cadmium(II) and lead (II) by spectrofluorimetric method after complexation with the Furosemide drug as a reagent using Triton X-114 as surfactant and quantitatively extracted into a small volume of the surfactant-rich phase after centrifugation. The optimum conditions, such as pH, concentration of reagent (drug) and surfactant (TritonX-114) , equilibration temperature and time on the cloud point extraction, were studied and optimized on the extraction efficiency. In this method limits of detection and quantification LOD and LOQ of Cd and Pb ion were (0.321,0.095) and (0.974,0.288) ng/mL respectively, and a linear calibration range of (6.75-50) ng/mL for Cd and (1.25-15) ng/mL for Pb . The proposed method has been successfully applied to the determination of Cd(II) ion , Pb(II) ion in milk samples with satisfactory results .

الاستخلاص بنقطة الغيمة الطيفي التفلوري لتقدير الكاديوم والرصاص الثنائي باستخدام دواء الفروسيمايد ككاشف تعقيدي

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الكلمات المفتاحية: الاستخلاص بنقطة الغيمة ، الطيف التفلوري ، تريتون اكس 114 ، إعادة التركيز ، فروسيمايد ، بنسل – هيلدراند

الخلاصة

في هذا البحث يصف طريقة فصل وإعادة تركيز لتقدير الكاديوم والرصاص بطريقة طيفية – تفلورية بعد تعقيده بواسطة دواء الفروسيمايد الذي استخدم ككاشف لتقدير هذه الايونات باستخدام التريتون اكس -114 كمادة سطح والاستخلاص الكمي في حجم صغير من الطبقة العضوية بعد فصله الى طورين عضوي ومائي بواسطة السنترفيوج. درست الظروف الفضلى مثل الدالة الحامضية ، تركيز الكاشف وتركيز التريتون اكس-114 ، درجة الحرارة والزمن المؤثرة على الاستخلاص بنقطة الغيمة للوصول الى كفاءة استخلاص عالية. في هذه الطريقة تم حساب كل من حد الكشف وحد التقدير والتي كانت مساوية الى (0.321,0.095) نانوغرام/مليتر لمعد الكاديوم ومساوية الى (0.974,0.288) نانوغرام /مليتر لمعد الرصاص، ومدى التقدير الذي يطاوع قانون بير كان مساوي الى (6.75-50) نانوغرام /مليتر و (1.25-15) نانوغرام / مليتر لكل من معقدي الكاديوم والرصاص على التوالي. الطريقة المقترحة استخدمت لتقدير الكاديوم والرصاص في نماذج من الحليب بدقة عالية.

1. INTRODUCTION

The Furosemide drug has a chemical formula is known as a 4-chloro-2-(furan-2-ylmethylamino)-5-sulfamoylbenzoic acid it is used as a ligand for the determination of cadmium and lead ions by cloud point extraction (CPE), Furosemide (FUR) it has been wide range for the diuretic and can be utilization for the treatment of heart failure; because (FUR) is made of the prevent the absorption of salts and fluid in kidney tube; therefore ,will increase the amount of urine (dieresis)⁽¹⁾ and the chemical structure of the drug is shown in figure 1⁽²⁾ .

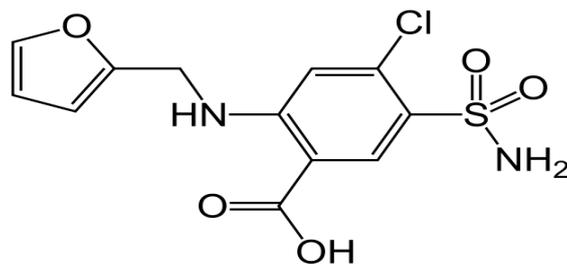


Fig.1. The chemical structure of Furosemide

Cadmium and lead are classified as a very toxic metals which its tend to be concentrated in humans and environmental systems. Absorption of these metals from the air in the local environment and intake from the diet are the major sources of human exposure. Cadmium has been known to damage organs such as the kidneys, liver and lungs (3), while the lead it's become demonstrated to be accumulated in bone and in some soft tissues, such as kidney, liver and the major damage to the brain(4).

