Article

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Evaluation of Optimal Parameters for Lead(II) Ion Extraction Using a Newly Azo Organic Reagent via Cloud Point Extraction Method

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Abstract

A new organic reagent [3-((2-chloro-4-hydroxyphenyl) diazenyl)-5nitrobenzene-1,2-diol] (CHPDND) was prepared and characterized by (IR, HNMR, 13C-NMR, C.H.N) used for spectrophotometric determination of Pb(II) ions in aqueous solution. The maximum wavelength of organic reagent λmax=410nm and for its complex with Pb(II) was 395nm. The study includes all optimal parameters for Pb(II) ions extraction as chelation complex by cloud point method such as pH_{ex}, temperature in addition to calculate thermodynamic data ΔH_{ex}, ΔG_{ex}, and ΔS_{ex}, heating time, surfactant volume, etc. Slope analysis method used for determine the probable structure of Pb(II)-(CHPDND) complex was 1:1. Applied method for spectrophotometric determination of Pb(II) in some environmental samples with LOD=0.219mg/L and LOQ=0.664mg/L and the results compared with AAS as standard method.

Keywords: Azo reagent, lead(II), cloud point extraction.

Introduction

Lead ion (Pb(II)) is one of dangerous pollutants because it is not biodegradable [1]. Any increase in lead content above these levels has a negative impact on human health and

causes a range of diseases, including learning disabilities, central nervous system defects, kidney, liver, and bone problems. All of these diseases are caused by the difficulty of its decomposition and accumulation in the bodies of living organisms[2]. Examples of lead estimation methods include atomic absorption spectrometry(AAS)[3], mass spectrometry[4], strip voltammetry[5], inductively coupled plasma emission spectrometry[6], flame atomic absorption spectroscopy[7] inductively coupled plasma optical emission spectroscopy (ICP-OES)[8], inductively coupled plasma mass spectrometry (ICP-MS)[9], Raman spectroscopy[10] electrothermal atomic absorption spectroscopy (ETAAS)[11], graphite furnace atomic absorption spectroscopy (GFAAS)[12] , laser desorption/ionization mass spectrometry (LDI-MS) electrochemical[13], voltammetry and high-performance liquid chromatography[14]. A heterogeneous azo dye was prepared in this study and proposed as a highly sensitive chromogenic reagent for the estimation of lead (II) ions[15], Green techniques were used to determine lead (II) ions[16], which represent a central axis in modern analytical chemistry among these techniques, we used in this study cloud point extraction, which is characterized by being an environmentally friendly method used for separation and pre-concentration.

CPE is characterized by being accurate [17], fast, selective, and inexpensive [18]. In addition, it uses small amounts of toxic organic solvents [19], there are still new and proposed steps regarding CPE [20]. This study describes the preparation and characterization of new chelate complex of Pb (II) were prepared by reacting this ion with the ligand [3-((2-chloro-4-hydoxy phenyl) diazenyl)-5nitrobenzen 1,2-diol].

Experimental part

Apparatus

For absorption measurements and optical studies, a Biochrom Libra S60 dual-beam spectrophotometer, 1cm quartz cell (UK) was used. pH measurements were performed using a pH-meter, 720, WTW 82362, for heating the solutions at a constant temperature an electrostatic water bath (Hamburg-90, England) was used. A&D balance (DOOl, CE,

HR 200) Japan, Elemental Analysis (C.H.N) C.H.N Analyzer EURO EA3000, FT-IR Shimadzu (8400 series, Japan) spectrophotometer across the range of 4000 to 400 cm^{-1}. Atomic absorption AA-500, Pg Instrument, UK.

Reagents and solution

Solution: All chemicals used were of high purity, as the chemicals were purchased from certified companies. In all experiments, distilled water was used to prepare the solutions. the standard Pb(II) solution (100µg/mL) was prepared by dissolving 1.59g of $Pb(NO_3)_2$ in 100 mL of distilled water, the ligand solution 1×10^{-3}M [3-((2-chloro-4-hydroxyphenyl) diazenyl)-5nitrobenzene-1,2-diol] was prepared by dissolving (0.00773 g) in 100 mL of distilled water, and any other working solutions were prepared by serial dilution with distilled water

Preparation and Characterization of azo compound (CHPDND)

The dark yellow reagent was prepared by dissolving (0.01mol, 1.43g) of 2-chloro-5-hydroxy aniline in 5 mL of distilled water and 2mL of concentrated hydrochloric acid, then the solution was cooled below (5 °C). To this mixture a solution of (0.01 mol, 1.028g) of sodium nitrate in 10 mL of distilled water was added drop wise at (0-5 °C) then, the mixture was stirred for 20 min. This diazonium solution was added drop wise to a 500 mL beaker containing (0.01 mol, 1.55g) of 4-nitrobenzene-1,2-diol dissolved (20 mL) ethanol, and (10mL) of NaOH (10%). The mixture was observed to turn dark yellow at pH~8 The mixture was left for 24 hours, then the precipitate was filtered, and washed with distilled water several times, dried, and recrystallized with absolute ethanol [21].

