Article

a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa,under the title:
(6'th Postgraduate Students Annual Conference) (PSAC2025).
which held for Tuesday, 15/4/2025.

A Novel Approach for the Rapid Detection of Sulfanilamide in Pure and Formulated Samples Using Cloud Point Extraction

Farah Abdul Adheem Razooqi and Safa Majeed Hameed

[1,2] Department of Chemistry, Faculty of Education for Women, University of Kufa, Al-Najaf, 54001, Iraq

Emails: [1] faraha.kerbal@student.uokufa.edu.iq

[2]Safaa.alhassani@uokufa.edu.iq

Abstract

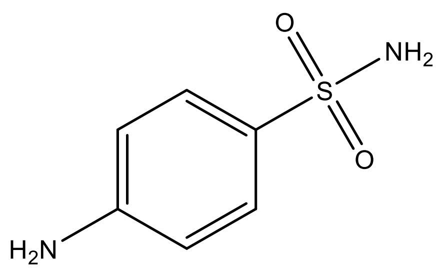
To determination of sulfanilamide (SNA) as a pure and pharmaceutical forms by easy, fast, and sensitive method. A liquid ion association complex of SNA formation by $NiCl_4^{-2}$ in the presence of nonionic surfactants TritonX-114 and mixture of (TritonX-114+Tween80) to form cloud point layer. The highest absorption at 277nm with both surfactants. The optimum conditions effect on the separation method was studied: concentration of HCl (0.8M), temperature (75℃), heating time (15min), the concentration of Ni (II) was 100μg.mL^{-1}, and (0.5mL) of both surfactants. The calibration curve was linear over the concentration range of

10-100μg.mL^{-1}with LOD of (0.984 μg.mL^{-1}) and LOQ of (2.98 μg.mL^{-1}) with TritonX-114 and LOD of (0.9936μg.mL^{-1}) and LOQ of (3.011μg.mL^{-1}) with mixture (TritonX-114+Tween80). SNA spectrophotometric determination was carried out by CPE and compared with HPLC. The results showed no significant differences between the two methods.

Keywords: Sulfanilamide, Ni (II), Liquid ion exchange, HPLC

Introduction

Sulfanilamide (SNA), also known as 4-aminobenzenesulfonamide, it's belong to a group of antibiotics called sulfonamide, it has molecular weight (172.21 g.mol^{-1}) and the melting point (165.5℃). [1]



4-aminobenzenesulfonamide (SNA)

Sulfanilamide is a white powder and medicinal compound used as antibacterial for the treatment of vulvovaginal candidiasis [2,3]. It's used as form a powder to treat surface infections or a topical cream as well as a pill for internal infections [4,5]. It's solubility in the binary solvents containing the water [6,7]. SNA was determined by different methods such as, Derivative Spectrophotometric [8,9], HPLC [10-13], Diazotization [14-16], flow injection system [17], TLC [18], Liquid-Liquid microextraction [19] colorimetric sensing [20], fluorescence spectroscopy [21], electrical sensing [22], voltametric determination [23]and solid phase extraction [24].

The current work proposed a new method for the sensitive and certain spectrophotometric detection of sulfanilamide by CPE-LIE using Ni (II) as $NiCl_4^{-2}$ to producing an ion pair association complex with HCl. The technique was useful for analyzing sulfanilamide as a pure and pharmaceutical preparation.

Experimental

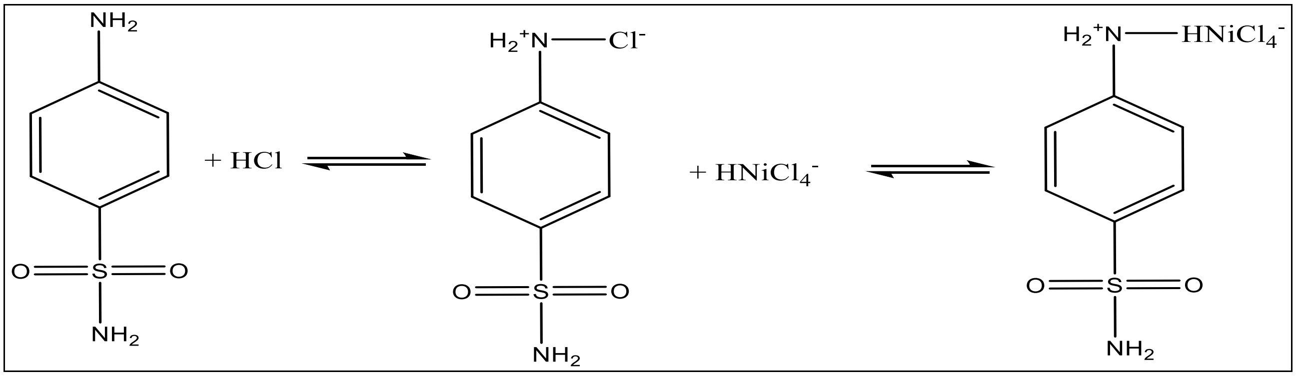
- **Instruments**: A UV- Vis double beam spectrophotometer model (Biochrom libra S60) (UK), an HPLC-column C18 system manufactured by Skam 2012 (Germany), Ultrasonic cleaner by (FUYANG) and Electrostatic water bath (Germany) were used
- **Reagents:** Sulfanilamide (MACKLIN) (99.5%), $NiCl_2.6H_2O$(Merck 99.8%), TritonX-114 (1%) (MACKLIN), Tween80(1%) (B.D.H). The standard Ni (II) solution (1mg.mL^{-1}) was prepared by dissolving (0.406 g) from $NiCl_2.6H_2O$ in 100mL of distilled water. sulfanilamide (100µg.mL^{-1}) were prepared by dissolving 0.01g of the substance in a small amount of distilled water and completing the volume to 100 mL with distilled water.
- **Synthesized Sulfanilamide drug sample**

Mix (0.025g) of sulfanilamide and (0.005g) each of glucose, sucrose, lactose, starch and vanillin, and dissolve in (10mL) ethanol, and filter using Whatman filter paper No. 41 [25].