Also, the presence of heavy metals as lead and cadmium even in low concentrations such as in milk samples leads to metabolic disorders with extremely serious consequences and causing a lot of one problems as it causes many health problems such as heart failure, cancer, weakness, and also affects the kidneys (5,6). Cloud point extraction (CPE) is a separation and preconcentration method(7). This method which has some advantages such as rapid, low cost, and has be a very wide application fields. One of these application is use for the determination of trace metal ions from different matrices in the recently years (8-10).

Cloud point extraction applied two different methods for separation of metal ions. In one method, is based on direct application of cloud point extraction procedure without presence any ligand while the other method is based on the metal ions are occurring complex formation with suitable ligand (11-13), therefore these separation and preconcentration were becomes an important and practical application of surfactants in analytical chemistry (14,15).

In this work, a spectrofluorimetric - cloud point extraction procedure described using as surfactant of Triton X-114 for separation / preconcentration of Cd (II) and Pb (II), ions and applied this method for the spectrofluorimetric method for the determination of these ions in milk samples.

2. EXPERIMENTAL

Instrumentation

Spectrofluorimeter (Shimadzu- Model RF-5301PC) was used for all the measurements in this methods for the determine the concentration of the cadmium (II) and lead (II). A digital pH meter (inolab 720 model WTW Germany) was used for all pH measurements. A thermo stated bath Thermo, balance (Circulator England) maintained as the desired temperature was used for the cloud point extraction experiments, a UV-visible 1800(spectrophotometer Shimadzu) and phase separation was assisted using a centrifuge(PLC 03 USA), AA-6300 Shimadzu Atomic Absorption spectrophotometer type GFA-EX7I Graphite Furnace.

Reagents and solutions

All the chemicals used were of analytical reagent grade, Stock solutions of Cd(II) and Pb(II) were prepared by dissolving appropriate amounts of $\text{Pb}(\text{NO}_3)_2$ and $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ in distilled water. The non-ionic surfactant, Triton X-114 was used without further purification. A (0.01) M solution of Furosemide stock solution was prepared by dissolving appropriate amounts of this reagent in some drops of 0.1 M NaOH and completely the volume by distilled water. Doubly distilled water was used throughout the entire study. The pipettes and vessels used for trace analysis were kept in 5% nitric acid for at least 24 h and subsequently washed three times with distilled water.

Cloud point extraction procedure (CPE)

For the CPE procedure, aliquots of 5 mL of a solution each individual containing of the Cd(II) and Pb(II), Triton X-114, Furosemide and a buffered solution at a suitable pH was kept in the thermostatic bath maintained at 60 °C at a period 20 min. Because the surfactant density, was 1.052 gm.L^{-1} , the surfactant-rich phase can settle through the aqueous phase, then the centrifuging for 20 min at 4000 rpm accelerated the phase separation, and then make a cooling in an ice bath, the surfactant rich phase became viscous and was still at the bottom of the centrifuge tube. Simply a syringe centered in the bottom of the its tube can readily discard the aqueous phases. In order to decrease the viscosity and facilitate sample handing prior to the spectrofluorimeter instrument, 3 mL of ethanol was added to the surfactant rich phase. The final solution was introduced into the spectrofluorimetry cuvette to complete its measurement of CPE.

Fluorescence measurements

A spectrofluorimetric method was used to enhancement the furosemide fluorescence spectrum intensity (Furosemide – Pb, Cd complexes) for the determination of its metal ions in all the experimental. The fluorescence intensity of its complexes were measured in the intensity solution at an excitation wavelength of 363 nm and an emission wavelength of 409 nm and calibration curve was constructed. The fluorescence intensity of its complexes is dissolved in ethanol after extraction was measured and the Cd(II) and Pb(II) were determined. All measurements were carried out at room temperature after the optimization for the temperature of its cloud point extraction for each ions, Fig.(2) show the excitation and the emission spectrum of Pb and Cd complexes.