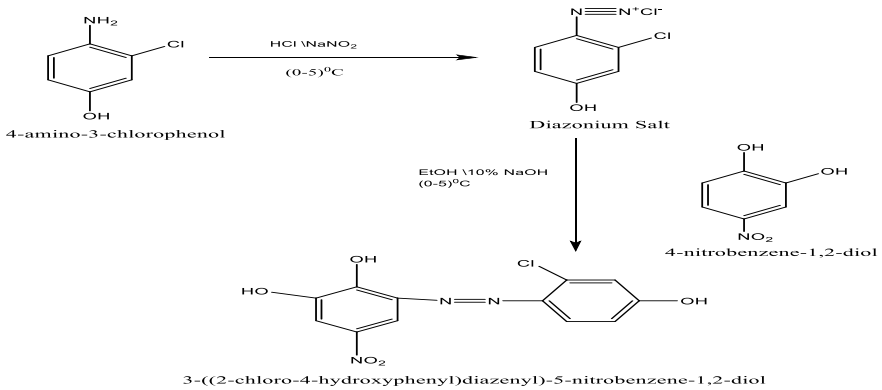


Figure (1): Preparation of azo compound

Physical Characteristics of CHPDND: when Pb(II) and the organized dark yellow reagent (CHPDND) react at the ideal concentration and pH, a complex with the formula [Pb(CHPDND)] is created. Table (1) explains the physical characteristics and outcomes obtained from C.H.N.O. testing. Based on the information obtained from spectral analysis, the molecular formula of the organic reagent has been proposed.

Table (1): Physical details of CHPDND

Empirical Formula (M.wt) g/mol	Color	MP. °C	Yield %	Found (Calc.) (%)		
				C	H	N
$C_{12}H_8ClN_3O_5$ M.wt=309.02	Dark Yellow	201-202 °C	85%	13.592 14.245	2.558 2.326	46.601 45.382

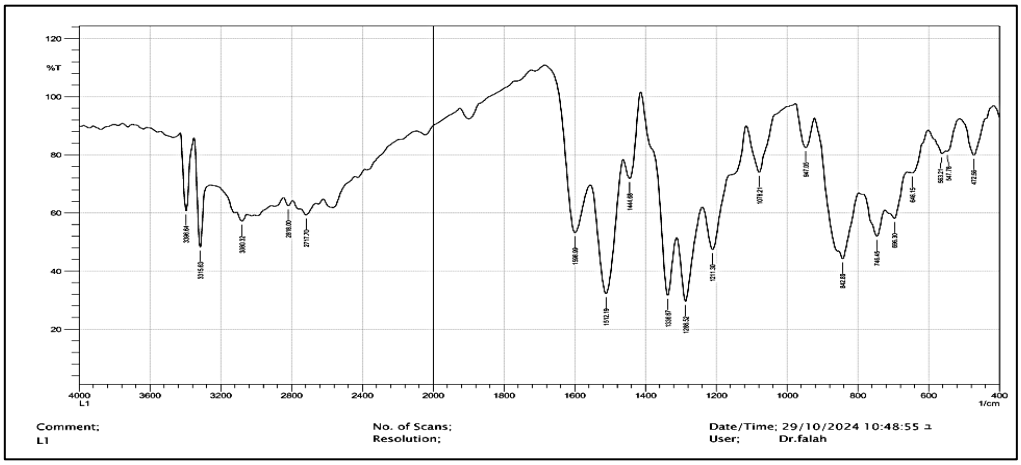


Figure (2): IR spectrum of the new organic detector (CHPDND)

IR Spectrum of CHPDND: The IR spectrum data of CHPDND was illustrated in Figure (2). The IR spectrum of the (CHPDND), exhibited a wide and strong intensity band at (3396-3315cm^{-1}), which was attributed to the stretching vibration of the hydroxyl group [22,23]. The band at (3080cm^{-1}) due to aromatic (C-H). Also the spectrum appear band at (1598.99 cm^{-1}) due to aromatic (C=C). The azo reagent showed a strong band at (1512 cm^{-1}) duo to (N=N) [24,25]. The band at (1568.02 cm^{-1}) assignable to the ν (C=N) vibration mode [24,26]. A medium intensity band at (1338.67cm^{-1}) returning to the (C-N) (carbon - nitrogen azo). Also the bands at (1286, 1336) cm^{-1} due to stretching

vibration of nitro group [27]. Finally, the band at (696.30cm^{-1})due to stretching vibration of(C-Cl) [27].

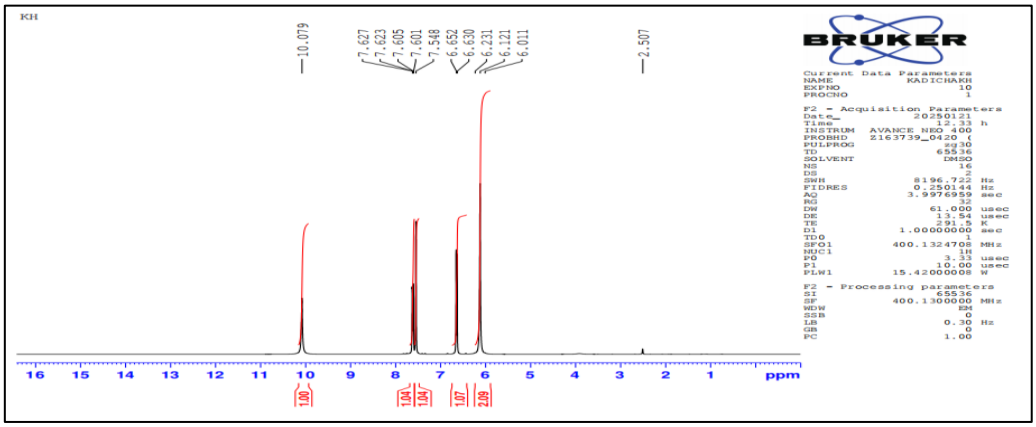


Figure (3): 1H NMR spectrum of the organic reagent (CHPDND) dissolved in DMSO

^{1}H-NMR Spectrum for CHPDND: The ^{1}H-NMR spectrum of (CHPDND) in DMSO Figure (3) was shows, δ (2.528) ppm DMSO, a multiplet peak appeared at δ(7.028-7.476) ppm which was attributed to chemical shift of protons in the aromatic rings [22,28]. A single peak at δ (9.883) ppm that have been as a result of chemical shifts of (N-H) protons. The single peak at δ (11.028) ppm were assigned to O-H protons [29, 30].