- **Extraction Procedure**: Prepared aqueous solutions with a volume of 10 ml containing (100µg.mL^{-1}) of sulfanilamide in HCl, and (100µg.mL^{-1}) of Ni (II), a suitable volume of 1% TritonX-114 or mixture of (TritonX-114+Tween80), that solutions were heated in electrostatic water bath for (75℃) and (15min), and then separated the cloud point layer (CPL) from aqueous solution, and dissolve it in (3mL) ethanol, then the absorbance was measured against ethanol as a blank at λ_{max}.

Results and Discussion

The mechanism of a nickel chloroanionic complex and sulfanilamide to produce ion pair association complex of SNA by LIE.



Selection of maximum wavelength

We measured the absorbance of the ion pair association complex from (200 - 500 nm), that shown in Fig.1 which represent the spectrum with Triton X-114, where λ_{max}= 277 nm. Fig.2 represent the spectrum with Triton X-114 +Tween 80, where λ_{max}= 277 nm.

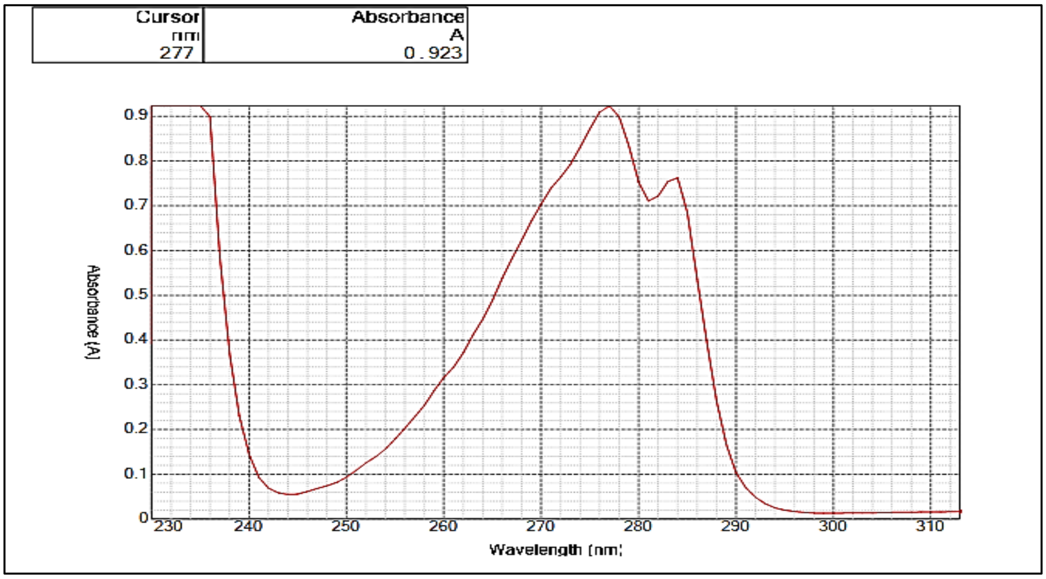
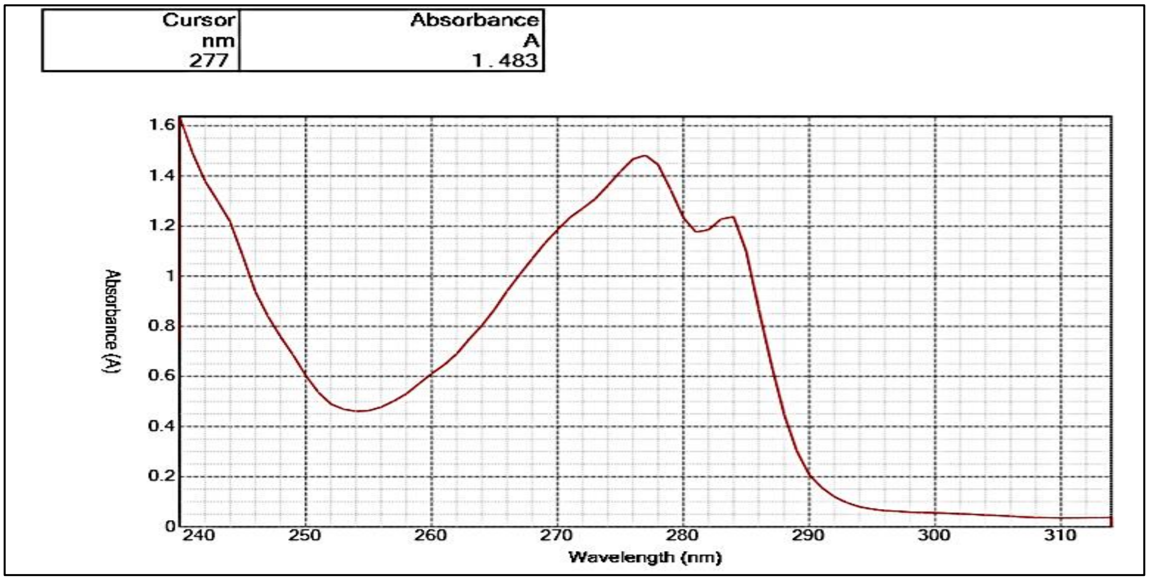


Fig (1): UV-Vis. Spectrum for ion association complex between SNA and $NiCl_4^{-2}$



with TritonX-114

Fig (2): UV-Vis. Spectrum for ion association complex between SNA and $NiCl_4^{-2}$ with TritonX-114+Tween80

Optimization of the Experimental Condition

Acidic media Effect:

The solutions with volume 10 mL were prepared, by mixed 100µg.mL^{-1} of SNA with 100µg.mL^{-1} of Ni (II) and different hydrochloric acid concentrations in the range (0.1-1.0 M) with appropriate volume of surfactants TritonX-114 or mixture (TritonX-114+Tween80), the results were as in Fig.3

