

Article

a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa and in cooperation with Hilla University College, under the title **(5'th Postgraduate Students Annual Conference) (PSAC2024)**, which held for Wednesday, **24/4/2024**.

Riboflavin Spectrophotometric Determination with Zn(II) as a Choro Complex Anion using Cloud Point Extraction and HPLC in Different Drugs

Hanaa Abdullah Mohammed and Safa Majeed Hameed

^{1,2} Department of Chemistry, Faculty of Education for Women, University of Kufa, Al-Najaf 54001, Iraq

Emails: ¹hanaaa.alghazali@student.uokufa.edu.iq

²Safaa.alhassani@uokufa.edu.iq

Abstract

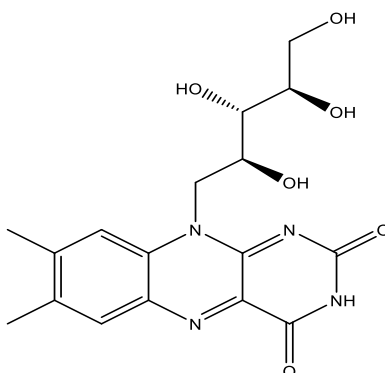
Because riboflavin is becoming increasingly significant in the food, biological, and pharmaceutical fields, it is critical to accurately detect its content to guide nutritional health, diagnose and treat related diseases, conduct pharmacological research on riboflavin, and oversee the quality of food and drugs. A thorough application cloud point extraction and, liquid ion exchange in addition to HPLC techniques for the determination of riboflavin in pharmaceutical domains was provided. To produce an ion-pair association complex for RF with Zn(II) from HCl media and in the presence of Triton X-100 as non-ionic surfactant, the approach uses a liquid ion exchange process. The maximum wavelength was 295 nm. The influence of the ideal conditions on the separation process was examined: 95°C temperature, 15 min. of heating, 100µg/mL Zn(III) concentration, and TritonX-100 as the best surfactant. Within the concentration limit 5-1000µg/mL, the calibration curve is linear, with

LOD of 6.07 $\mu\text{g/mL}$ and LOQ of 18.38 $\mu\text{g/mL}$. HPLC applied to compare the results with cloud point extraction and, liquid ion exchange in RF determination, they were identical.

Keywords: Riboflavin, Zinc(II), Liquid ion exchange, HPLC

Introduction:

Riboflavin (RF), 7,8-dimethyl-10-[(2S,3S,4R)-2,3,4,5-tetrahydroxypentyl] benzo[g] pteridine-2,4-dione^[1]:



Riboflavin (vitamin B2) is a water-soluble, precursor of flavoprotein. It also acts as a cofactor in other key enzymatic reactions, including fatty acid oxidation and the Krebs cycle. The use of riboflavin in an average daily dose of 100 mg^[2,3]. Several analytical techniques have been employed for quantifying riboflavin, including sensors^[4,5], high-performance liquid chromatographic methods (HPLC)^[6-8], solid phase extraction^[9,10], and electrochemical detection methods for the determination of riboflavin^[11-14]. The current work proposed a new approach for the sensitive and specific spectrophotometric detection of RF using Zn (II) as a chloroanion for producing an ion pair association complex from hydrochloric acid medium. The technique was useful for analyzing RF in several pharmaceutical samples. Additionally, the accuracy of the point cloud extraction method was compared using the HPLC methodology as a standard method.

