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Separation and Micro Determination of Zinc(II) and Cadmium(II) in Food Samples Using Cloud Point Extraction method

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Abstract

The cloud point extraction technique has become increasingly popular in recent years for trace metal separation and preconcentration. When heated to a specific temperature, cloud point extraction utilizes the property of nonionic surfactants in aqueous solutions to generate micelles and become turbid (so-called cloud point temperature). For analytical chemists, developing a simple and selective technology for the separation and determination of metals and medicinal drugs is a critical concern. Therefore, a sensitive, accurate, and green cloud point extraction (CPE) procedure was developed for the micro-determination of metal cations like zinc (II) and cadmium (II) in food samples. Triton X–114 and 1-(4-(Phenyldiazenyl) phenyl) azo naphthalene-2-ol (Sudan III) were used as extractants. Sudan III forms an ion-pair association complex with metal ions when the pH is 9. In the presence of 5 ppm zinc (II) or 4 ppm cadmium (II) in an aqueous solution, the maximum extraction efficiency should be achieved. In order to create Cloud Point Temperature (CPT) quantitatively, the extraction applications in this approach required heating at 85°C for 20 minutes. In this work, the impacts of different surfactants, pH, stoichiometry, and various organic reagents on interferences as well as spectrophotometric determination were explored. The linearity ranges of zinc (II) and cadmium (II) were 0.25-700 and 0.25-400 ppm, respectively. The results show low detection limits of 0.035 and 0.042 ppm for zinc (II) and cadmium (II), respectively. Also, the quantification limits for zinc (II) and cadmium (II) are 0.116 and 0.140 ppm, respectively.

Keywords: Cadmium(II), Cloud Point Extraction, Food Samples, Sudan III, Zinc(II).

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الفصل والتقدير لأيوني الخارصين والكادميوم من عينات غذائية باستخدام طريقة استخلاص نقطة الغيمة

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الخلاصة

تم تطبيق تقنية استخلاص نقطة الغيمة (CPE)وهي تقنية حساسة ودقيقة وغير ملوثة لتحديد الكاتيونات المعدنية الدقيقة الزنك (II) والكادميوم ((II) في عينات الغذاء. تم استخدام المادة النشطة سطحيا Triton X – 114 مع 1–(4– (Phenyldiazenyl) phenyl) azo naphthalene–2–ol (Sudan III) كمستخلص عند الرقم الهيدروجيني = 9 . يتم دمج السودان الثالث مع أيونات المعادن لتكوين معقد ترابط أيوني لذلك يجب أن تكون كفاءة الاستخلاص المثلى في وجود 5ppm من الزنك (II) أو4 ppm من الكادميوم (II) في محلول مائي. بالإضافة إلى تأثير درجة الحرارة يظهر أن 60 درجة مئوية كانت درجة حرارة نقطة الغيمة المثلى (CPT)مع 20 دقيقة لأقصى كفاءة استخلاص لكل من الأيونات المعدنية. يتضمن البحث دراسات أخرى مثل الأس الهيدروجيني وأنواع وأحجام المواد الخافضة للتوتر السطحي وقياس العناصر المتكافئة وتحديد الطيف الضوئي لهذه الأيونات المعدنية في عينات الطعام. أظهرت النتائج حدود كشف منخفضة للزنك والكادميوم 0.035و 0.042 جزء بالمليون على التوالي. تم الحصول على حدود قياس للزنك والكادميوم مقدارها 0.116 0.140,جزء بالمليون على التوالي

1. Introduction

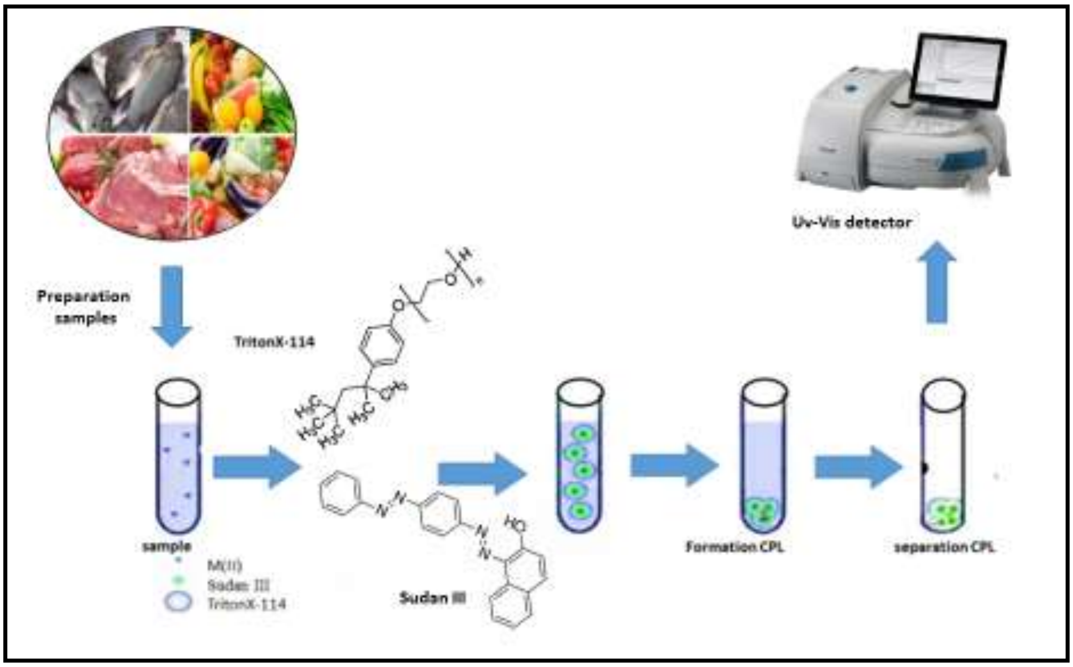
Due to discharges from industrial wastes, Urban, agricultural and sewage, heavy metal contamination has been noted as an issue in the coastal environment. Marine organisms can absorb metal ions through a number of mechanisms, including breathing, uptake, and ingestion. Essential metals, such as copper, iron, copper, manganese, zinc, play a significant part in biological processes, but non-essential metals, such as mercury, lead, and cadmium, are poisonous even in tiny amounts. When metal consumption is overly high, the essential metals might cause hazardous effects. Because fish and mussels make up such a large portion of the human diet, it's no surprise that several research on metal accumulation in various fish species have been conducted. Zinc deficiency can cause sickness because it is one of the most significant components of enzyme cellular function [1]. As a consequence, it has been investigated in various studies for use in water and food samples [2]. Zinc was extracted in real samples using the chemical reagent 1-(2-Pyridylazo)-2-naphthol (PAN) in Triton X-100 media with an effective CPE technique [3]. When employing a novel ligand 3- [(2-Pyridyl azo)]-1-nitroso-2-naphthol (PANN) and an ionic surfactant Triton X-100 to assess zinc from acidic conditions in many samples, the CPE application procedure produces a low detection limit of D.L = 0.0292 ppm [4].