Extraction method

The solution was prepared under optimum conditions by taking 100µg/mL of lead (II) with 1×10^{-4} M of the prepared reagent and adding 0.5mL of TritonX-114, then the pH was adjusted to pH~8, then completed the volume to 10 mL with distilled water, then the solution was heated in an electrostatic water bath at a specified temperature and time, after which the cloud point layer (CPL) with the smallest size and highest density was separated and dissolved in 3 mL of ethanol, then measure the absorbance of the alcoholic solution at λ_{max} = 395 nm used ethanol as a blank. The aqueous solution was treated with dithizone reagent and its absorbance was measured at λ_{max} = 520nm versus CCl_4 as blank [31]. Then we return to the calibration curve in Figure (4) to find out the amount of Pb(II) ions that is still present in the aqueous solution after the separation process, then subtract the amount of Pb(II) ions remaining from the initial value to find

the amount transferred to the CPL, then calculate the distribution ratio D from these amounts as follows [32]:

$$D = \frac{[Pb^{+2}]cpi}{[Pb^{+2}]aq}$$

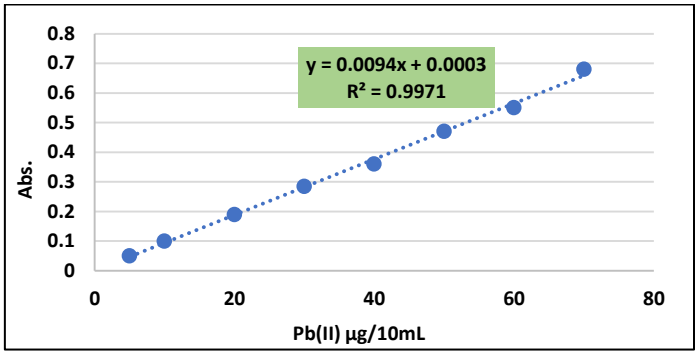


Figure (4): Calibration curve of Pb(II) using Dithiazone spectrophotometric method

Results and Discussion

Spectroscopic study: Aqueous solution with volume 10 mL containing 100 µg of Pb(II) ion, 1×10^{-4} M of the CHPDND, then adjusted pH to 8 and 0.5 mL of TritonX-114 surfactant were prepared. Then, the UV-visible spectrum of the alcoholic solution taken against a sample prepared in the same way without Pb(II) ions[34]. The results were shown in Figures (5, 6).

Figure (5): UV-visible spectrum of the organic reagent CHPDND

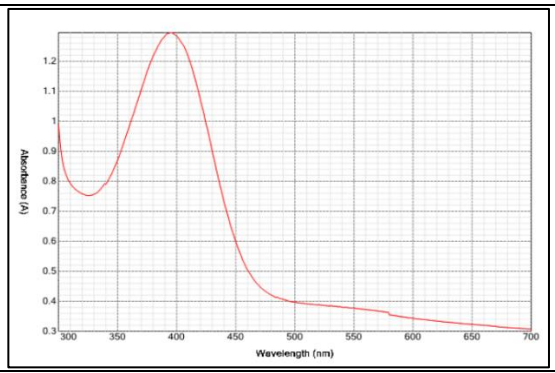


Figure (6): UV-visible spectrum of the chelated complex of Pb(II) ions and CHPDND

The spectrum showed that the wavelength of maximum absorption was λ_{max} = 410 nm for organic reagent CHPDND and λ_{max} = 395 nm for its complex with Pb(II). This

wavelength was used to measure the absorption of alcoholic solutions in subsequent experiments.

Study of the effective parameters of the CPE method

Effects of pH

As in the CPE procedure, the effects of different pHs in the range (5-11), in the presence of the surfactant 1%TritonX-114 (0.5mL), and 100 µg of lead (II) ions and 1×10^{-4} M of the CHPDND and completing the volume to 10 mL, the solutions were heated in a water bath for 15 min at 70 °C, until the CPL was formed, and then the CPL was separated from the aqueous phase, and dissolved in 3 mL of ethanol and aqueous phase treated with Dithiazone[35], completed the experiment as in the previous main steps in extraction methods. The results were shown as shown in the Figures (7 and 8).

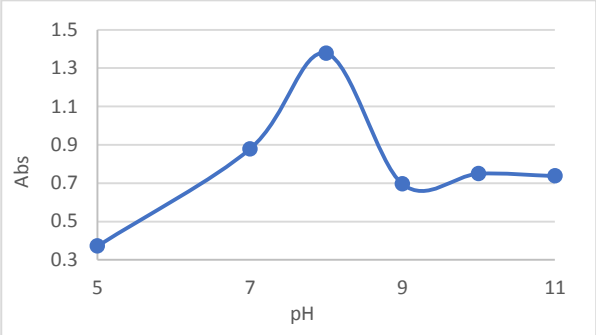


Figure (7): - Effect of pH on the absorption of the metal complex Pb (II) with the CHPDND

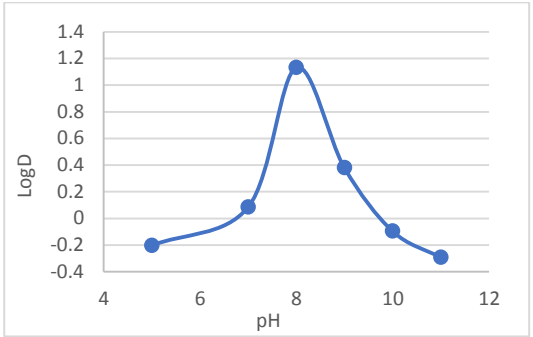


Figure (8): - Effect of pH on the absorption of D-values of Pb (II) complex with the CHPDND

The results are shown in Figures (7 and 8) indicates that pH~8 to extract the complex enhanced by Pb (II) and CHPDND as a chelating complex yielded the highest extraction efficiency and D values. This pH facilitates the rapid formation of the chelating complex and affects the direction of the equilibrium formation of the chelating species. Whether the concentration is higher or lower than the ideal, it affects the rate of reversible dissociation and reduces the concentration of the extracted chelating species.