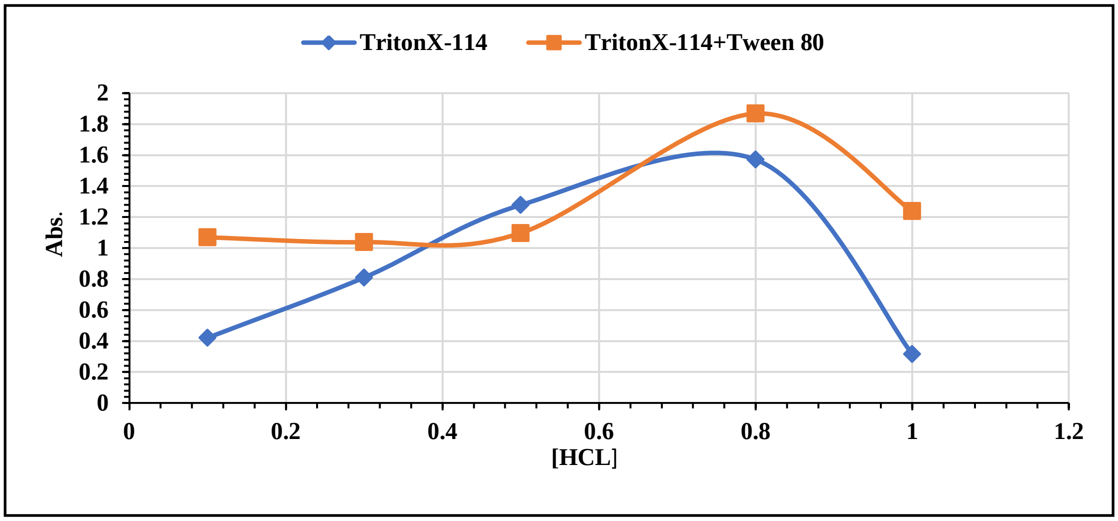


Fig (3): Effect of HCl concentration on the absorbance of ion association complex

According to the LIE, the optimum value of HCl concentration was (0.8M), this concentration gave highest absorbance, which means highest efficiency, this concentration allowed for the best speed formation of the chloroanion complex of Ni (II) and complex concentration will increase [26], as indicate by scheme (1)

Effect of Temperature

The impact of temperature on the formation of CPL was studied by extracting of sulfanilamide at different temperatures from 70 to 90 ℃ using TritonX-114 or TritonX-114 +Tween80, the result as in Fig .4

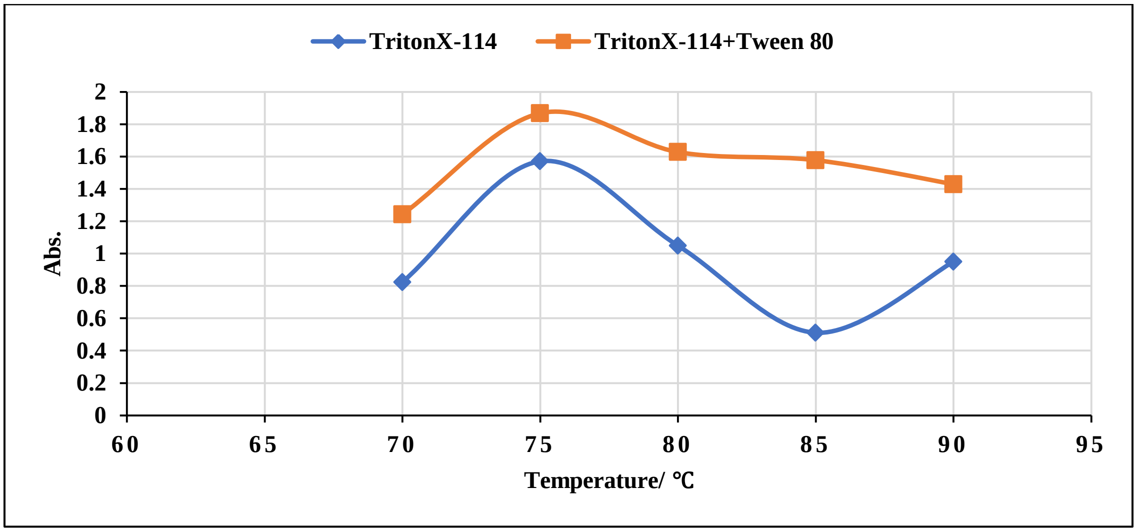


Fig. (4): Temperature effect on the absorbance of ion association complex

The results show the optimum temperature in the both surfactants were (75℃) giving higher extraction efficiency of sulfanilamide according to the LIE. This optimum temperature clarified the necessary energy for best gathering micelles of surfactant to formation CPL [27].

Impact of Heating Time

Extracted sulfanilamide through prepared a set of solutions containing Ni (II) and optimum concentration of HCl and appropriate volume of surfactants at 75℃ and different heating time (5-25) min. the results are presented in Fig.5

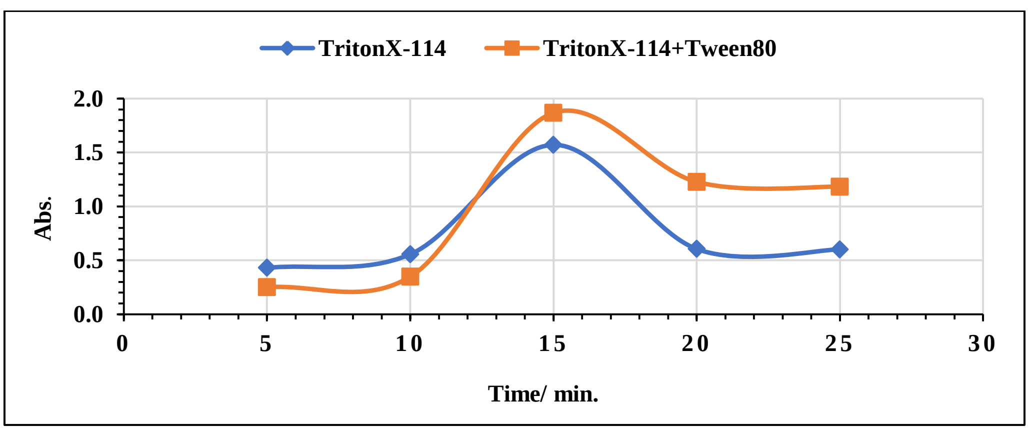


Fig (5): Variation of the absorbance ion association complex with heating time

According to the results, the best heating time for extracted sulfanilamide in both surfactants is (15min), this time gives the best micelle aggregation and highest absorption value, therefore the heating time is the kinetic aspect of the extraction process that contributes to the formation of the CPL with ideal specifications [28].