Experimental

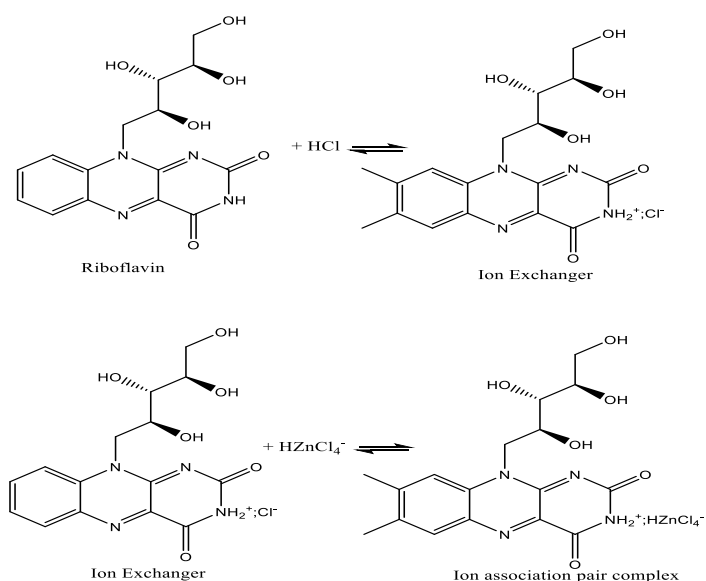
- **Equipment:** UV-Vis double beam was a Biochrom libra S60 spectrophotometer model from the UK, the HPLC-column C18 apparatus from Skam 2012 (Germany), and an electrostatic water bath from Germany.
- **Materials and solution preparation:** The 1% TritonX-100 (Alpha Chemika) and riboflavin (100 $\mu\text{g/mL}$) were made by dissolving 0.01g (Merck 99.96%) in 100 mL of distilled water, and any other working solutions were made by serially diluting with distilled water to the proper volume. The standard Zn(II) solution (1000

mg/L) was made by dissolving 0.21g of $ZnCl_4$ (Merck 99.8%) in 100 mL of distilled water.

- **Drug Samples preparation**^[15]: weighed the tablet and Instant powder, grinding it in a mortar, and then weighing the solid powder again, dissolved it in distilled water in volumetric flasks, filtering out the insoluble solids with Whatman #1 filter paper.
- **Extraction Procedure**: An appropriate volume of 1% TritonX-100 as a non-ionic surfactant was added after preparing aqueous solutions with a volume of 10 mL containing 100 μ g/mL of riboflavin and 100 μ g/mL of Zn(II) in HCl media. The cloud point layer was isolated from the aqueous solution and dissolved in 5 mL ethanol after the solutions were heated in an electrostatic water bath for the appropriate amount of time and temperature. At λ_{max} , the absorbance was measured using ethanol as a blank.

Results and Discussion

The mechanism by which a zinc chloroanionic complex and riboflavin produce an ion pair association complex according to liquid ion exchange method:



Scheme (1): Ion pair association complex between Riboflavin and $HZnCl_4^-$

Select the maximum wavelength

The UV-Vis spectrum of Riboflavin and $HZnCl_4^-$ the complex was obtained by measuring the absorbance in the range (200-500) nm against ethanol as a blank; the result appears in Fig (1), where $\lambda_{max}=295$ nm.

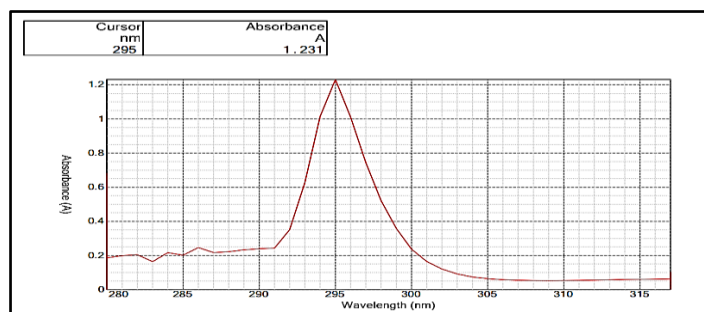
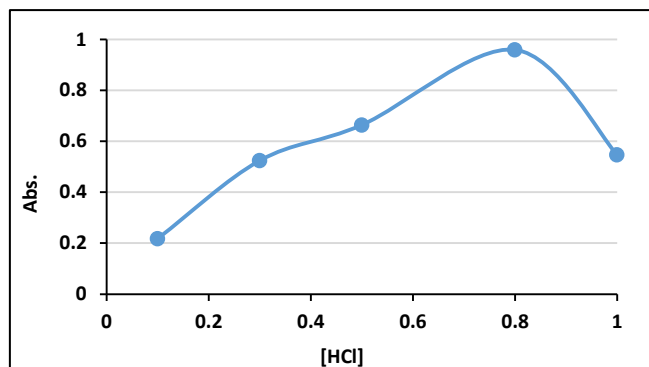