Effect of Temperature

Several 10 mL aqueous solutions including 100 µg Pb(II) ion, pH~8, 1×10^{-4} M CHPDND, and 0.5 mL 1%Triton X-114 were prepared. These solutions were then heated in an electrostatic water bath at the range (70-85)°C for 15 min. until CPL created and the work concluded using the usual procedure. The results are shown in Figures (9), (10).

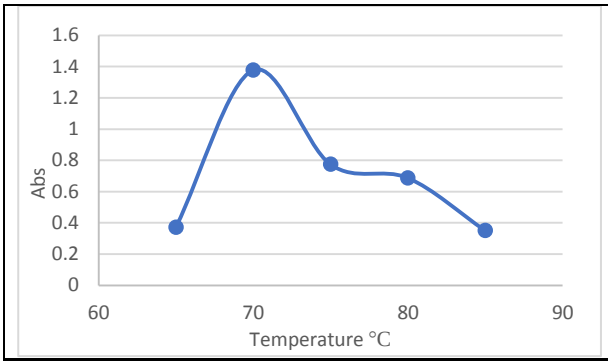


Figure (9): - Effect of temperature on the formation of the CPL containing the chelating complex

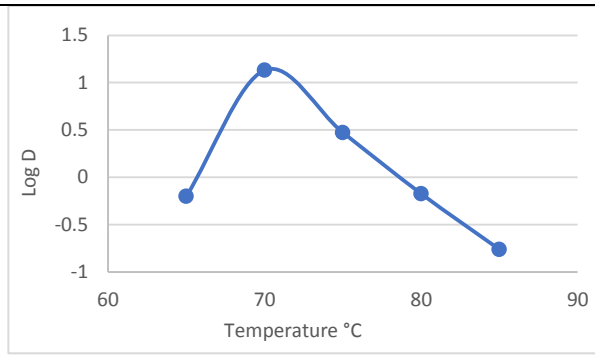


Figure (10): Temperature=*f*[D]

The results showed that the optimum extraction temperature at 70°C gave high extraction efficiency, at this temperature the CPL forming in the best properties and chelating complex was transferred with maximum quantity [36]. According to the thermodynamic relationship, the extraction constant K_{ex} was calculated from the values and the results were shown in the Figure (11)

$$K_{ex}=\frac{D}{[Pb]_{aq.}[CHPDND]_{org.}}$$

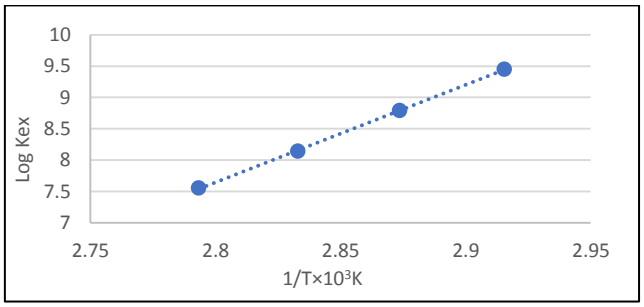


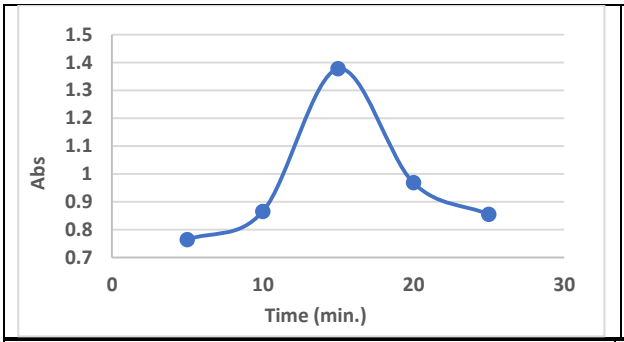
Figure (11): $K_{ex}=f$ [T]

Table (2): Thermodynamic Data for Pb(II) extraction

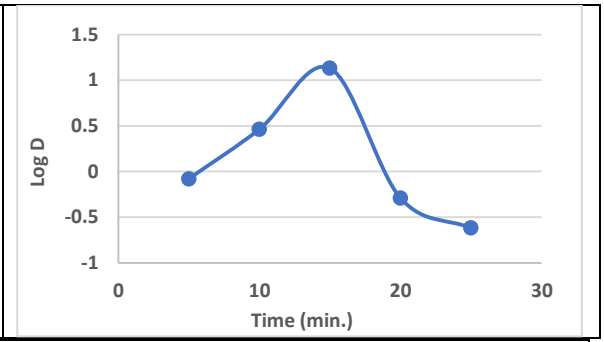
ΔH_{ex} kJ/mol	ΔG_{ex} kJ/mol	ΔS_{ex} J/mol.K
-297.71	-62.038	1048.816

Effect of heating time

Timing is critical; too long reduces extraction efficiency, and too short does not achieve the intended equilibrium, the same general steps were repeated, which included preparing several 10 mL aqueous solutions containing 100 µg of Pb(II) ion, pH~8, 1×10^{-4} M of CHPDND, and 0.5 mL 1%TX-114 to determine the optimum heating time to extract the CPL containing the chelated complex with the highest efficiency[37]. The results as in Figures (12 and13).