Cadmium (Cd) is an extremely hazardous heavy metal that has become widespread in the environment during the last few decades. Cd (II) is a hazardous heavy metal that poses a serious health risk to humans. Cd has a long half-life (10-30 years) and can enter the body through the atmosphere, soil, water, and food. It primarily accumulates in the kidneys, liver, bone, and other tissues, causing irreversible harm to the target tissues.

Cd (II) is a hazardous metal that has no biological rules, although it can accumulate in the kidneys and liver when exposed to it from any source. Many cadmium experts have used the CPE approach, which produces good, accurate, and sensitive results [5-8].

A variety of methods have been used for this purpose, including liquid-ion exchange (LIE) [9,10], solvent extraction (SE) [11], flame atomic absorption spectroscopy (FAAS) [12], spectrophotometric study [13], liquid chromatography [14], kinetic spectrophotometry [15], and cloud point extraction (CPE) [16]. However, The CPE is a sensitive preconcentration and isolation procedure for abundant analytes with several advantages over traditional solvent extraction [17–19]. CPE is a quick, cost-effective, fast, precise, and selective process. It is defined as a method of green extraction since harmful organic solvents are not used [20–22]. Depending on the surfactant characteristics, micelles form after heating above a fixed temperature (CPT) and using a small amount of salt (salting-out phenomenon) [23]. The solution in CPE is divided into two layers: an aqueous layer (AL) and a cloud point layer (CPL), which is rich in surfactants and typically contains analytes. The type and concentration of surfactant used to form CPL are two critical reagents in separation [24–29]. In CPE, the analytes must be hydrophobic or be converted into hydrophobic molecules for surfactant coupling. Also, direct extraction of metal ions is impossible unless they are changed into special species (more hydrophobic) before CPE by complexation with a special reagent. However Organic chemicals (phenol, ethers, azo dyes, and pharmaceutical substances) may be extracted by CPE without chelation [30–33].

The purpose of this study was to determine the amount of Zn (II) and accumulate Cd (II) in food samples, as well as to investigate the impact of contamination on green vegetables, fruits, legumes, and seafood. It's also important to use a cheap, easy, and safe method to preconcentrate and determine ultra-low Zn (II) and Cd (II) concentrations in practical experiments. Prior to spectrophotometric determination, a CPE technique was used to preconcentrate Zn (II) and Cd (II) using Triton X-114 micelle medium and organic reagent Sudan III. The technique for determining samples was effectively implemented. Scheme 1 abstracted the procedure.



Scheme 1. Graphic Abstract of Applied Procedure.

2. Experimental

2.1. Instrumentation

For spectrophotometric studies, absorbance measurement and adjustment temperature were used Shimadzu UV-Vis spectrophotometer (UV-1700) made in Japan, electrostatic bath (Hamburg-90) made in England, respectively. So that pH-meter (model E163694), from Germany and a flame atomic absorption spectrophotometer, a Shimadzu type (AA-6800 design, Kyoto, Japan), with a hollow cathode lamp operating at 12 mA, were also used in this work.

2.2. Solutions

Providing a stock solution (1000 g/mL), 0.1 g of Zinc metal was dissolved in 10 mL hydrochloric acid (0.5 M) and the solution was completed to 100 mL in a volumetric flask. Using a volumetric flask, make a 1000 g/mL stock solution for Cd (II), dissolve 0.1632 g of $CdCl_2$, add Concentrated HCl (2 mL), and complete with 100 mL distill water. Another working solution can be made by diluting with distilled water in a volumetric flask of suitable size.

2.3. Food Samples Preparation

Food samples were collected at Al-Kufa, Iraq. After that, the samples were dried in an electric oven at 120°C and ground to a fine mesh. A 10 g mixture was cooked for 4 hours on a hot plate in a silica crucible, then transported to a furnace for nightly heating at 650°C. The residue was cooled, then processed with 10.0 mL concentrated HNO_3 and 3 mL 30% H_2O_2 before being maintained in a furnace for 3 hours at the same temperature to ensure that no traces of organic compounds are remained. The final residue was treated with 5 mL of concentrated HCl and 3 mL of 70% $HClO_4$ before being heated to evaporate the fumes and convert all of the metals to their proper ions [42]. In addition, blank digestions were carried out. The above-mentioned preconcentration process was then applied.