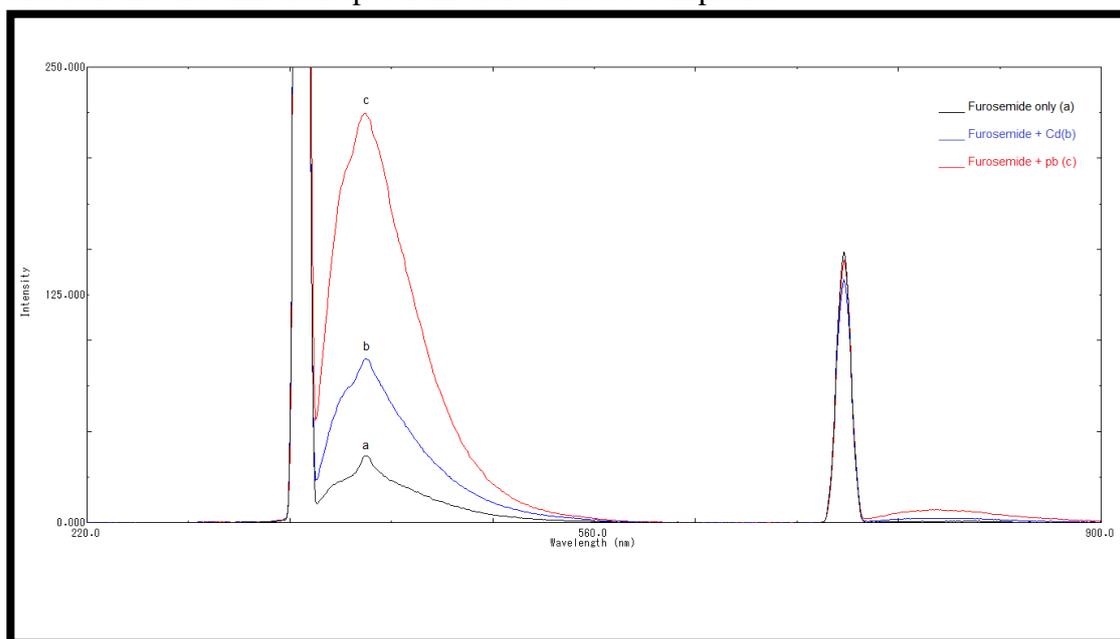


Fig. 2. Excitation and emission spectrum of Furosemide and its Cd(II), pb(II) complexes.

Results and discussion

Under the optimum experimental conditions which are represented in the coming results. Cd(II) and Pb(II) reacts with Furosemide to form hydrophobic complexes, which is subsequently trapped in surfactant micelles.

Effect of pH

Cloud point extraction of cadmium and lead species were prepared at different buffered pH solutions. The separation of metal ions by CPE involves prior formation of a complex with sufficient hydrophobic and the Furosemide drug to be extracted into the small volume of the surfactant-rich phase. The pH of the solution was optimized for the non-ionic CPE in order to obtain the optimum signal for the selected emission spectrofluorimetry, the pH was adjusted to the desired value by addition of buffer solution. Following the experimental process described in the procedure section it was found that the extraction efficiency is almost dependent on pH conditions for the pH range of (4-5.5) for each complex. (Fig. 3) giving a plateau in the area of its value. This behavior is anticipated due to the hydrophobic, non-ionizable nature of the analytes.