Figure (1): The UV-Vis. Spectrum of RF and HZnCl_4^- ion association complex
Improvement of the Experimental Conditions

Acidic Media Effect:

As in the extraction procedure, the impacts of various amounts of HCl in the range (0.1-1.0M) were examined.; the results are displayed in Fig (2):



Figure(2): Concentration of hydrochloric acid as a function for absorbance

The ideal HCl concentration was 0.8 M, which allowed for the formation of the chloroanion complex of Zn(II) and a liquid ion exchanger of riboflavin, as indicated by the scheme (1). Reaching the referable equilibrium is difficult at any HCl concentration, whether it is greater or less than the optimal value ^[16].

Suitable Temperature

Temperature was investigated to show its significance in developing CPL by extracting riboflavin at various temperatures (70-95°C) throughout the extraction procedure. The findings are displayed in Fig. (3):

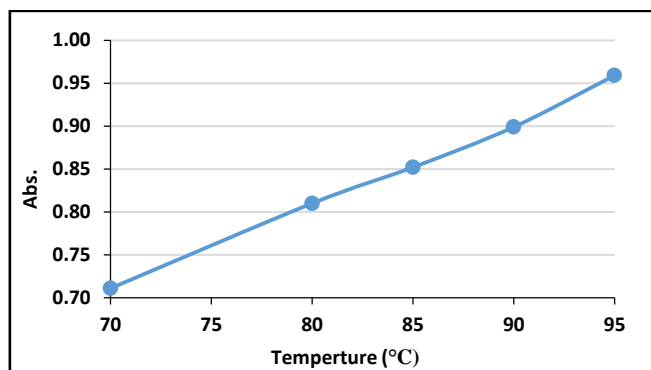


Figure (3): Temperature's influence on the absorbance of the ion association complex

The results of the liquid ion exchange method showed that 95°C was the ideal temperature for riboflavin extraction, resulting in a greater extraction efficiency. The energy needed for appropriate surfactant aggregation micelles to produce CPL, the ion pair association complex between riboflavin and HZnCl_4^- , is explained quantitatively by this temperature^[17].

Impact of Heating Time

Extracted riboflavin at various heating times by the extraction procedure. The following results are shown in Fig. (4):

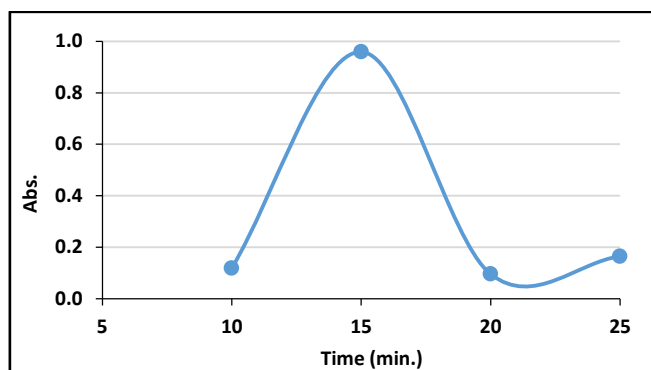
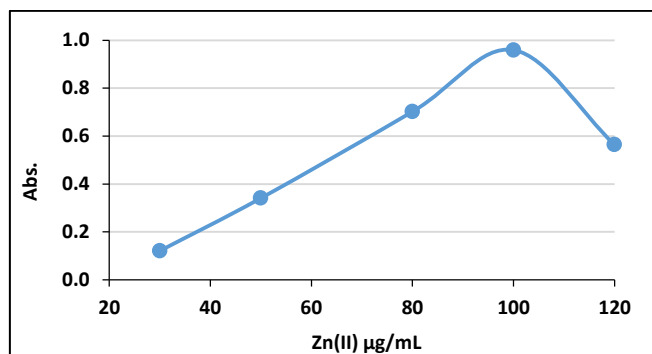