2.4. General Method

Preparation of 10 mL aqueous solutions containing the optimal amount of metal ions Zn (II) or Cd (II), pH=9 for both metal ions, $1x10^{-4}$ M Sudan III, and 0.5 mL (0.13 %/v) Triton X-114. The solutions were heated in electrostatic water bath at 60°C for 20 minutes for formation CPL, then separated CPL from aqueous solution, and dissolved in 5 mL ethanol. After that, compare the absorbance of dissolved CPL against a blank made in the same way but without the investigated metal ions at λ_{max} = 460 nm for Zn (II) and λ_{max} = 389 nm for Cd (II). Return to the calibration curve (Figure 1) to calculate the remaining quantity to every metal ion in aqueous solution for each metal ion treated using the dithizone spectrophotometer method [29]. As soon as the ion pair association is complete, subtract the quantity from the actual amount of every metal ion in aqueous solution to determine the transfer amount to CPL. the calculate distribution ratio (D) by relation below equation (1):

$$D = \frac{[M(II)]CPL}{[M(II)]aq} \quad ...(1) \quad M = \mathrm{Zn(II)\ or\ Cd(II)}.$$

3. **Results and Discussion**

Extraction of Zn (II) and Cd (II) ions according to the process outlined in the general approach and evaluation of effective parameters were the focus of the spectrometric experiments. Analytical statistics were also computed. Figure 1 shows the dithizone technique calibration curve for two metals, which can be used to calculate the amounts of metals in aqueous solution after preconcentration.

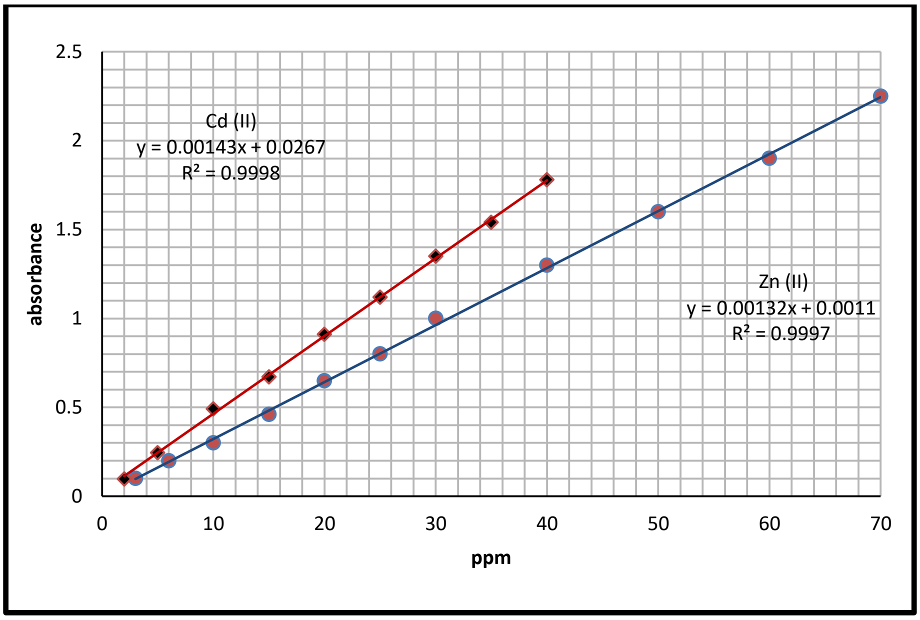


Figure 1: Calibration Curve for Dithizone Method for Extraction Zinc (II) and Cadmium (II).

3.1. Extraction Method Parameters

3.1.1. pH Effect

Two aqueous solution series (10 mL) with 0.5 mL (0.13%) Triton X–114, 1×10^{-4} M Sudan III at various pH were used, with the first series containing 5 ppm Zn (II) and the second series including 4 ppm Cd (II) These solutions were heated at the ideal temperature in an electrostatic water bath till CPL formation. The CPL for each ion was separated from aqueous solution and completed the work as described in the detailed process. Figures 2 and 3 show the results, that the pH=9 was the best value for both metal ion extraction efficiencies. This optimum pH value gives best chance for binding metal ion with organic reagent Sudan III for formation a higher concentration of ion pair association complex. Any pH value which less or greater than the optimal point will reduce extraction efficiency by reducing the impact of metal ions forming ion pair association complexes. When the pH value is higher than the optimal value, the hydroxide ions will contribute to the creation of stable species with the metal ion. The formation of an ion pair association complex with metal ions whose distribution is in the aqueous phase is achievable for hydroxide ions.

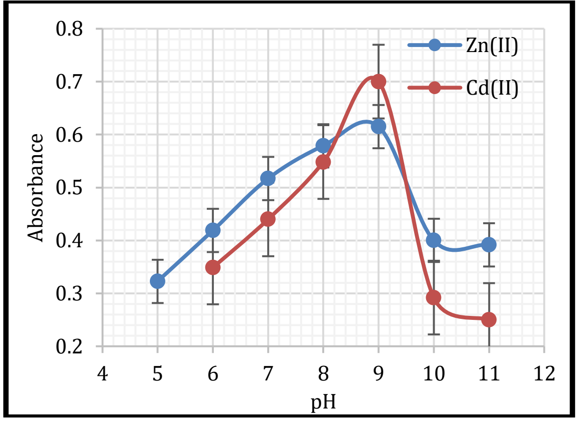


Figure 2: pH Effect on CPL at Applied Method

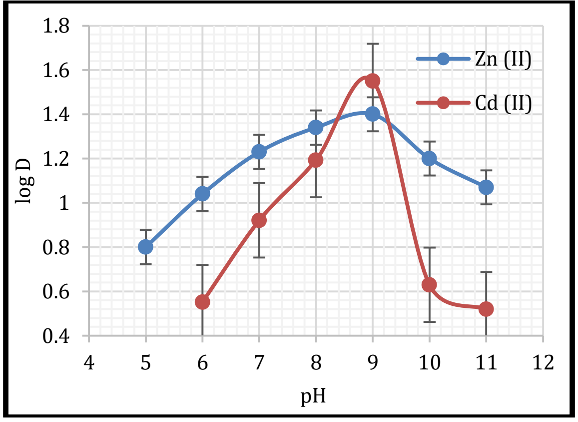


Figure 3: pH Effect on Extraction Efficiency for Each Metal Ion

3.1.2. surfactant kind Effect

According to the comprehensive approach, metal ions under investigation were extracted at optimum conditions, in the presence of different surfactants, and the results were as shown in Table 1. The results reveal that varying extraction efficiency exists for both metal ions in the presence of different surfactants because each surfactant has a different micelle structure and ability to form CPL, implying that extraction efficiency varies. Triton X-114 was used for the experiments because it provided the best absorbance and distribution ratio values.