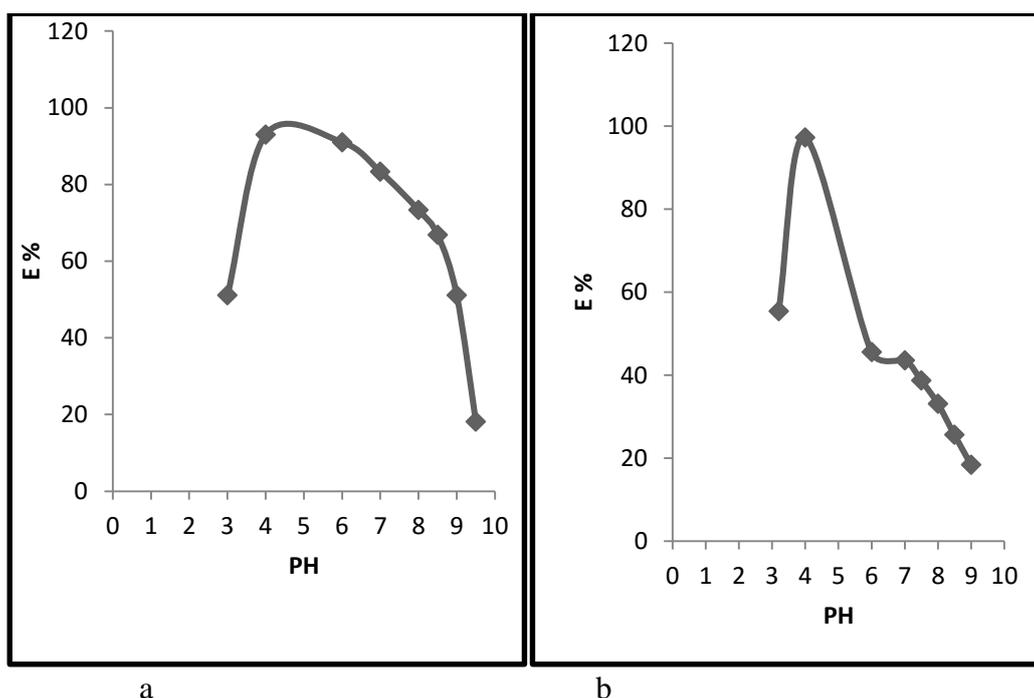


Fig. 3. Effect of pH on the extraction percent of (a) Pb(II), (b) Cd(II) - Furosemide complexes.

Effect of reagent concentration(drug) on the cloud point extraction

The effect of Furosemide drug concentration on the determination of cadmium (II) and lead (II) were investigated by changing the volume of (10^{-3} M) drug in the range of (0.1–1) mL. The signal intensity was found increased up to the Furosemide drug volume of (0.8,0.4) mL for the cadmium (II) and lead (II) respectively, and attributed to near

complete extraction of each complexes. A volume of above value was therefore chosen as the optimum volume for the subsequent studies shown in Fig.(4).

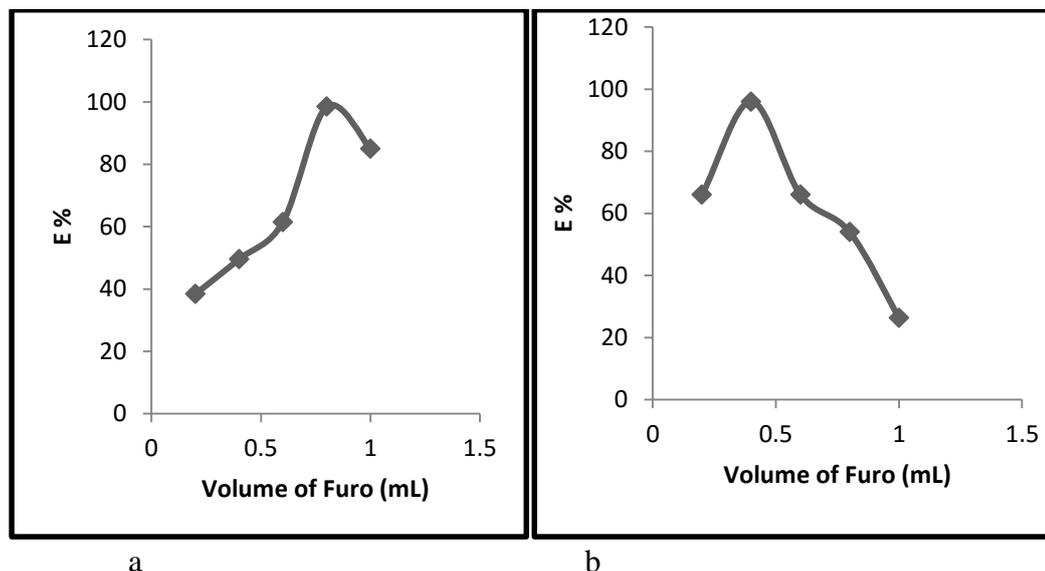


Fig. 4. Effect the volume of Furosemide on the extraction percent of (a) Pb(II), (b) Cd(II) - Furosemide complexes.

Effect the volume of Triton X-114

A best CPE method would be that which maximizes the extraction efficiency through minimizing the phase volume ratio, thus maximizing its volume factor. Triton X-114 was chosen for the formation of the surfactant-rich phase due to its have a high density and low cloud point temperature of the surfactant-rich phase by which phase separation will be done then by centrifugation. Extraction of cadmium(II) and lead (II) in the presence of TritonX-114 of was investigated within the range of (0.01–0.4) mL which is shown in Fig. 5. The optimum surfactant volume used for the Cd(II) and pb(II) was (0.25)mL for each ions . So, the concentration of TritonX-114 was chosen as its above volume for each its elements in order to achieve the optimal surfactant concentration in conjunction with the highest possible extraction efficiency.