Figure(12): - The effect of heating time on the formation of CPL containing the chelating complex.



Figure(13):-heating time=*f* [D]

The results indicated that heating for 15 min is the best way to increase the extraction efficiency. The kinetics of the extraction process was represented by the heating duration, which helps in the micelles to aggregate well upon complete drying to create a CPL with larger surface area that can accommodate more chelating.

Effect surfactant volume

A number of 10 mL aqueous solutions were prepared, each containing 100 µg of Pb(II) ion, pH~8, 1×10^{-4} M of CHPDND, and varying amounts of the surfactant 1%TX-114. Then applied the same steps of the previous experiment to form the CPL. Then heated all these solutions in an electrostatic water bath at 70 °C for 15 minutes. The experiment was completed with the same general steps and the results are shown in Figs 14 and 15.

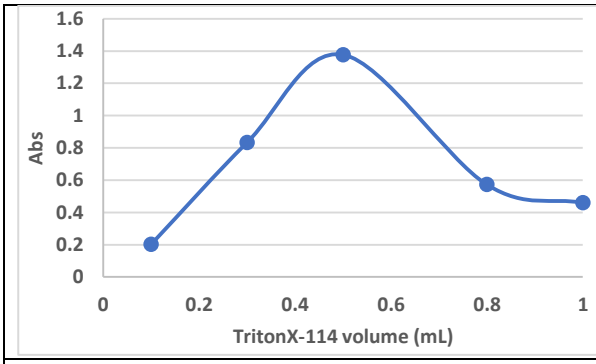
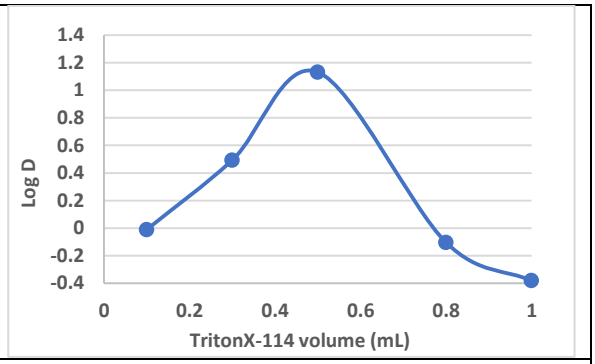


Figure (14): -Effect surfactant volume

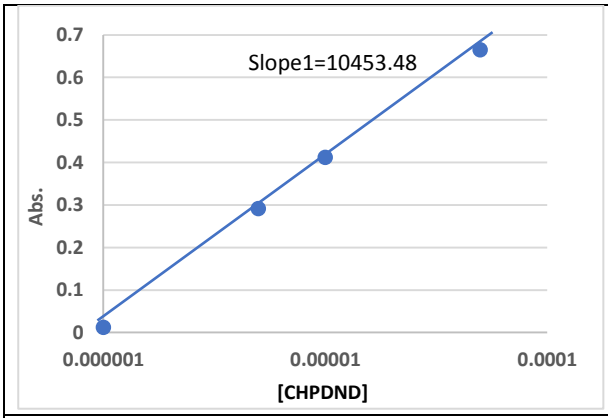


Figure(15):-Surfactant volume=*f*[D]

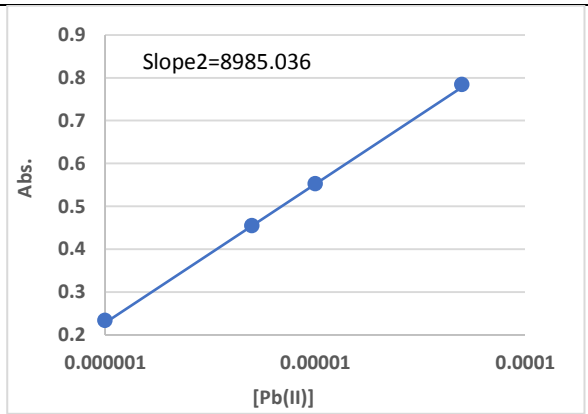
The results showed that the optimum volume of 0.5 mL of surfactant 1%TritonX-114 produced the highest extraction efficiency because it helped to establish the best thermodynamic and kinetic equilibrium of the aggregate micelles to form a good CPL. This volume was the smallest and highest density, meaning that it had a large surface area that could accommodate a large amount of chelating complex [38].

Stoichiometry of Pb(II) chelated complex

Two spectroscopic methods used for this purpose which is Slope analysis and Slope ratio, the solution prepared according to extraction method and at optimum condition [39], the results were as in Figure (16).



Figure(16): Change in reagent concentration(CHPDND)



Figure(17): Change in metal concentration Pb(II)

$$\text{Slope ratio} = \frac{\text{Slope 1}}{\text{Slope2}} = \frac{10453.48}{8985.036} = 1.163$$