Table 1: Effect of Surfactant Kinds on Extraction Efficiency of Zn (II) and Cd (II) Metal Ions.

Surfactant	Extraction of Zn (II)		Extraction of Cd (II)	
	Absorbance	**D**	**Absorbance**	**D**
Tween 20	0.415	40.11	0.422	44.15
Tween 40	0.325	53.56	0.378	32.74
Tween 80	0.356	56.98	0.587	80.89
Triton X-114	0.615	90.32	0.732	95.56

3.1.3. Surfactant Volume Effect

Preparing two series of 10mL aqueous solutions at pH=9, 5 ppm Zn (II), and 4 ppm Cd (II), with increasing volumes of surfactant Triton X-114 and all ideal conditions for examining the influence of Triton X-114 volume, yielded the findings shown in Figures 4 and 5. The results show that 0.5mL Triton X-114 was the optimum volume for higher extraction efficiency for both metal ions, and that this volume is suitable for reaching critical micelles concentration (CMC) state, in order to form the best CPL suitable for excellent extraction. Any volume less or more than the optimum value is not suitable for reaching CMC state, and thus gives lower extraction efficiency [42].

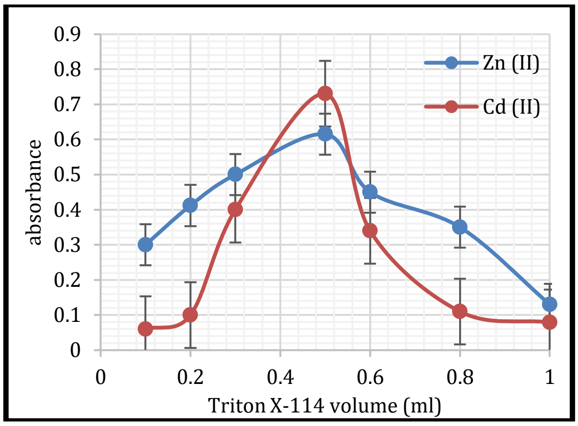


Figure 4: Impact of Surfactant Amount on Excellent CPL Formation.

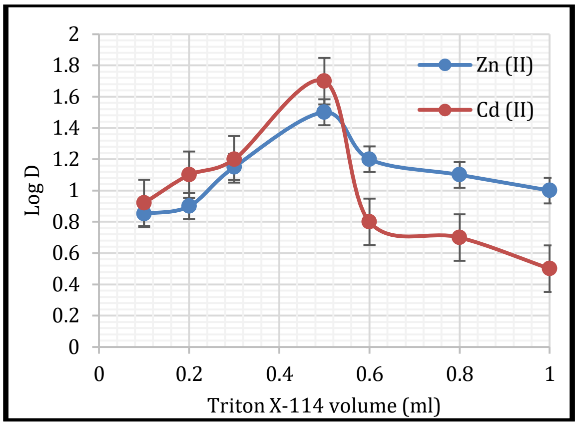


Figure 5: Impact of Surfactant Amount on Extraction Efficiency.

3.1.4. Temperature and Heating Time Effect

Zn (II) and Cd (II) were extracted at optimal conditions for a predetermined duration at rising temperatures till the creation of CPL, according to the overall procedure. As a result, at each temperature, CPL was isolated from the aqueous phase, and the process was completed, providing the results shown in Figures 6 and 7. T=60 °C was reported to be the optimum temperature for maximum extraction efficiency for both ions examined, according to the findings. When it comes to CPL production and extraction, this temperature achieves the strongest thermodynamic equilibrium [43].

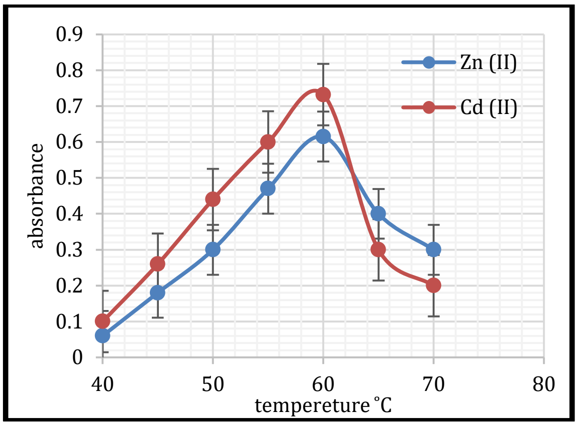


Figure 6 Effect of Temperature on Formation and Stability of CPL.

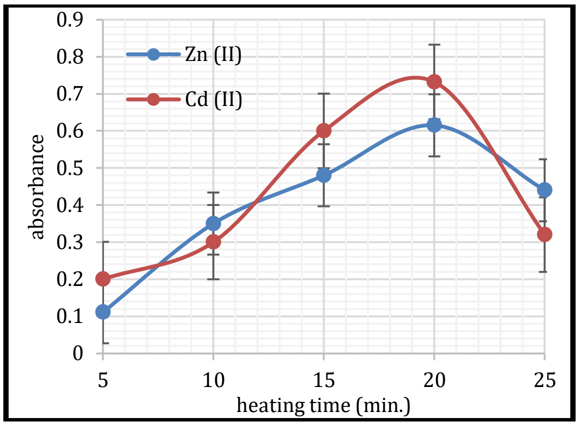


Figure 7 Effect of Heating Time on Formation and Quality CPL

After measuring the extraction content K_{ex} at each temperature, Figure 8 was created by plotting the logarithm value K_{ex} versus 1/T K. As shown in Table 3, which was utilized to calculate the thermodynamic date for each metal ion [42]. The optimum heating period for excellent extraction efficiency of both metal ions was 20 minutes.