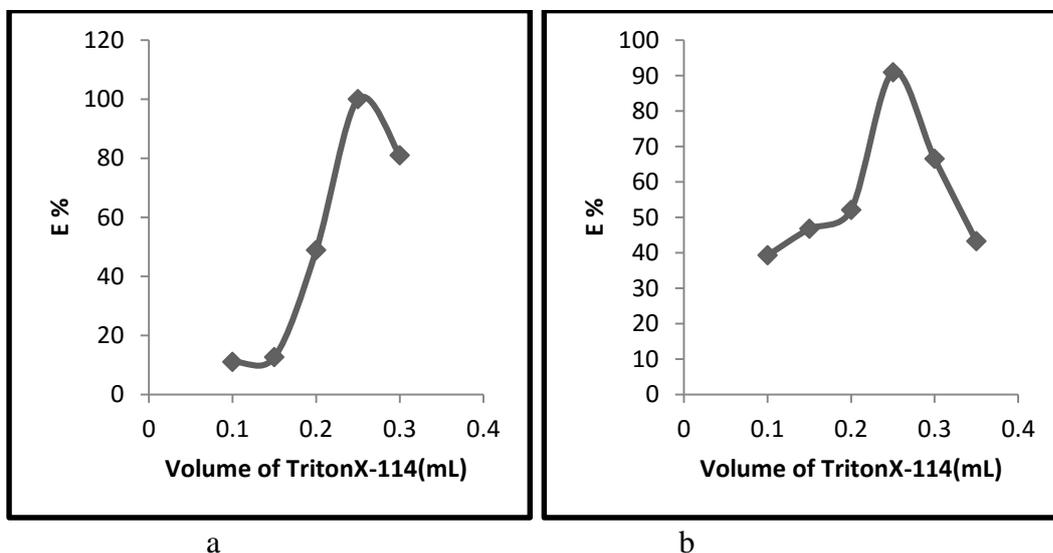


Fig. 5. Effect the volume of TritonX-114 on the extraction percent of (a) pb(II), (b) Cd(II) - Furosemide complexes.

Effect of the equilibrium temperature and time

In the method of cloud point extraction system, temperature of extraction is very important parameter for micelle as occurring. The effect of equilibration temperature and time incubation in the CPE system was investigated at 30 to 80 °C. At temperatures lower than 30 °C the separation of the two phases at the cloud point temperature was not complete. After 60 °C, the signal intensity was approximately same value or decrease because this complexes will be dissociated at high temperature (Fig. 6) and (Fig.7). The incubation time in the water bath was kept between 20 and 25 min. for each Cd(II) and lead Pb(II) complexes respectively which is sufficient for the completion separation into two phase aqueous and organic phase of these processes.

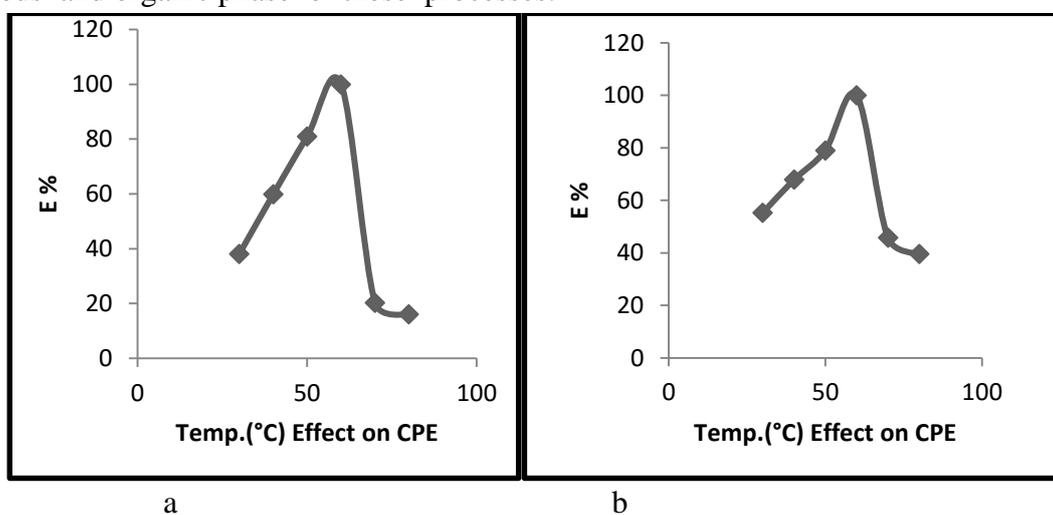


Fig. 6. Effect of temperature on the extraction percent of (a) pb(II), (b) Cd(II) - Furosemide complexes.