Figure (4): Absorbance in dependence on Heating Time

The results show that 15 minutes was the ideal heating time for greater extraction efficiency because it forms the optimal CPL for quantitatively extracting the ion pair association complex. Diffusion of micelles results from reduced extraction efficiency over the ideal heating time^[17].

Influence of Concentration of Zinc (II)

Based on the preceding extraction procedure, the recovered riboflavin from 10 mL aqueous solutions has an increased Zn (II) content. The results are presented in Figs. (5).



Figure(5): Zn (II) concentration's effect on the formation of ion association complex

The ideal quantity for higher extraction efficiency was 100 µg/mL. According to the mass action law, concentrations above the optimum value reduce extraction efficiency. However, to achieve the optimal extraction equilibrium, concentrations below the optimal value are inappropriate^[18].

Stoichiometry

Under ideal conditions, apply the slope analysis approach as in the extraction procedure to determine the most likely structure of the ion pair association complex extracted into CPL. The extracted complex's expected structure appears in Figs. 6 and 7:

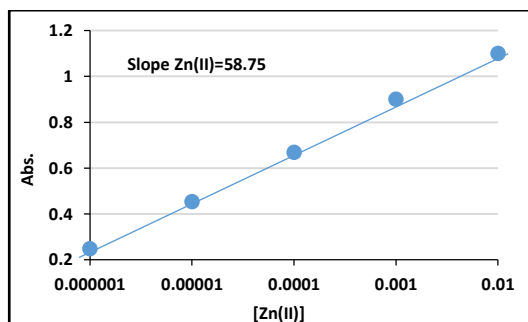


Figure 6: Abs.=f(Zn(II))

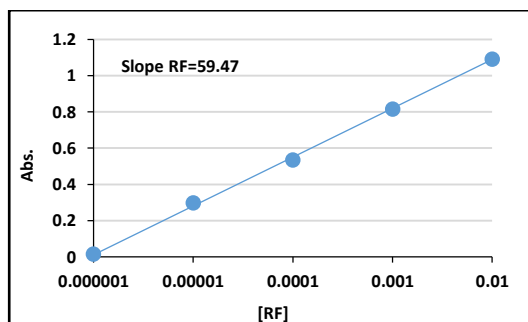


Figure 7: Abs.=f(RF)

The slope ratio values of the two straight lines in Figs. 6 and 7 were (1.012) and ratio 1:1, respectively, thus presenting a better illustration of the expected structure of the ion pair association complex extracted into the CPL of RF and Zn(II).

Importance of Organic Solvent

extracted riboflavin at optimal conditions from 10 mL aqueous solutions, and then the CPL dissolved in various organic solvents; equation 1 depicts the effect of the dielectric constant (ϵ_r), which can be expressed in relation to Gati and Szalay^[19].

$$\Delta\tilde{V} = \left[(a - b) \left(n^2 - 2 \frac{1}{n^2} + 1 \right) \right] + b \left(\frac{D - 1}{D + 1} \right) \dots \dots \dots (1)$$

The results are as in (Table 1):

Table 1. The organic solvent's maximum wavelength and dielectric constant

<i>Solvent</i>	λ_{max} (nm)	<i>Dielectric constant</i> (ϵ_r)	$(D-1/D+1)$
<i>Pentane</i>	285	1.84	0.296
<i>Benzene</i>	290	2.28	0.390
<i>Ethyl acetate</i>	277	6.02	0.715
<i>2-Butanol</i>	291	15.8	0.881
<i>2-Propanol</i>	238	18.3	0.896
<i>Ethanol</i>	295	24.3	0.921
<i>Ethylene glycol</i>	231	37.7	0.948