Impact of Surfactant Volume

Aqueous solutions were prepared by applying in the general method, extracted sulfanilamide has been made in the presence of increasing the volume of 1% TritonX-114 or (TritonX-114 + Tween 80) with optimum temperature and heating time. The results were as in Fig. 6

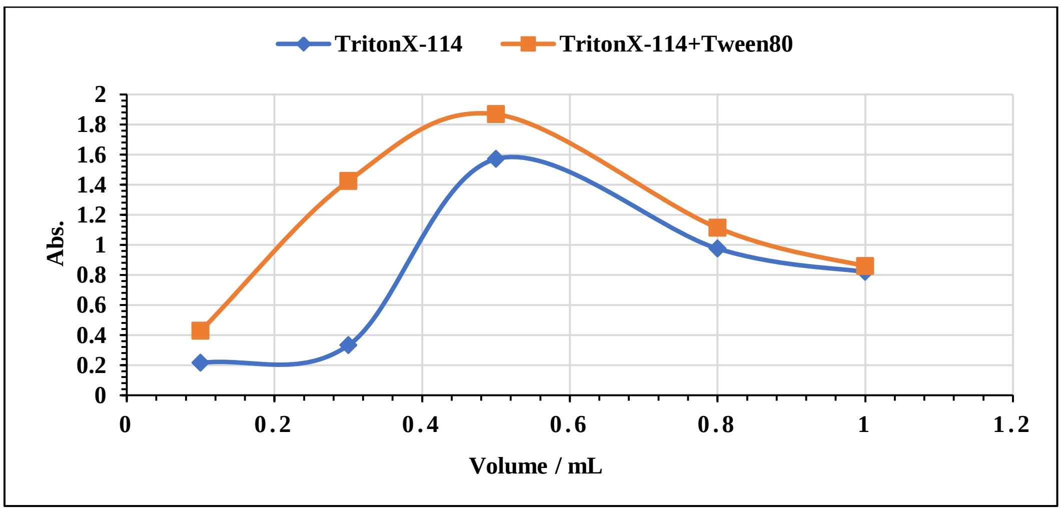


Fig (6): Effect of surfactant volume on CPL formation

The results showed that the optimum volume of the both surfactants were (0.5 mL). This optimum volume represented critical micelles concentration CMC to be the best cloud point layer with the highest density and smallest size, which is a new phase suitable for the extraction process [29].

Impact of metal ion concentration

The concentration of Ni (II) ion has an important role in the extraction process, it's of the ionic association complex which trans to CPL. According to the general procedure, (10 mL) aqueous solutions contained of increasing concentration of Ni (II). The results are presented in Fig. 7

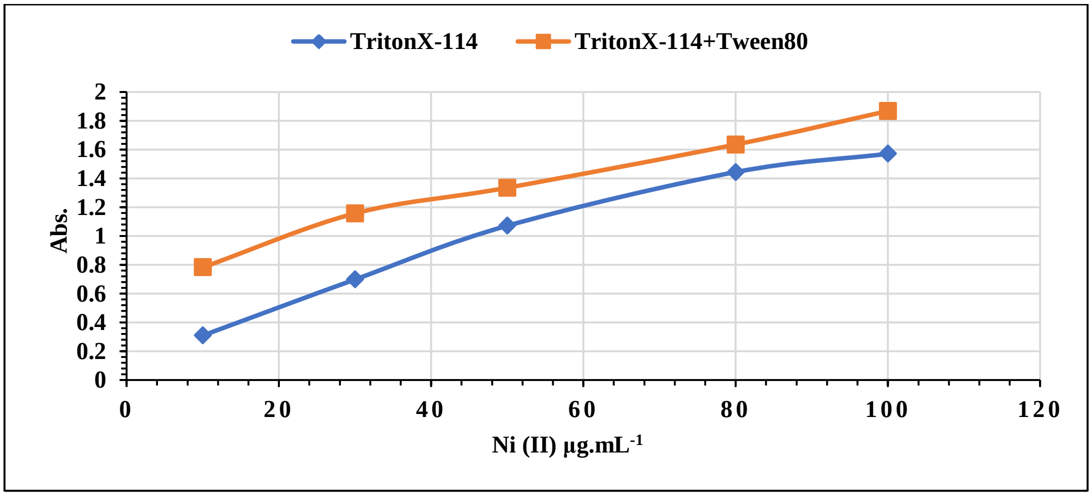


Fig. (7): Effect of Ni (II) concentration ion association complex formation

The concentration of 100µg.10mL^{-1}was the optimum quantity for both surfactants, the concentration of Ni (II) is a thermodynamic value that plays a role in the thermodynamic equilibrium for the formation of the ionic association complex extracted to the CPL [30].

Surfactant Type

To demonstrate the surfactants type effect on the efficiency of the separation process and formation CPL, aqueous solutions containing optimum concentrations of Ni (II) and HCl and sulfanilamide were taken at optimum conditions in different surfactants [31]. Some of the surfactants were used for e.x. Triton X-100, Tween 20, Tween80.The results were shown in Table 1

Table (1): Effect of the Surfactants used on the maximum wave length of ionic association complexes

Surfactant	MW(g/mol) monomer	MW(g/mol) micelle	CMC (Mm) 25℃	Cloud Point (℃)	Aggregation NO.	λ_{max} nm
TritonX-100	647	>62.500	0.2-0.9	64	90-150	277
TritonX-114	537	80.550	0.21	23	140-180	277
Tween20	1228	>36.840	0.06	95	20-50	-
Tween80	1310	>52.400	0.01	65-85	40-60	232
SDS	289	17302-28.800	7-10	>100	50-150	-
CTAB	364.45	25.515	1.00	30	75-120	-
TritonX-114+ Tween 80	-	-	-	-	-	277
TritonX-114+ Tween 20	-	-	-	-	-	277
TritonX-100+ Tween 20	-	-	-	-	-	277
TritonX-100+ Tween 80	-	-	-	-	-	277
TritonX-114+ CTAB	-	-	-	-	-	277

Impact of electrolytes

The presence of electrolyte salts in the aqueous solution more hydrophilic than Ni (II), so they will contribute to the destruction of the ion shell of Ni (II) and formation of free ion and ionic association complex formation which more stable and contributes significantly to raising the extraction efficiency, therefore, a group of the results were shown in Table 2

Table 2: Effect of different electrolytes on extraction efficiency

Electrolyte salt	**Tritonx-114**	**Tritonx-114+Tween80**
	Abs.at λ_{max}=277nm	**Abs.at λ_{max}=277nm**
With out	1.571	1.868
NaCl	1.624	1.953
KCl	1.554	1.385
$NaNO_3$	1.510	1.968
KNO_3	1.449	1.876
$MgCl_2.6H_2O$	0.648	0.668
$BaCl_2.2H_2O$	0.766	0.567
$AlCl_3$	0.714	0.940
NH_4Cl	1.149	1.197

Impact of interferences

To work in the field of spectroscopic estimation, the effect of interference substances precent with the analyte substance in the models must be studies to determine the extent of their effect on the separation process, as well as to determine the selectivity of the method [32]. The results were shown in Table 3.