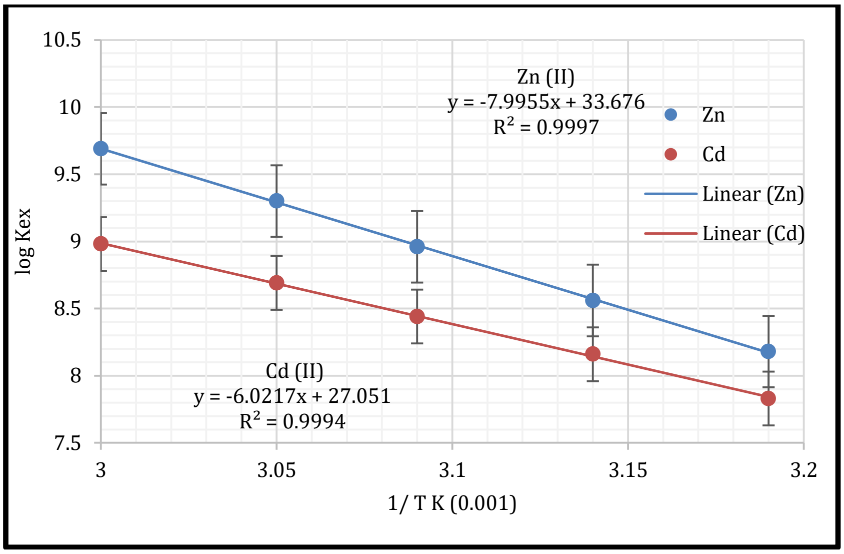


Figure 8: Effect of Temperature on Extraction Content K_{ex}

$$slope = \frac{-\Delta H_{ex}}{2.303\ R} \qquad \dots(2)$$

$$\Delta G_{ex} - RT\ Ln\ K_{ex} \qquad \dots(3)$$

$$\Delta G_{ex} = \Delta H_{ez} - T\Delta S_{ex} \qquad \dots.(4)$$

Table 2: Thermodynamic Data for Cloud Point Extraction Method

Metals	ΔH_{ex} K.J.mole^{-1}	ΔG_{ex} K.J.mole^{-1}	ΔS_{ex} J.mole^{-1}K^{-1}
Zn(II)	0.152	-60.475	**181.930**
Cd(II)	0.115	-57.246	**172.26**

3.2. Stoichiometry

The structure of the extracted ion pair association complex was determined using two spectrophotometric methods, slope mole ratio and slope analysis, as illustrated in equations 5 and 6 and Figures 9 and 10. The results suggest that the extracted ions pair association complex for both metal ions has a more likely structure, with Sudan (III) (1:1) to the ion.

$$\textbf{slope ratio } Zn(II) = \frac{\mathbf{5902.3}}{\mathbf{5862.7}} = \mathbf{1.00} \ldots (5)$$

[Sudan III–Zn]$^{+}$; anion

$$\textbf{slope ratio } Cd(II) = \frac{\mathbf{7568.2}}{\mathbf{6399.8}} = \mathbf{1.18} \ldots (6)$$

[Sudan III-Cd]$^{+}$; anion

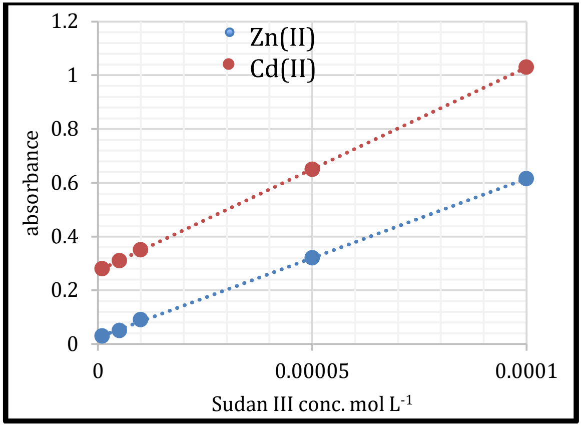


Figure 9: Slope Mole Ratio Method Relation Sudan III for Zn (II) and Cd (II)

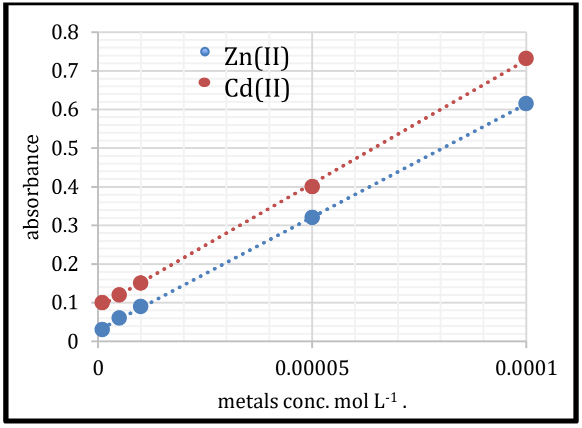


Figure 10: Effect of Metal in Calculator Slope Ratio

3.3. Food Samples Determination

After constructing a calibration curve of the metals, Zn (II) and Cd (II) metal ions were determined extensively under optimal conditions [43]. Table 3 also lists the analytical parameters that were measured.

In different positions, all nine food samples were taken from Al-Kufa markets. The figures given are the average of three samples examined. Table 5 highlights the results obtained through the use of two methods: CPE and FAAS. These results suggest that the technique may be used to determine the levels of zinc (II) and cadmium (II) in food samples. The recovery of metals applied to the samples demonstrates the efficacy of the suggested procedure. For this work, we also acquired a low detection limit and higher preconcentration factors.

Table 3: Analytical Parameters of Applied Method.