When Plotted λ_{max} against $(D-1)/(D+1)$, the results in Fig. 8 are as follow:

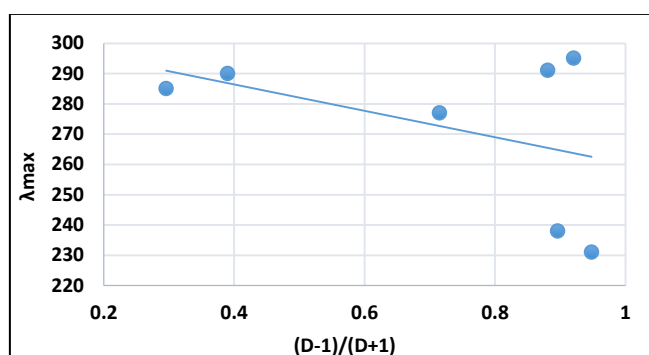


Fig. 8: A relationship between an organic solvent's dielectric constant and λ_{max}

Calibration Curve for CPE-ILE method

Riboflavin with Zn(II) as a chloroanion complex can be determined spectrophotometrically using a practical and specific method combining cloud point extraction with liquid ion exchange. The calibration curve was prepared at $\lambda_{max}=295\text{nm}$ for the extraction procedure under ideal conditions (Figure 9).

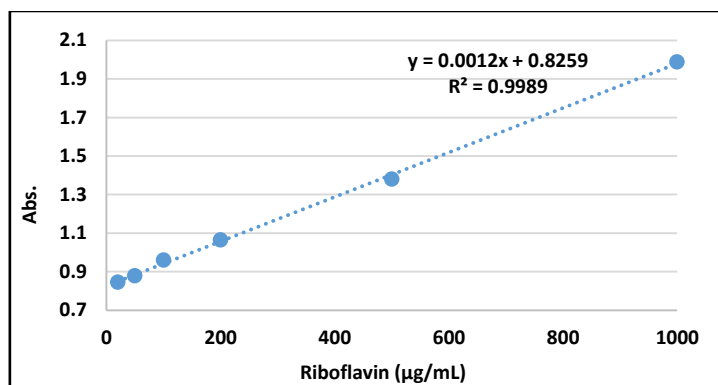


Figure 9: Calibration curve of Riboflavin with Zn(II) using CPE-ILE

Table (2): Analytical parameters for the determination of Riboflavin

Parameter	Zn(II)
λ_{max} (nm)	295
Bear's law obey (ppm)	5-1000
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.434×10^3
Limit of Detection (μgmL^{-1})	6.07
Limit of Quantity (μgmL^{-1})	18.38

HPLC analysis

The HPLC analytical program, as well as the chromatograms of the riboflavin standard and samples displayed as in Figures (11–14), are utilized to determine the amount of riboflavin in pharmaceuticals for comparison with the CPE-LIE method.

Column C18
Flow rate 1mL/min.
UV 254nm
mobile phase 70% Buffer (Sodium salt of hexane sulphonic acid):30%Methanol