Table 3: Effect of interferences on the spectroscopic estimation of SNA

Interferences	Abs.at λ_{max}=277nm	%Error
Without	1.571	0.000
Glucose	1.624	0.034
Sucrose	1.560	-0.039
Lactose	1.991	0.276
Ascorbic acid	1.363	-0.315
Benzoic acid	1.476	0.083
Calcium chloride dehydrate	1.620	-0.0122
Cobalt (II)chloride dehydrate	1.311	-0.191
Sodium chloride	1.640	0.111
Sodium Sulfate	1.466	0.118
Starch	1.626	0.109
Vanillin	1.880	0.156

Stoichiometry

Slope analysis was used at the optimum conditions to selected the more possible structure of the ion pair association complex extracted of sulfanilamide in to the cloud point layer. The results shown in Figures 8,9

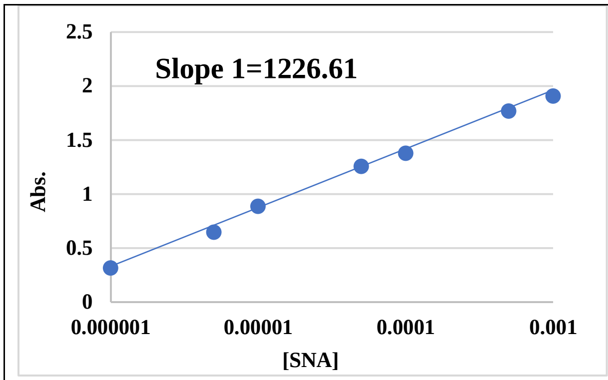


Fig. (8): Abs. = f [SNA]

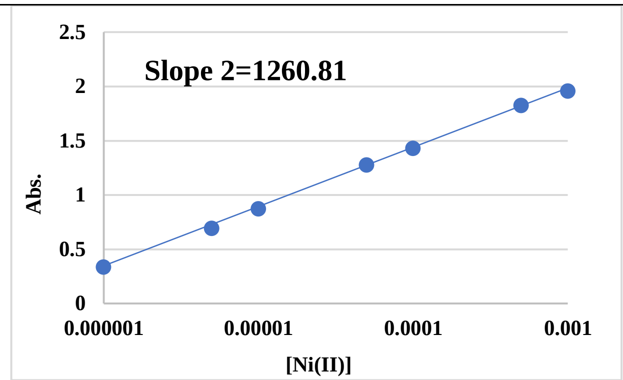


Fig. (9): Abs. = f [Ni (II)]

$$Slope\ ratio = \frac{Slope\ 1}{Slope\ 2} = \frac{1226.61}{1260.81} = 0.973$$

Evaluation of kinetic method (Initial Rate method)

To find the reaction kinetics, absorption curves versus time were prepared for the extracting SNA with Ni (II) at optimum conditions, the absorption-time curves, which were measured for 2hour at interval 30 min.at 75℃, it's following the pseudo first order to obey the following equation:

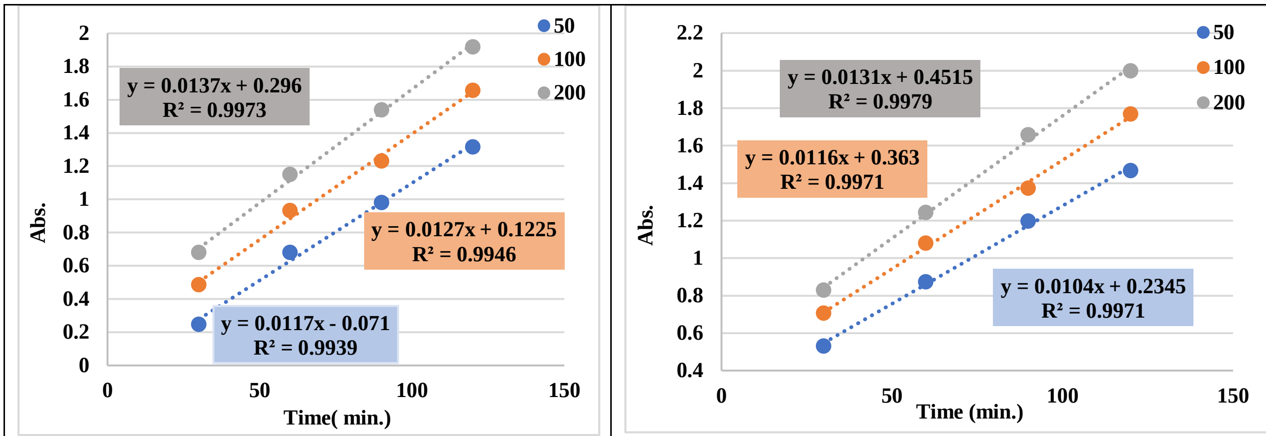
$$\boldsymbol{V} = \frac{\Delta\ \boldsymbol{A}}{\Delta \boldsymbol{t}} = \acute{K}\ \boldsymbol{C}^{n}$$

Where is V: the rate reaction, A: the absorbance, t: the heating time, Ḱ: the pseudo-first order rate constant and C: the molar concentration of SNA.