Parameters	Zn (II)	Cd (II)
Regression equation correlation coefficient	$y = 0.3308x + 0.0228$ $R^2 = 0.9998$	$y = 0.2704x + 0.0518$ $R^2 = 0.9997$
Linear range (ppm)	0.25- 700	0.25- 400
Reproducibility (RSD%) n = 3	1.2	2.1
Detection limit (ppm)	0.035	0.042
Quantification Limit (ppm)	0.116	0.140
Preconcentration factor	250	180

The characteristics of the employed methodology are compared to the previously published techniques of the Triton X-114 micellar system (CPE) for metal determination with various detection systems [34–41], with the findings shown in Table 4. According to the analysis, the produced application has a wide dynamic range, low detection and quantity limits, and a higher preconcentration factor.

Table 4: Comparison of recently CPE used Triton-X114 as surfactant with different detection procedures and the applied procedure for determination of studied ions

Metal ion	Organic reagents	Sample volume (mL)	L.D ppm	Applied method	P F[a]	Ref.
Zn (II)	8-hydroxyquinoline	25	1.2	CPE/UV-Vis	10	[34]
Zn (II)	PAR	10	51.7	MCPE / UV-Vis	2.58	[35]
Zn (II) **Cd (II)**	PAR	-	1.1 1.0	CPE/ ICP-OES	9.4 10.1	[36]
Zn (II) **Cd (II)**	PAN	-	0.02 ±0.001	CPE / FAAS	-	[37]
Zn (II) **Cd (II)**	8-hydroxyquinoline	25	1.9 2.0	CPE/ICP OES	2	[38]
Cd (II)	VBB^+	5	0.337	CPE/FAAS	35	[39]
Cd (II)	DPTH	10	0.95	CPE/FAAS	10.5	[40]
Cd (II)	PAN	0.01	0.0178	CPE/TS-FF-AAS	55.5	[41]
Zn (II) **Cd (II)**	Sudan III	10	0.035 0.042	CPE / UV-Vis	250 180	This work

[a] The ratio of the slope of preconcentration samples to that obtained without preconcentration is determined as the Preconcentration factor.

ICP OES; inductively coupled plasma optical emission spectrometry, TS-FF-AAS; thermos pray flame furnace atomic absorption spectrometry.

PAR; 4-(2-pyridylazo)-resorcinol, VBB^+; Victoria blue B, DPTH; 1, 5-bis(di-2-pyridylmethylene) thiocarbonohydrazide.

Table 5: Determination Zn (II) and Cd (II) Ions in Food Samples by Application CPE and FAAS Methods.

No.	Samples	Added Concentration ppm	Found Concentration Zn (II) ppm± RSD	FAAS	Found Concentration Cd (II) ppm ± RSD	FAAS
1.	Celery leaf	0 10 20	- 10.35±0.01 20.35±0.20	- 9.90±0.40	- 10.11±0.01 20.15±0.11	- 11.25±0.14
2.	Chickpeas	0 10 20	- 11.205±0.01 20.315±0.07	- 11.341±0.20	- 10.96±0.03 19.00±0.01	- 9.955±0.11
3.	Fish	0 10 20	- 10.36±0.20 21.05±0.44	- 10.955±0.20	- 10.05±0.01 20.15±0.11	- 11.15±0.51
4.	Lentil	0 10 20	- 12.38±0.21 22.45±0.01	- 12.450±0.70	- 10.08±0.02 20.05±0.71	- 11.15±0.71
5.	Limon	0 10 20	- 13.32±0.12 23.75±0.55	- 12.55±0.11	- 09.12±0.08 19.55±0.71	- 10.20±0.20
6.	Liver	0 10 20	- 11.40±0.22 21.45±0.01	- 12.37±0.20	- 10.85±0.01 20.15±0.41	- 11.15±0.41
7.	Orange	0 10 20	- 12.31±0.11 23.05±0.41	- 12.35±0.77	- 10.05±0.20 2.125±0.01	- 9.95±0.21
8.	Parsley leaf	0 10 20	- 11.71±0.22 21.15±0.01	- 11.350±0.20	- 10.18±0.02 20.15±0.01	- 9.55±0.11
9.	Peaches	0 10 20	- 10.41±0.22 21.35±0.79	- 10.25±0.11	- 10.15±0.06 19.15±0.71	- 10.10±0.70

4. Conclusions

The new approach comes with a number of benefits, such as affordability, environmental friendliness, safety, and high extraction efficiency. Additionally, it provides RSD values, an excellent preconcentration factor, and a low detection limit. As a consequence of the study's findings, there was no contamination in the samples that were examined. Triton X-114's properties as a lipophilic surfactant and its capacity to resist the electrolytes created by related ions make CPE possible. Sudan III makes extracted complexes more lipophilic, facilitating their easier transfer to the cloud point layer. Preconcentration variables are quite high compared to another research. Additionally, the relatively low detection limits enable greater extraction sensitivity and the use of this method for the detection of extremely small metal amounts.

5. Acknowledgements

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6. conflict of interest

The authors declare that they have no conflicts of interest.

References

[1] W. Maret, "The metals in the biological periodic system of the elements: concepts and conjectures," *Int. J. Mol. Sci.*, vol. 17, no. 1, pp. 66, 2016.

[2] B. Alyüz, S. Veli, "Kinetics and equilibrium studies for the removal of nickel and zinc from aqueous solutions by ion exchange resins," *J. Hazard. Mater.*, vol. 167, no. (1–3), pp. 482–488, 2009.

[3] N. D. Jaffer, "Cloud-point technique for extraction and spectral estimation of zinc (II) in different samples," *Biochem. Cell. Arch*., vol. 19, no. 2, pp. 4683-4690, 2019.

[4] S.K. Jawad, and E. A. Azooz, "A new Approach for Separation, Extraction and Determination of Zinc in Different Samples using Cloud-Point Extraction Coupled with spectrophotometry," *FIRE J. Science and Technology*, vol. 3, pp.261-273, 2015.