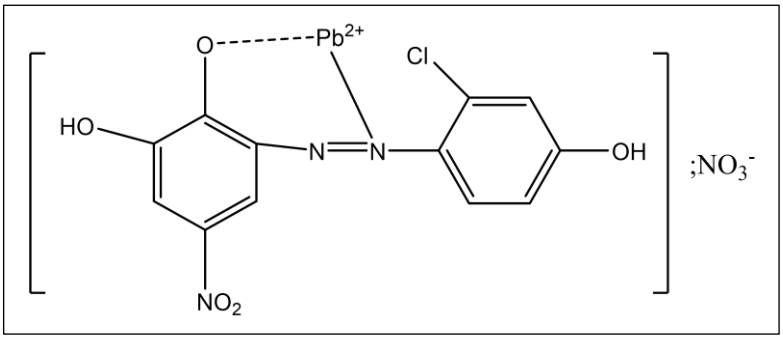
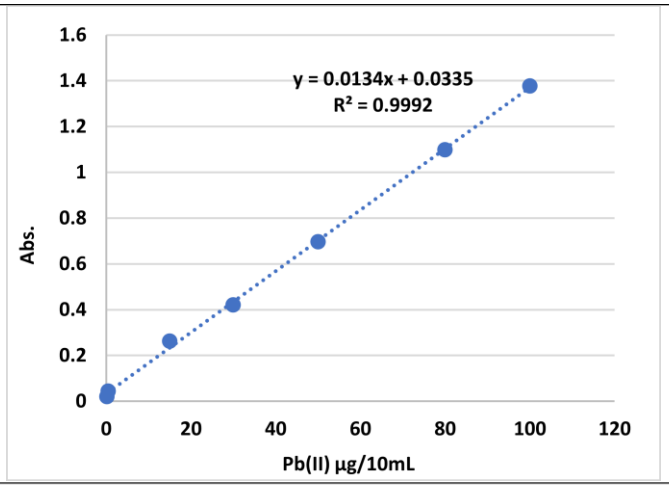


Figure (18): Probable structure of Pb(II) complex

Applied method of extraction on Pb(II) determination

1- **Calibration curve of Pb(II)**

Prepared series solutions contain increased amounts of Pb(II) in the range (0.2-100µg/10mL) at optimum conditions and treated according to general extraction method, the results were as in Figure (19) and Table (3):



Figure(19): Calibration curve of Pb(II)

Table (3): Figures of merit for the determination of lead (II) ion (CHPDND) using CPL

λ_{max}(nm)	395
Bear's law obey (ppm)	0.2-100
Slope	0.0134
Intercept	0.0335
R^2	0.9992
Molar absorptivity $(L.mol^{-1}.cm^{-1})$	27.712×10^3
Limited of Detection $(\mu g.ml^{-1})$	0.219
Limited of Quantity $(\mu g.ml^{-1})$	0.664

2- **Lead (II) Determination:**

Several food samples collected from Iraqi markets to determine Pb(II) amounts using cloud point extraction, at optimum conditions, samples prepared according to wet digestion method [40] then treated as in general method and add the suitable

masking agent. The results compared with atomic absorption method as in Table (4):

Table (4): Determination of Lead in environmental samples

No.	Samples	Measured Concentration	
		CPE method±SD	AAS method±SD
1.	Fenugreek	0.251±0.01	0.249±0.01
2.	Dill	0.288±0.01	0.286±0.02
3.	Radish	0.283±0.02	0.286±0.01
4.	Plant leave	0.365±0.01	0.364±0.02
5.	Beef	0.321±0.01	0.325±0.01

Although lead is not a necessary ingredient for plants, it can contaminate vegetables during planting through the air, soil, and water [41]. In general, it was thought to be safe to utilize vegetables grown in soils with lead levels < 300 ppm because vegetables don't absorb a lot of lead from the soil. Lead contamination in soil or dust deposits on vegetables pose a greater risk than lead absorption by vegetables, even in cases when soil levels are greater than 300 parts per million [42]. The WHO has certified that leafy vegetables (0.3µg/g) have the highest lead levels for human health [42]. According to these findings, burning leaded gasoline releases lead particles into the atmosphere, which can land on vegetables sold on the street and near busy intersections. When dust is transported by the wind, some lead particles settle on the soil and subsequently infect vegetables [43]. For Iraqis, animal species like beef, sheep, poultry, fish, etc. are the primary source of meat. Because meat contains carbs, proteins, lipids, fatty acids, and minerals, it has a high nutritional value and plays a major part in human nutrition. Humans and animals received minerals through the food chain[43].

In comparison to the maximum lead levels approved by the FAO/WHO for meat (0.2 mg/kg) [44]. Lead levels in farm animals typically indicate the extent of environmental pollution and the animals' food patterns. When lead is transported from soil to plants, it

may end up in farm animal feed. However, the deposition of particles on plant surfaces is the cause of the significant lead contamination of feed [45].

Conclusion

Extraction efficiency in the spectrophotometric determination of Pb(II) ions with the organic reagent CHPDND reached promising levels through optimal pH and temperature settings. The analysis of chelation complex formation together with thermodynamic data calculations has delivered valuable understandings about the process. Through comparison with Atomic Absorption Spectrometry as the standard procedure, this method demonstrated its dependability for analyzing environmental samples.

References

[1] Odar, M. O. (2021). Adsorption and determination of Lead in water and human urine samples based on Zn2 (BDC) 2 (DABCO) MOF as polycaprolactone nanocomposite by suspension micro solid phase extraction coupled to UV–VIS spectroscopy. *Analytical Methods in Environmental Chemistry Journal*, *4*(03), 5-20

[2] Jalu, R. G., Chamada, T. A., & Kasirajan, R. (2021). Calcium oxide nanoparticles synthesis from hen eggshells for removal of lead (Pb (II)) from aqueous solution. *Environmental Challenges*, *4*, 100193.

[3] Zaman, B. T., Erulaş, A. F., Chormey, D. S., & Bakirdere, S. (2020). Combination of stearic acid coated magnetic nanoparticle based sonication assisted dispersive solid phase extraction and slotted quartz tube-flame atomic absorption spectrophotometry for the accurate and sensitive determination of lead in red pepper samples and assessment of green profile. *Food chemistry*, *303*, 125396.