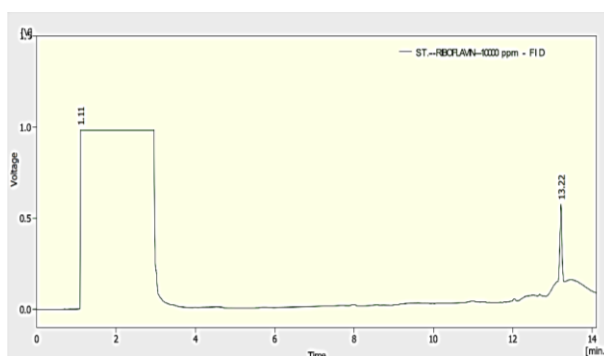


Figure 10. Chromatogram of RF Standard 1000ppm

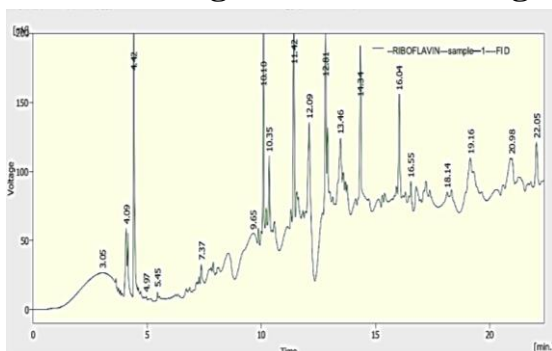


Figure 11. Chromatogram of Immuntive Lemon with Vitamin C+Zinc (Instant powder)

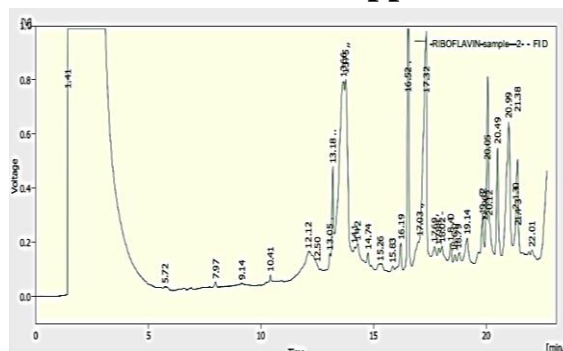


Figure 12. Chromatogram of B-2 (Capsule)

Table (3): Riboflavin amount in different drugs by two methods CPE-ILE method and HPLC method

Name	Company	Measured Concentration	
		UV method	HPLC method
<i>Immuntive Lemon with Vitamin C +Zinc (Instant powder)</i>	<i>Queisser in Germany</i>	<i>100.789</i>	<i>100.855</i>
<i>B-2 (Capsule)</i>	<i>Now</i>	<i>394.083</i>	<i>396.380</i>

Conclusion

CPE-ILE method used for Riboflavin spectrophotometric determination using Zinc (II) cloroanion complex from aqueous solution, then from different drugs, the CPE-ILE method appear good result compared with HPLC results. The ion pair association complex of Riboflavin with $ZnCl_4^{2-}$ has the structure 1:1.

References

- [1] Timothy J. Wiegand. [2024]: Riboflavin, Encyclopedia of Toxicology (4th Ed.), Academic Press, Pages 291-294.
- [2] Pozdnyakov Dmitry. [2021]: Chapter 2 - Perspectives of pharmacological correction and evaluation of mitochondrial dysfunction in neurodegenerative and ischemic brain lesions, Studies in Natural Products Chemistry, Elsevier, Volume 69, pp. 65-98.
- [3] Goh, L. L., Lee, Y., Tan, E. S., Lim, J. S. C., Lim, C. W., & Dalan, R. [2018]: Patient with multiple acyl-CoA dehydrogenase deficiency disease and ETFDH mutations benefits from riboflavin therapy: a case report. *BMC Medical Genomics*, 11(1), pp. 1-8.
- [4] Wang, Z., Zhang, L., Hao, Y., Dong, W., Liu, Y., Song, S., ... & Gong, X. [2021]: Ratiometric fluorescent sensors for sequential on-off-on determination of riboflavin, Ag⁺ and l-cysteine based on NPCl-doped carbon quantum dots. *Analytica Chimica Acta*, pp.1144, 1-13.
- [5] Çimen, D., & Denizli, A. [2020]: Development of rapid, sensitive, and effective plasmonic nanosensor for the detection of vitamins in infant formula and milk samples. *Photonic Sensors*, 10, pp. 316-332.
- [6] Laverroux, S., Picard, F., Andueza, D., & Graulet, B. [2021]: Vitamin B2 concentration in cow milk: Quantification by a new UHPLC method and prediction by visible and near-infrared spectral analysis. *Food Chemistry*, 342, pp. 128310.
- [7] Lin, Y., Chen, J. H., He, R., Tang, B., Jiang, L., & Zhang, X. [2020]: A fully validated high-throughput liquid chromatography tandem mass spectrometry