[5] M. Khan, T.G. Kazi, H.I. Afridi, M. Bilal, A. Akhtar, N. Ullah, S. Khan, S. Talpur, "Application of ultrasonically modified cloud point extraction method for simultaneous enrichment of cadmium and lead in sera of different types of gallstone patients," *Ultrason. Sonochem*. vol. 39, pp. 313–320, 2017.

[6] M. Bilal, T.G. Kazi, H.I. Afridi, M.B. Arain, J.A. Baig, M. Khan, N. Khan, "Application of conventional and modified cloud point extraction for simultaneous enrichment of cadmium, lead and copper in lake water and fish muscles", *J. Ind. Eng. Chem*., vol. 40, pp. 137–144, 2016.

[7] E. A. Azooz, H. S. A. Al-Wani, M. S. Gburi, E. H. B. Al-Muhanna, "Recent Modified Air-Assisted Liquid-Liquid Micro-Extraction Applications for Medicines and Organic Compounds in Various Samples: A Review". Open Chem., vol. 20, no. 1, pp. 525-540, 2022.

[8] M. Ghaedi, A. Shokrollahi, K. Niknam, E. Niknam, A. Najibi, M. Soylak, "Cloud point extraction and flame atomic absorption spectrometric determination of cadmium (II), lead (II), palladium (II) and silver (I) in environmental samples," *J. Hazard. Mater*., vol. 168, no. (2–3), pp. 1022–1027, 2009.

[9] E. A. Azooz, F. A. Wannas, R. K. Ridha, S. K. Jawad, E. A. J. Al-Mulla, "A Green Approach for Micro Determination of Silver(I) in Water and Soil Samples Using Vitamin C," *Anal. Bioanal. Chem. Res.,* vol. 9, no.2, pp.133-140, 2022.

[10] A. Matsuoka, and K. Mae, "Liquid–liquid extraction performance of circulation-extraction method using a microchannel device, "*Solvent Extraction and Ion Exchange*, vol. 39, no. 7, pp. 785-805, 2021.

[11] F. A. Wannas, E. A. Azooz and S. K. Jawad, "Liquid Ion Exchange with Spectrophotometric Method for Separation and Determination W(VI)," *Jour of Adv. Research in Dynamical & Control Systems*, vol. 11, 07-special issue, pp. 260-269, 2019.

[12] S. Kori, "Cloud point extraction coupled with back extraction: a green methodology in analytical chemistry," *Forensic Sciences Research*, vol. 6, no. 1, pp. 19-33, 2021.

[13] R. K. Ridha, E. A. Azooz, and S. S. Taresh, "Rapid Palladium Preconcentration and Spectrophotometric Determination in Water and Soil Samples," *Anal. Bioanal. Chem. Res.,* Vol. 9, No. 3, pp. 251-258, 2022.

[14] M. G. A. Al-Mosawy, E. A. J. Al-Mulla, and M. J. Mohamad, "Bentonite-Based Nano Organic Clay Using Chalcone and Azo Dye as Organophilic Reagents," Nano *Biomed. Eng.,* vol. 9, no.2, , pp. 124-128, 2017.

[15] A. N. Naser, M. A. Kasim, A. K. Zainab, "New approach for determination of sulfadiazine in pharmaceutical preparations using 4(4-sulphophenylazo) pyrogallol: Kinetic spectrophotometric method," *Spectrochim Acta A Mol Biomol Spectrosc*, vol. 201, pp. 267–280, 2018.

[16] W. I. Mortada, "Recent developments and applications of cloud point extraction: A critical review," *Microchemical J.*, vol. 157, pp. 105055, 2020.

[17] E. A. Azooz, J. R. Moslim and S. K. Jawad, "Cloud Point Extraction Methodology for Separation, Extraction and Preconcentration of Mn (VII) Coupled with Spectroscopy for Determination in

Different Samples," *Biochem. Cell. Arch*. vol. 20, no. 1, pp. 2641-2648, 2020.

[18] E. J. Sahan, "Evaluation of Zinc, Copper, and Lead Levels in The Blood of Breast Cancer Women in Baghdad City," *Iraqi Journal of Science*, Vol. 63, No. 1, pp: 1-8, 2022.

[19] S. K. Jawad, M. U. Kadhium, and E. A. Azooz, "Separation and Spectrophotometric Determination of Iron (III) and Mercury (II) via Cloud Point Extraction with New Azo-Derivative," *Eur. J. Anal Chem.*, vol. 13, no. 5, pp. 1-11 em48, 2018.

[20] M. G. A. Korn, J. B. Andrade, D. S. de Jesus, V. A. Lemos, M. L.S.F. Bandeira, W. N.L. Santos, M. A. Bezerra, F. A. C. Amorim, A. S. Souza, and S. L.C. Ferreira, "Separation and preconcentration procedures for the determination of lead using spectrometric techniques: A review," *Talanta*, vol. 69, no. 1, pp. 16-24, 2006.

[21] E. A. Azooz, R. K. Ridha, and H. A. Abdulridha, "The Fundamentals and Recent Applications of Micellar System Extraction for Nanomaterials and Bioactive Molecules: A Review," *Nano Biomed. Eng.*, vol. 13, no. 3, pp. 264-278, 2021.

[22] M. K. Aruoah, and S. A.-K. Al-Jowari, "The Effects of Zinc and Vitamin C Supplementation on the Glycemic Profile in Type 2 Diabetic Patients," *Iraqi Journal of Science*, vol. 63, no. 1,pp. 70-76, 2022.

[23] E. A. Azooz, J. R. Moslim, S. M. Hameed, S. K. Jawad, E. A. J. Al-Mulla, "Aspirin in Food Samples for Separation and Micro Determination of Copper (II) using Cloud Point Extraction/Solvation Method," *Nano Biomedicine and Engineering*, vol. 13, no. 1, pp. 62-71, 2021.