[4] Zhang, N., Peng, H., Wang, S., & Hu, B. (2011). Fast and selective magnetic solid phase extraction of trace Cd, Mn and Pb in environmental and biological samples and their determination by ICP-MS. *Microchimica Acta*, *175*, 121-128

[5] Antunović, V., Tripković, T., Tomašević, B., Baošić, R., Jelić, D., & Lolić, A. (2021). Voltammetric determination of lead and copper in wine by modified glassy carbon electrode. *Analytical Sciences*, *37*(2), 353-358.

[6] Yan, C., Yang, X., Li, Z., Liu, Y., Yang, S., Deng, Q., & Wen, X. (2021). Switchable hydrophilicity solvent-based preconcentration for ICP-OES determination of trace lead in environmental samples. *Microchemical Journal*, *168*, 106529.

[7] Cui, Y., Cui, F., Wang, L., Zhang, Q., Xue, W., Jing, F., & Sun, J. (2008). Determination of lead in Yellow River using ammonium molybdate as a molecular probe by resonance light scattering technique. *Journal of luminescence*, *128*(10), 1719-1724.

[8] Guo, Z., Chen, P., Yin, L., Zuo, M., Chen, Q., El-Seedi, H. R., & Zou, X. (2022). Determination of lead in food by surface-enhanced Raman spectroscopy with aptamer regulating gold nanoparticles reduction. *Food Control*, *132*, 108498.

[9] Acar, O., Özvatan, S., & Ilim, M. (2005). Determination of cadmium, copper, iron, manganese, lead and zinc in lichens and botanic samples by electrothermal and flame atomic absorption spectrometry. *Turkish Journal of Chemistry*, *29*(4), 335-344.

[10] Chaikhan, P., Udnan, Y., Ampiah-Bonney, R. J., & Chaiyasith, W. C. (2021). Air-assisted solvent terminated dispersive liquid–liquid microextraction (AA-ST-DLLME) for the determination of lead in water and beverage samples by graphite furnace atomic absorption spectrometry. *Microchemical Journal*, *162*, 105828.

[11] Wu, H. Y., Unnikrishnan, B., & Huang, C. C. (2014). Membrane-based detection of lead ions in seawater, urine and drinking straws through laser desorption/ionization. *Sensors and Actuators B: Chemical*, *203*, 880-886.

[12] Mohsen, S., & Hoidy, W. H. (2020). Spectrophotometric Determination of Cobalt (II) and Lead (II) Using (1, 5-Dimethyl-2-Phenyl-4-((2, 3, 4-Trihydroxy Phenyl) Diazenyl)-1H-Pyrazol-3 (2H)-One) as Organic Reagent: Using It as Antimicrobial and Antioxidants. *Nano Biomedicine & Engineering*, *12*(2).

[13] Sethy, N. K., Arif, Z., Mishra, P. K., & Kumar, P. (2020). Green synthesis of TiO2 nanoparticles from Syzygium cumini extract for photo-catalytic removal of lead (Pb) in explosive industrial wastewater. *Green Processing and Synthesis*, *9*(1), 171-181.

[14] Mohammed, R. J., & Hameed, S. M. (2024). Green Cloud Point Extraction Coupled for Separation and Determination of Erythrosine in Various Samples Compared to the HPLC Technique. *Methods*, *19*(3), 132-138.

[15] Hameed, S. M., Hussain, S. A., & Abdulzahra, M. A. (2024). Determination of Zinc and Magnesium in Food Samples via Cloud Point Extraction using Brilliant Green as a Reagent. *Methods & Objects of Chemical Analysis/Metody & Obekty Himičeskogo Analiza*, *19*(2).

[16] Semysim, F. A., Shabaa, G. J., Azooz, E. A., & Snigur, D. (2024). Alternative green solvents in cloud point extraction methods: recent developments, challenges, and greenness evaluation. *Trends in Environmental Analytical Chemistry*, e00250.

[17] Azooz, E. A., Moslim, J. R., Hameed, S. M., Jawad, S. K., & Al-Mulla, E. A. J. (2021). Aspirin in food samples for separation and micro determination of

copper (II) using cloud point extraction/solvation method. *Nano Biomed. Eng*, *13*(1), 62-71.

[18] AbdulKareem, E. A., Al-Murshedi, A. Y., Ridha, R. K., & Azooz, E. A. (2024). Optimization and greenness assessments of the supramolecular solvent-assisted cloud point extraction method for copper spectrophotometric determination in water and food samples. *Green Analytical Chemistry*, *11*, 100181.

[19] Fatimah, L. A. Z., & Khdeeja, J. A. (2023). Green flow injection spectrophotometric system for lead ion (II) evaluation in vegetables samples using new azo reagent. *Analytical Science and Technology*, *36*(1), 1-11.

[20] Azooz, E. A., Shabaa, G. J., Al-Muhanna, E. H. B., Al-Mulla, E. A. J., & Mortada, W. I. (2023). Displacement cloud point extraction procedure for preconcentration of iron (III) in water and fruit samples prior to spectrophotometric determination. *Bulletin of the Chemical Society of Ethiopia*, *37*(1), 1-10.

[21] Hammood, Z. A., Ali, I. R., Ali, N. H., & Jawad, S. K. (2021). Liquid ion exchange methodology for extraction Cr (VI) using azo derivative compound. *Materials Today: Proceedings*, *43*, 2156-2161.

[22] Vi dya v. G.; Sadasivan V.; Meena S. S. and Bhatt P .,Synthesis, Spectral and Biological Studies of Complexes with Bidentate Azodye Ligand Derived from Resorcinol and 1-Amino-2-Naphthol-4-Sulphonic Acid, *Orient. J, of Chem.,* 34(1), 45-54 (2018).