The logarithmic form of the above equation is as follows:

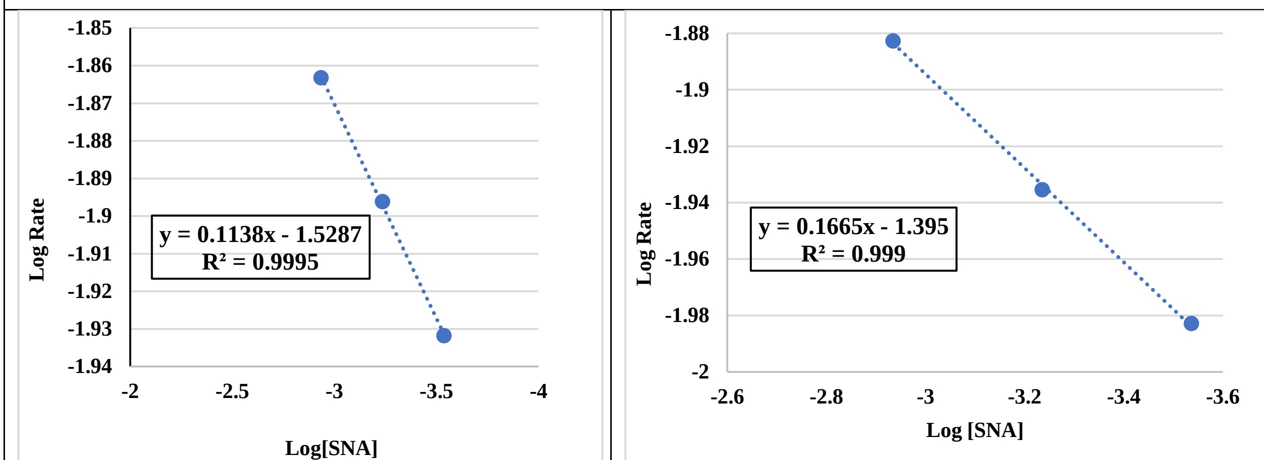
$$\log \boldsymbol{V} = \frac{\Delta \boldsymbol{A}}{\Delta \boldsymbol{t}} = \log \acute{K} + \boldsymbol{n}\ \log \boldsymbol{C}$$

The results shown in Figures 10,11 and Table 4



TritonX-114 | **TritonX-114 + Tween 80**

Fig. (10): Curves of absorption - time



TritonX-114 | **TritonX-114 + Tween 80**

Fig. (11): Graphs of the log Rate of the reaction for the initial rate method vs. log [SNA]

Table 4: Analytical information for the initial rate method

surfactant	Linear range(M)	Least square $\log V = \log \acute{K} + n \log C$		Correlation Coefficient (R^2)	LOD (M)	LOQ (M)
		Intercept ($\log \acute{K}$)	Slope (n)			

TritonX-114	**$2.9\times10^{-4} – 1.2\times10^{-3}$**	**- 1.5287**	**0.1138**	**0.9995**	**0.984**	**2.98**
TritonX-114 +Tween80	**$2.9\times10^{-4} – 1.2\times10^{-3}$**	**- 1.395**	**0.1665**	**0.9990**	**0.9936**	**3.011**

The rate constant of the kinetic reaction (Ḱ) of SNA with Ni (II) using TritonX-114 and mixture of TritonX-114+Tween 80, the value of (n) in the regression equation was 0.1138 and 0.1665 with TritonX-114 and TritonX-114+Tween 80 respectively, confirming that the reaction of SNA with Ni (II) was in the pseudo first order.

Calibration Curve for CPE-LIE Construction

The Cloud point extraction was joined with the LIE method as a selective and sensitive method used for spectrophotometric determination of SNA with Ni (II). Prepared calibration curve at $\lambda_{max} = 277nm$ as a basic operation at optimum condition Fig. (12) and Table5

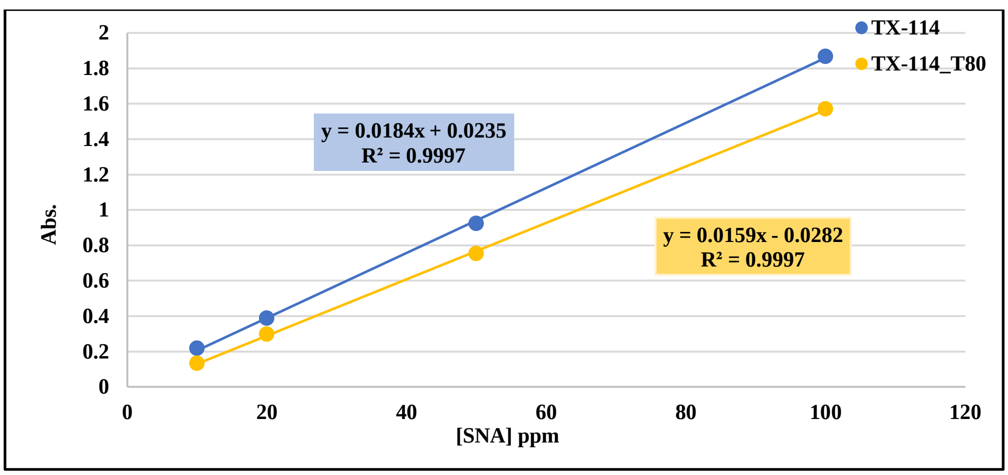


Fig. (12): Sulfanilamide's calibration curve using CPE-LIE method

Table 5: Analytical parameters for calibration curves of sulfanilamide using TritonX-114 and mixture of TritonX-114+Tween80

Parameter	TritonX-114	TritonX-114+Tween80
λ_{max}(nm)	277	277
Bear's law obey (ppm)	10-100	10-100
Slope	0.0182	0.0159
Intercept	0.0298	0.0282
R^2	0.9980	0.9997
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	3134.743	2746.766
Limited of Detection ($\mu g.ml^{-1}$)	2.882	3.085
Limited of Quantity ($\mu g.ml^{-1}$)	8.734	9.349

HPLC analysis

The HPLC analysis program as well as the chromatograms of the sulfanilamide standard and prepared sample shown as in Figures 13,14and Table 7is represented to determine the amount of pharmaceutical mixture of sulfanilamide for compare with CPE-LIE method.