- method for automatic extraction and quantitative determination of endogenous nutritional biomarkers in dried blood spot samples. *Journal of Chromatography A*, 1622,pp. 461092.
- [8] Diniz, M., Dias, N., Andrade, F., Paulo, B., & Ferreira, A. [2019]: Isotope dilution method for determination of vitamin B2 in human plasma using liquid chromatography–tandem mass spectrometry. *Journal of Chromatography B*, 1113,pp.14-19.
- [9] Xie, L., Huang, J., Han, Q., Song, Y., Liu, P., & Kang, X. [2019]: Solid phase extraction with Polypyrrole nanofibers for simultaneously determination of three water-soluble vitamins in urine. *Journal of Chromatography A*, 1589,pp. 30-38.
- [10] Hu, K., Lv, Y., Ye, F., Chen, T., & Zhao, S. [2019]: Boric-acid-functionalized covalent organic framework for specific enrichment and direct detection of cis-diol-containing compounds by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Analytical chemistry*, 91(9),pp. 6353-6362.
- [11] Sukumar, S., Rudrasenan, A., & Padmanabhan Nambiar, D. [2020]: Green-synthesized rice-shaped copper oxide nanoparticles using *Caesalpinia bonducella* seed extract and their applications. *ACS omega*, 5(2),pp. 1040-1051.
- [12] Manjunatha, J. G., Raril, C., Hareesha, N., Charithra, M. M., Pushpanjali, P. A., Tigari, G., ... & Gowda, J. [2020]: Electrochemical fabrication of poly (niacin) modified graphite paste electrode and its application for the detection of riboflavin. *The Open Chemical Engineering Journal*, 14(1).
- [13] Dokur, E., Gorduk, O., & Sahin, Y. [2020]: Selective electrochemical sensing of riboflavin based on functionalized multi-walled carbon nanotube/gold nanoparticle/pencil graphite electrode. *ECS Journal of Solid State Science and Technology*, 9(12),pp.121003.
- [14] Hareesha, N., & Manjunatha, J. G. [2020]: A simple and low-cost poly (dl-phenylalanine) modified carbon sensor for the improved electrochemical analysis of Riboflavin. *Journal of Science: Advanced Materials and Devices*, 5(4),pp.502-511.
- [15] Bartzatt, R., & Wol, T. [2014]: Detection and assay of vitamin B-2 (riboflavin) in alkaline borate buffer with UV/Visible spectrophotometry. *International scholarly research notices*, 2014
- [16] Hussain, S. A., & Hameed, S. M. [2019]: Solvation Methodology for Separation and Micro Determination of Lead (II) In Different Sample. *ARNP Journal of Engineering and Applied Sciences*, 14(5),pp. 1005-1011.
- [17] Safa Majeed Hameed, Marwa Sabah Abdulwahid. [2022]: Extraction and Preconcentration of Cd (II) by using Crown Ether DB18C6. *Research*

Journal of Pharmacy and Technology.15(6),pp.2451-4. doi: 10.52711/0974-360X.2022.00409.

- [18] 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. Journal of Medicinal and Chemical Sciences, 6(3),pp.656-667. doi: 10.26655/JMCHEMSCI.2023.3.22
- [19] Mutar, M. M., & Ali, H. M. [2021]: Preparation, Characterization and Analytical Studies of novel Azo dyes and Diazo dyes. In Journal of Physics: Conference Series (Vol. 2063, No. 1, p. 012023). IOP Publishing.