[23] Çanakçı D., and Serin S., Synthesis of New Azo Dye Polymers Based On Naphthol By Oxidative Polycondensation: Antimicrobial Activity and Fastness Studies, *J. of Polym. Rese.*, 27(1), 1-23 (2020).

[24] Al-Muhanaa S. S.; Al-Khafagy A. H., Preparation and Biological Activities of New Heterocyclic Azo Ligand and Some of Its Chelate Complexes, *J. Nano Biomed. Eng.,* 10(1), 46-55 (2018).

[25] Hadi M. A. and Kareem I. K., Synthesis and Characterization of Some Transition Metal Complexes with New Azo- Schiff Base Ligand 3,4-bis(((1E,2E)-2-((2-((4-((Z)-(3-Hydroxyphenyl)Diazenyl)Naphthalen-1-yl)amino)ethyl)imino)-1,2-Diphenyl ethylidene)Amino) Phenyl) (phenyl)Methanone, *Egypt.J.Chem.,* 63(1), 301-313 (2020).

[26] Abdul karem L. K. and Mahdi S. H., Spectroscopic, Structural and Antibacterial Activity of Mixed Ligand Complexes from Schiff Base with Anthranilic Acid, *J. Phys.: Conf. Ser.* 1234, 1-13(2019).

[27] Hameed, G. F., Wadday, F., Farhan, M. A. A., & Hussain, S. A. (2021). Synthesis, Spectroscopic characterization and bactericidal valuation of some metal (II) complexes with new Tridentate Heterocyclic Azo Ligand Type (NNO) Donor. Egyptian Journal of Chemistry, 64(3), 1333-1345.

[28] Z.H. Falih, Z.A. Hammood. New Preparation and Characterization of Reagent 1-EDBBA Derived from Para-Amino Benzoic Acid: Impact of Solvent and Limitation Isosbestic Point Study. Adv. J. Chem. A, 2025, 8(3), 533-544.

[29] Jaffer, N. D. Ghafil, R. A. A. (2023). Preparation, investigation and studying the (physical, spectral and biological activity) of new monomers and heterocyclic compounds containing nitrogen and oxygen. *Periodicals of Engineering and Natural Sciences*, *11*(2), 284-296.

[30] Apotrosoaei M.; Vasincu I. M.; Dragan M.; Buron F.; Routier S and Profire L., Design, Synthesis and the Biological Evaluation of New 1, 3-Thiazolidine-4-ones Based on the 4-Amino-2, 3-dimethyl-1-phenyl-3-pyrazolin-5-one Scaffold, *J.of molecu.,* 19 (2014).

[31] Z. Marczenko and M. Balcerzak. 2000. Separation, preconcentration and spectrophotometry in inorganic analysis, 1st ed. Amesterdam: ELSEVIER.

[32] Moslim, J. R., Hameed, S. M., & Jawad, S. K. (2021). Cloud point extraction with liquid ion exchange for separation and determination of zinc (II).

[33] Hameed, S. M., Hussain, S. A., & Al-Khkany, A. J. (2022). Extraction for Mo (VI), W (VI) and Mn (VII) as Oxyanions by Incorporation Cloud Point with Liquid Ion Exchange Methods. Research Journal of Pharmacy and Technology, 15(8), 3685-3689.

[34] Hameed, S. M., & Abdulwahid, M. S. (2022). Extraction and Preconcentration of Cd (II) by using Crown Ether DB18C6. Research Journal of Pharmacy and Technology, 15(6), 2451-2454.

[35] Hussain, S. A., Hameed, S. M., Abed, A. S., & Mahdi, N. S. (2023). Separation, Pre-concentration, and Determination of Al (III) by Cloud Point Extraction as a Compact Method with Liquid Ion Exchange.

[36] Hameed, S. M., Hussain, S. A., & Abdulzahra, M. A. (2024). Determination of Zinc and Magnesium in Food Samples via Cloud Point Extraction using Brilliant Green as a Reagent. Methods & Objects of Chemical Analysis/Metody & Obekty Himičeskogo Analiza, 19(2).

[37] Azooz, E. A., Wannas, F. A., & Jawad, S. K. (2021). Developed cloud point extraction coupled with onium system for separation and determination cobalt in biological samples. Research Journal of Pharmacy and Technology, 14(2), 594-598.

[38] Jawad, S. K., & Ridha, R. K. (2019). Cloud point extraction coupled with liquid ion exchange for separation and determination Mn (VII) in real samples. Research Journal of Pharmacy and Technology, 12(10), 4861-4866.

[39] Fatimah, L. A. Z., & Khdeeja, J. A. (2023). Green flow injection spectrophotometric system for lead ion (II) evaluation in vegetables samples using new azo reagent. Analytical Science and Technology, 36(1), 1-11.

[40] El Hosry, L., Sok, N., Richa, R., Al Mashtoub, L., Cayot, P., & Bou-Maroun, E. (2023). Sample Preparation and Analytical Techniques in the Determination of Trace Elements in Food: A Review. Foods (Basel, Switzerland), 12(4), 895.

[41] Lokeshappa, B., Shivpuri, K., Tripathi, V. and Dikshit, A. K. (2012). J., Food and public Health, 2(1), pp: 24-29.

[42] Rosen,C.J. (2002). Communication and Educational Technology Services, University of Minnesota Extension.

[43] UNEP. (2000). Earth Scan Publications Ltd.: London, UK; pp:132-133.

[44] Chio, Y.Y. (2011). "International/National standards for heavy metals in food". Government laboratory (Australia).

[45] Beckert, F. W. (1978). Environmental Protection Agency, Office of Research and Development, Environmental Monitoring and Support Laboratory.