Table 6: Chromatographic parameter used for the determination of sulfanilamide

Parameter	Optimized HPLC conditions
Column	C18
Flow rate	0.5mL

UV.	275 nm
Mobile phase	Me CN:$H_2\ O$ 80: 20
Add	0.005% H_2SO_4

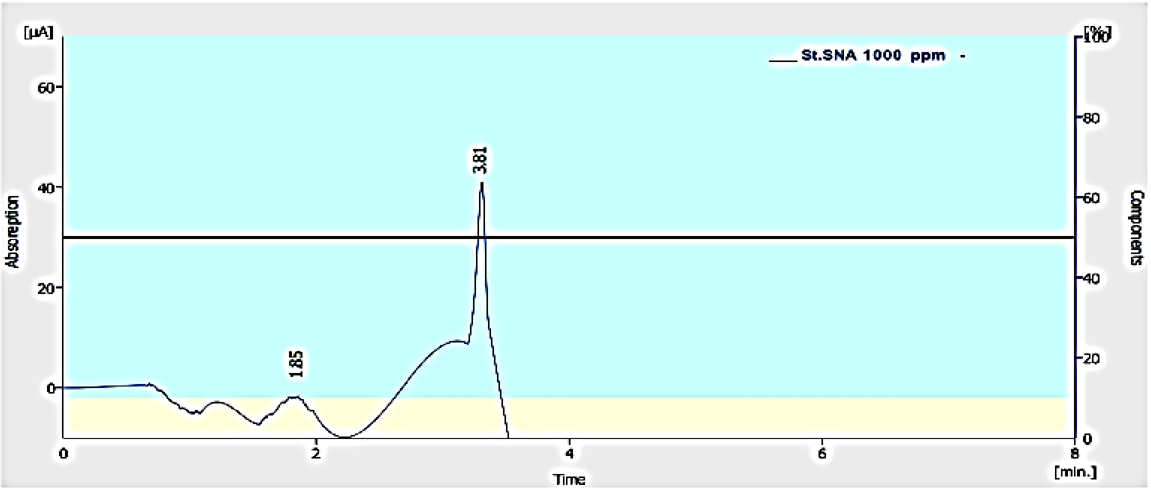


Fig. (13): Chromatogram of SNA standard 1000ppm

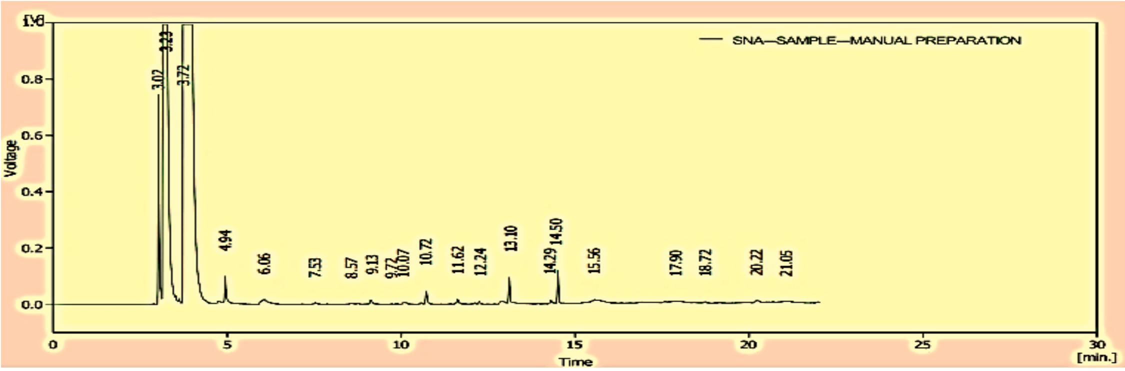


Fig. (14): Chromatogram of the manual preparation sample of SNA

Table 7: Sulfanilamide amount in the pharmaceutical preparation by two methods CPE-LIE method and HPLC method

SNA concentration	Measured Concentration		Recovery%	
	CPE-LIE method	HPLC method	CPE-LIE method	HPLC method
100µg.mL^{-1}	98.97	98.89	%98.97	%98.89

The above table's data demonstrated how well the suggested procedure worked to determine the amount of sulfanilamide in the pharmaceutical preparation that included it.

Conclusion

A simply and sensitive method was used to estimate sulfanilamide in a pure form and manual preparation sample using the cloud point extraction method which be fast, economical, environmentally friendly and highly efficient. It can be used with liquid ion exchange using Ni (II)ion and study their optimum conditions to give the best extraction efficiency.

Reference

[1] Khalaf, H. S., Al-Haidari, A. M. A., Mohammed, A. K., & Dikran, S. B. (2015). Spectrophotometric Determination of Sulfanilamide in Pure and in Synthetic Sample based on Condensation Reaction Method.

[2] Ochu, R. C., Okoro, U. C., Conradie, J., & Ugwu, D. I. (2024). New antibacterial, antifungal and antioxidant agents bearing sulfonamide. *European Journal of Medicinal Chemistry Reports*, *10*, 100136.

[3] Al- Farraj, E. S., Anazy, M. M., Alturiqi, A. S., & Ammar, R. A. (2021). Solution Behaviour of Ternary Complexes of Cobalt (II) Involving

Sulfanilamide and Dicarboxylic acids. *International Journal of Electrochemical Science*, *16*(1), 150944

[4] Dinić, A., Nikolić, V., Nikolić, L., Ilić-Stojanović, S., Najman, S., Urošević, M., & Gajić, I. (2023). Modified Sulfanilamide Release from Intelligent Poly (N-isopropylacrylamide) Hydrogels. *Pharmaceutics*, *15*(6), 1749.

[5] Tadi, K. K., Motghare, R. V., & Ganesh, V. (2014). Electrochemical detection of sulfanilamide using pencil graphite electrode based on molecular imprinting technology. *Electroanalysis*, *26*(11), 2328-2336.

[6] Cysewski, P., Przybyłek, M., & Jeliński, T. (2024). Predicting sulfanilamide solubility in the binary mixtures using a reference solvent approach. *Polimery w Medycynie*, *54*(1), 27-34.

[7] Jeliński, T., Bugalska, N., Koszucka, K., Przybyłek, M., & Cysewski, P. (2020). Solubility of sulfanilamide in binary solvents containing water: Measurements and prediction using Buchowski-Ksiazczak solubility model. *Journal of Molecular Liquids*, *319*, 114342.