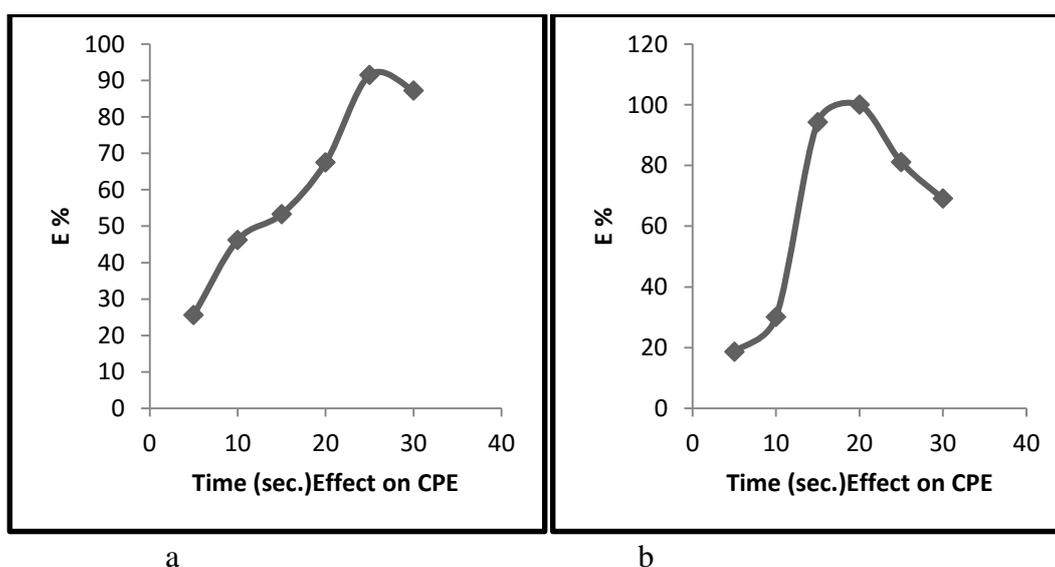


Fig. 7. Effect of time incubation on the extraction percent of (a) pb(II), (b) Cd(II) - Furosemide complexes.

Determination of stoichiometry and stability constants of the complexes

The stoichiometry and stability constant of the Furosemide–cadmium and lead complexes were determined by Benesi–Hildebrand method⁽¹⁶⁻¹⁸⁾.

In the case of a 1 : 1 complex, the following equation is applicable:

$$1/F-F_0 = 1/(F_{\infty}-F_0)K[Me]_0 + 1/(F_{\infty}-F_0) \quad (1)$$

In this line, a graph of $1/F-F_0$ versus $1/[Me]_0$ was constructed where F is the observed fluorescence at each concentration tested, F_0 is the fluorescence intensity of analyte in the absence or without found of metal ion, and $[Me]_0$ is the concentration of metal ion (cadmium and lead). A linear plot is required for this double reciprocal plot in order to conclude 1 : 1 stoichiometry. While when the case where 2 : 1 stoichiometry is predominant, the applicable equation is become and applied is :

$$1/F-F_0 = 1/(F_{\infty}-F_0)K[Me]_0^2 + 1/(F_{\infty}-F_0) \quad (2)$$

Also when the stoichiometry of the complex is 2 : 1 complex, by using the continuous variation method⁽¹⁸⁾, a straight line would be obtained when $1/(F-F_0)$ is plotted against $1/[Me]_0^2$. To calculate the stability constant of the complexes, an aliquot of the furosemide to give a final concentration of 1.0×10^{-3} M was placed in a 5 mL calibrated flask and different amounts of metals were added to give a final concentration between 0.3×10^{-6} – 8.0×10^{-6} M. After the addition of 1 mL of buffer solution and optimum volume of TritonX-114 to the solutions and final diluted to volume with distilled water and made a cloud point extraction to make a two phases, at last the organic phase contain a small amount of the surfactant rich phase is obtained and the fluorescence intensity was measured at $\lambda_{em.}=409$ nm, and the stability constant is determined by dividing the intercept on the slope as a visible emission^(20,21), this method is made at 50, 60 C° because this degree gives a best extraction of the complex, as shown in fig.(8) that a pure the pb–Furosemide is 1 : 1 and the Cd–Furosemide complex is be 1:2.