[24] M. M. El-Ajaily, H. B. Alhaj, A. A. Erdeni, T. H. Al-Noor and S. H. Al-Mabrouk, "Removal of Cadmium(II) ion from aqueous solutions by the outer layer of Onion" *IOP Conf. Series: Earth and Environmental Science*, vol. 790, pp.012011, 2021.

[25] E. A. Azooz, F. A. Wannas and S. K. Jawad, "Developed Cloud Point Extraction Coupled with Onium System for Separation and Determination Cobalt in Biological Samples," *Res. J. Pharm. Tech.*, vol. 14, no. 2, PP. 594-598, 2021.

[26] N. P. Milchev, F. Genç, P. V. Rachev, V. B. Delchev, V. Andruch, K. B.Gavazov, " An environmentally friendly cloud point extraction–spectrophotometric determination of trace vanadium using a novel reagent," *J. Molecular Liquids,* Vol. 334, pp. 116086, 2021.

[27] S. K. Jawad, M. U. Kadhium and E. A. Azooz, "Application Cloud Point Extraction Method Joined with Liquid Ion Exchange for Selective Determination of $Fe^{3+}$and Hg^{2+} In Real Samples," *J. Eng. Appl. Sci.*, vol. 14, no. 11, pp. 3514-3521, 2019.

[28] M. Xie, W. Liu, X. Hao, et al. "Cloud point extraction combined with flame atomic absorption spectrometry for the determination of copper, iron and zinc using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol-tetraphenylborate ion-pairing agent." *Chem. Pap.*, vol. 75, pp. 5849–5859 2021.

[29] Z. Habibi, and F. Bamdad, " Simultaneous Determination of Traces of Cobalt and Iron Ions after Pre-concentration by Surface-active Ionic Liquid-assisted Cloud Point Microextraction" *Anal. Bioanal. Chem. Res.*, vol. 9, no. 3, pp. 243-250, 2022.

[30] S. K. Jawad, M. O. Kadhim, and E. A. Azooz, "Incorporation of onium system with cloud point extraction and determination of iron (III) and mercury (II) in different samples," *Orient. J. Chem.*, vol. 33, no. 4, pp. 1879–1889, 2017.

[31] X. P. Yang, Z. H. Jia, X. C. Yang, N. Luo and X. J. Liao, "Determination of Trace Zinc in water, soil and rabbit blood samples using Cloud Point extraction coupled with Ultra Violet-Visible spectrophotometry," *Applied Ecology and Environmental Research*, vol. 15, no. 3, pp. 537-548, 2016.

[32] W.I. Mortada, M. M. Hassanien, A. F. Donia, A. A. Shokeir, "Application of cloud point extraction for cadmium in biological samples of occupationally exposed workers: relation between cadmium exposure and renal lesion," *Biol. Trace Elem. Res.*, Vol. 168, no. 2, pp. 303–310, 2015..

[33] H. Wu, G. Y. Zhao, L. M. Du, "Determination of ofloxacin and gatifloxacin by mixed micelle-mediated cloud point extraction-fluorimetry combined methodology," *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.* Vol. 75, no. 5, pp. 1624–1628, 2010.

[34] A. B. Tabrizi, "Cloud point extraction and spectrofluorometric determination of aluminum and zinc in foodstuffs and water samples," *Food Chemistry*, vol. 100, pp. 1698, 2007.

[35] E. Ghasemi and M. Kaykhaii, "Determination of Zinc, Copper, and Mercury in Water Samples by Using Novel Micro Cloud Point Extraction and UV-Vis Spectrophotometry," *Eurasian Journal of Analytical Chemistry*, vol. 12, no. 4, pp. 313-324, 2017.

[36] E.L. Silva, P.D.S. Roldan and M.F. Giné, "Simultaneous preconcentration of copper, zinc, cadmium, and nickel in water samples by cloud point extraction using 4-(2- pyridyl azo)-resorcinol and their determination by inductively coupled plasma optic emission spectrometry," *J. Hazard. Mater*., vol. 171, pp. 1133–1138, 2009.

[37] J. Guo, L. Li, T. Li, G. Ren, P. Wang, J. Liu, Y. Zhang, "Determination of copper, zinc and cadmium by cloud point extraction-flame atomic absorption spectrometry in red wine," *J. of Chinese Institute of Food Science and Technology*, vol. 16, no. 7, pp. 252-258, 2016.

[38] F. L. F. Silva, W. O. Matos, G. S. Lopes, "Determination of cadmium, cobalt, copper, lead, nickel and zinc content in saline produced water from the petroleum industry by ICP OES after cloud point extraction," *Analytical methods,* vol. 23, pp. 1-29, 2015.

[39] R. Gurkan and N. Altunay, "Determination of trace cadmium in nonalcoholic beverages by coupling cloud point extraction with spectrophotometry," *Food Nutr. Sci*., vol. 63, pp. 253-260, 2013.

[40] C. B. Ojeda, F. S. Rojas and J.M. Cano Pavón, "Preconcentration of cadmium in environ-mental samples by cloud point extraction and de-termination by FAAS," *Am. J. Anal. Chem*., vol. 1, pp. 127-134, 2010.

[41] H.C. Rezend, C.C. Nascentes and N.M.M. Coe-lho, "Cloud point extraction for determination of cadmium in soft drinks by thermospray flame furnace atomic absorption spectrometry," *Microchem. J*., vol. 97, pp. 118–121, 2011.

[42] W. I. Mortada, E. A. Azooz, "Microextraction of metal ions based on solidification of a floating drop: basics and recent updates," *Trends in Environ. Anal. Chem.*, vol. 34, pp. e00163, 2022.

[43] E. A. Azooz, G. J. Shabaa, E. A. J. Al-Mulla, "Methodology for Preconcentration and Determination of Silver in Aqueous Samples using Cloud Point Extraction," *Braz. J. Anal. Chem*. vol. 35, no. 9, pp. 39-48, 2022.