[8] Darweesh, S. A. (2016). Simultaneous determination of Sulfanilamide and Furosemide by using derivative spectrophotometry. *Ibn AL-Haitham Journal For Pure and Applied Sciences*, *29*(2), 240-253.

[9] Khalil, R. A., Jalil, A. H., & Abd-Alrazzak, A. Y. (2009). Application of a Schiff base derived from sulfanilamide as an acid-base indicator. *Journal of the Iranian Chemical Society*, *6*, 345-352.

[10] Aher, P., Surana, K., Ahire, E., Patil, D., Sonawane, D., & Mahajan, S. (2023). Development and validation of RP-HPLC method for quantitative determination of 4-amino benzene sulphonamide in sulphonamide hydrochloride. *Trends in Sciences*, *20*(6), 5209-5209.

[11] El-Ragehy, N. A., Hegazy, M. A., Tawfik, S. A., & Sedik, G. A. (2021). Validated chromatographic methods for the simultaneous

determination of a ternary mixture of sulfacetamide sodium and two of its official impurities; sulfanilamide and dapsone. *Acta Chromatographica*.

[12] Mahmoud, T. Y., Jarallah, A. L., & Hamza, I. S. (2023). Analysis of Chloramphenicol, Sulphamethoxazole, and Sulfanilamide Drugs by Reversed-Phase High-Performance Liquid Chromatography with Fluorescent Detector. *Al-Esraa University College Journal for Medical Sciences*, *4*(5), 103-120.

[13] Çelik, S. Y., Solak, K., & Mavi, A. (2023). Sulfanilamide Modified magnetic nanoparticles for purification of Carbonic anhydrase from bovine blood. *Applied Biochemistry and Biotechnology*, *195*(6), 3900-3913.

[14] Ahmad, N. M., & Mohammed, I. S. (2024). Azo Coupling Reaction for Spectrophotometric Determination of Sulfanilamide Using β-Naphthol as a Reagent.

[15] Gassim, M. A. G., Asmaa, A. A. R., & Khalid, A. (2019). Spectrophotometric Determination of Sulfanilamide in Pure and Synthetic Sample by New Derivative from Pyrazoline Derived from 2, 4–Dinitro Phenyl Hydrazine.

[16] Kamoon, R. A., Al-Mudhafar, M. M. J., & Omar, T. N. (2020). Synthesis, Characterization and Antimicrobial Evaluation of New Azo Compounds Derived from Sulfonamides and Isatin Schiff Base. *Int J Chronic Dis Ther*, *10*(1), 150-155.

[17] de Faria, L. V., Lisboa, T. P., Matias, T. A., de Sousa, R. A., Matos, M. A. C., Munoz, R. A. A., & Matos, R. C. (2021). Use of reduced graphene oxide for sensitive determination of sulfanilamide in synthetic biological fluids and environmental samples by batch injection analysis. *Journal of Electroanalytical Chemistry*, *892*, 115298.

[18] Kilinc, E., Gumgum, B., Hamamci, C., & Aydin, F. (2009). Stability-indicating high performance thin layer chromatographic determination of sulfanilamide in human urine. *Journal of Analytical Chemistry*, *64*, 714-720.

[19] Bekele, E. T., Dube, S., & Nindi, M. M. (2022). Dispersive Liquid-Liquid Microextraction for the Determination of Sulfonamide Residues in Egg Samples.

[20] Le, A. V., Huynh, T. T., Truong, D. P., Nguyen, D. T., Dinh, V. P., & Cheng, S. H. (2023). Modifier-free gold nanoparticle colorimetric sensing for detecting sulfanilamide in the presence of dopamine. *New Journal of Chemistry*, *47*(37), 17540-17548.

[21] Pal, A., Gaba, R., & Soni, S. (2019). Absorption and fluorescence spectral studies of the interaction of sulpha drugs

[22] Vanoni, C. R., Winiarski, J. P., Nagurniak, G. R., Magosso, H. A., & Jost, C. L. (2019). A novel electrochemical sensor based on silsesquioxane/nickel (II) phthalocyanine for the determination of sulfanilamide in clinical and drug samples. *Electroanalysis*, *31*(5), 867-875

[23] de Moura Junior, F. G., Veloso, W. B., de Oliveira Junior, J. A., Kraatz, H. B., da Silva, I. S., & Dantas, L. M. F. (2021). Voltammetric determination of sulfanilamide using a cobalt phthalocyanine

[24] Errayess, S. A., Lahcen, A. A., Idrissi, L., Marcoaldi, C., Chiavarini, S., & Amine, A. (2017). A sensitive method for the determination of Sulfonamides in seawater samples by Solid Phase Extraction and UV–Visible spectrophotometry. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *181*, 276-285.

[25] Abdullah, S. H., Al-rashidy, A. A. M., & Ahmed, A. M. K. (2024). Spectrophotometric determination of sulfanilamide in pharmaceutical

preparations by Schiff reaction. *Journal of Education and Scientific Studies*, *5*(23).

[26] Wannas, F. A., Azooz, E. A., & Jawad, S. K. (2019). Liquid ion exchange with spectrophotometric method for separation and determination W (VI). *Jour Adv Res Dynamical & Control Syst*, *11*(7), 260-96.

[27] 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).

[28] Mohammed, H. A., & Hameed, S. M. (2024). Aspirin Spectrophotometric Determination using Cloud Point Extraction and HPLC in Several Pharmaceuticals Sold in Iraqi Markets. *Methods & Objects of Chemical Analysis/Metody & Obekty Himičeskogo Analiza*, *19*(3).

[29] Habibi, Z., & Bamdad, F. (2022). Simultaneous determination of traces of cobalt and iron ions after pre-concentration by surface-active ionic liquid-assisted cloud point microextraction. *Analytical and Bioanalytical Chemistry Research*, *9*(3), 243-250.

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

[31] Kori, S. (2021). Cloud point extraction coupled with back extraction: a green methodology in analytical chemistry. *Forensic sciences research*, *6*(1), 19-33.

[32] Almansrawi, S. A., & Alrufaie, M. M. (2023). Spectrophotometric Determination of Sulfanilamide Using Oxidative Coupling Reaction with the Presence of The Peroxidase Enzyme. *Egyptian Academic Journal of Biological Sciences. C, Physiology and Molecular Biology*, *15*(2), 573-583.