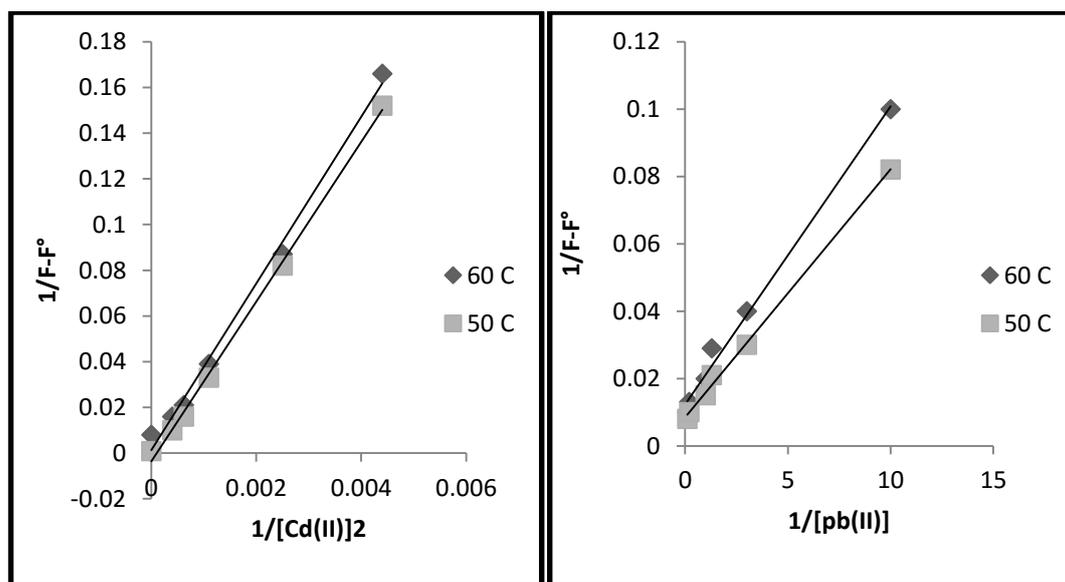


Fig. 8. Benesi-Hildebrand s plots for 1:2 and 1:1 furosemide –cadmium ,lead

respectively .

Calibration Graph for these complexes

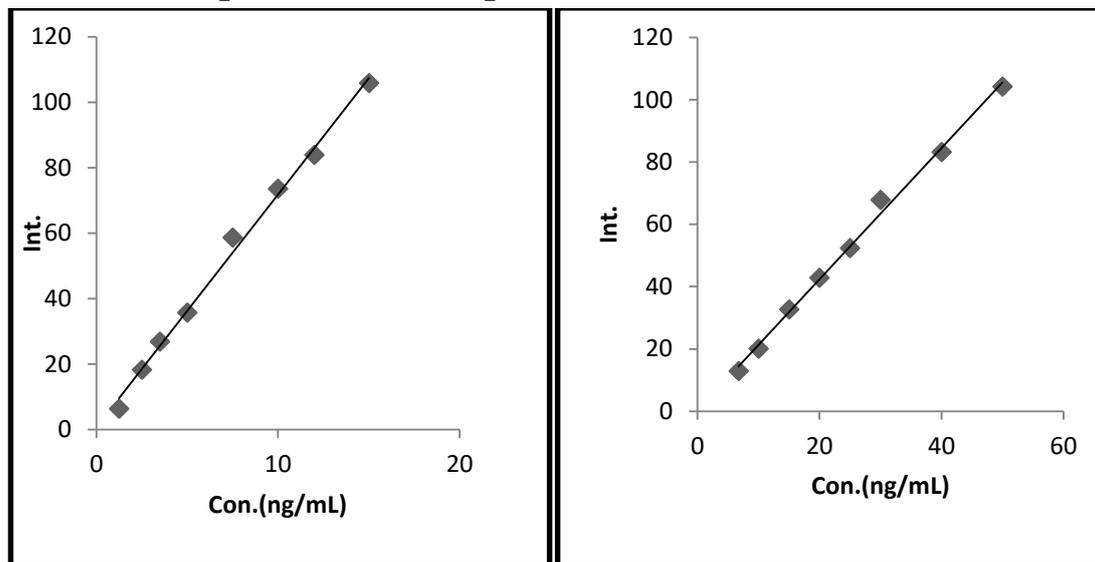


Fig. 9. Calibration graph , (a) pb(II), (b) Cd(II) - Furosemide complexes.

Thermodynamic Parameters

The dependence of the stability constant value on the temperature, a thermodynamic methods was considered to be responsible for this interaction. The thermodynamic parameters: enthalpy changes(ΔH), entropy changes(ΔS), and free energy changes(ΔG) are the main evidences to determine the binding mode. The free energy change (ΔG) can be estimated from the following equation, based on the binding constant at different temperatures.

$$\Delta G = -2.303RT \log K \quad (3)$$

Where R is the gas constant, T is the experimental temperature, K is the stability constant at the corresponding temperature. From the value of stability constant at different temperatures, the enthalpy changes can be calculated by using equation (4):

$$\log K_2 / K_1 = 2.303R \Delta H [1 / T_1 - 1 / T_2] \quad (4)$$

The entropy changes can be calculated by using equation (5):

$$\Delta G = \Delta H - T \Delta S. \quad (5)$$

The stability constant of Pb, Cd complexes and other thermodynamic parameter are shown in table (1) ⁽²²⁾.

Table 1. Stability constant and other thermodynamic parameter

Metal - complex	Log K		- ΔG ,KJ/mol		ΔH ,KJ/mol	ΔS ,KJ/mol K	
	50 °C	60 °C	50 °C	60 °C		50 °C	60 °C
Cd ²⁺	10.8	11.02	66.82	70.29	46.803	0.351	0.351
Pb ²⁺	5.86	5.944	36.25	37.91	17.29	0.165	0.165

From the results in table (1) the ΔH indicating that the extraction reactions are endothermic and the negative values of ΔG dictates that the solubilization are spontaneous phenomena.

Method validation of the spectrofluorimetric determination of Cd(II) and pb(II) by cloud point extraction procedure .

Table 2.Statistical data of calibration graph using proposed method

Parameter	Value of Cd(II)	Value of pb(II)
λ_{ex} (nm)	363	363
λ_{em} (nm)	409	409
Regression equation	$y=2.109x+0.125$	$y=7.118x+0.701$
Correlation coefficient (r)	0.994	0.995
Concentration rang (ng mL ⁻¹)	6.75-50	1.25-15
LOD (ng mL ⁻¹)	0.321	0.095
LOQ (ng mL ⁻¹)	0.974	0.288
Enrichment factor	42.34	62.44

Digestion and Determination of Lead and cadmium :

When the samples were to be analyzed, the milks were made the pH below 4.6 and this pH is made to separate the casein and fat. Each sample was centrifuged at 2000 rpm for 10 min, supernatant mixed with 5ml of 65% HNO₃ and heated to 90°C, after which 20 ml of de-ionized water was added. Lead and cadmium was measured after making the optimum condition of a cloud point extraction by spectrofluorimetric and atomic absorption ,table 3 is shown this approximately results^(23,24) .

Table 3.Determination of Cd(II) and pb(II) in different solution of cow milk

No.	Fluorescent Pb ng/mL	Atomic Absorption Pb	Fluorescent Cd ng/mL	Atomic Absorption Cd
1-	19.80	20.01	1.62	1.66
2-	28.90	29.21	2.42	2.61
3-	25.20	25.61	1.02	1.21
4-	40.12	40.07	4.12	4.60
5-	34.0	34.32	0.91	0.84

Conclusions

A simple spectrofluorimetric process for the determination of cadmium (II) and lead (II) was developed. Optimization of the parameters produced a stable and highly

fluorescence intensity for its ions – furosemide complex. This methodology resulted in a low value of LOD and LOQ its shown in above table for its ions. Simple and rapid method by sensitive value of spectrofluorimetric determination of its ions.

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