Speciation of Sulfamethoxazole in Pharmaceuticals via Ni(II) Complexation by Coupled CPE–LIE Techniques

Farah Abdul Adheem Razooqi and Safa Majeed Hameed*

Department of Chemistry, Faculty of Education for Women, University of Kufa, Al-Najaf, 54001, Iraq;
*e-mail: Safaa.alhassani@uokufa.edu.iq

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A sensitive, simple, and efficient spectrophotometric method was developed for the determination of sulfamethoxazole (SMX) in pure and pharmaceutical formulations. The approach employs liquid ion exchange based on the formation of an ion-pair complex between SMX and nickel(II) ions in an acidic hydrochloric acid medium, combined with cloud point extraction (CPE) using nonionic surfactants Triton X-114 and Tween 80. Systematic optimization identified maximum absorbance wavelengths at 277 nm for Triton X-114 alone and at 291 nm for the surfactant mixture (Triton X-114 + Tween 80). Optimal extraction conditions included 0.5 M HCl, Ni^{2+} concentration of 100 μg mL^{-1}, extraction temperatures of 75°C (Triton X-114) and 80°C (surfactant mixture), and a heating duration of 15 min. Reaction kinetics evaluated via the initial rate method demonstrated rapid and efficient complex formation. The linear calibration curves ranged from 10 to 100 μg mL^{-1}, with limits of detection (LOD) and quantification (LOQ) of 13.3 μg mL^{-1} and 40.4 μg mL^{-1} (Triton X-114) and 13.4 μg mL^{-1} and 40.6 μg mL^{-1} (surfactant mixture), respectively. The proposed method is straightforward, reliable, and suitable for routine analysis, offering practical utility for quality control in pharmaceutical laboratories.

Keywords: sulfamethoxazole, liquid ion exchange, cloud point extraction, nickel, nonionic surfactants

Introduction

Sulfamethoxazole (SMX) is a synthetic antimicrobial agent belonging to the sulfonamide class, chemically designated as 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide. Its molecular formula is $C_{10}H_{11}N_3O_3S$, with a molar mass of 253.28 g mol^{-1} and a melting point of 169°C. SMX appears as a white crystalline powder, characterized by its poor solubility in water, limited solubility in benzene and chloroform, and high solubility in ethanol and acetone.

Due to its broad-spectrum bacteriostatic activity, SMX is extensively applied in the treatment of a wide range of bacterial infections such as urinary tract infections, bronchitis, and prostatitis. It is also used in veterinary medicine both for therapeutic purposes and as a feed additive to enhance growth and prevent diseases in livestock. Despite its clinical efficacy, SMX is recognized for its environmental persistence and low biodegradability. It undergoes direct photo-transformation under sunlight, yet its presence in aquatic environments remains a concern due to continuous pharmaceutical and agricultural inputs [3]. As such, there is a pressing need for sensitive and sustainable methods to monitor and remove SMX from environmental matrices such as wastewater and urine [5–7].

Over the past decades, various analytical approaches have been developed for SMX determination, including UV-Vis spectrophotometry [8,9], flow injection analysis [10,11], diazotization-based methods [13,14], fluorescence spectroscopy [15], laser-induced photochemical reactions [16], high-performance liquid chromatography (HPLC) [17], solid-phase microextraction techniques [12,18], thin-layer chromatography (TLC) [19], and electroanalytical procedures [20]. Among these, cloud point extraction (CPE) has emerged as an attractive green chemistry alternative, particularly for trace-level detection of pharmaceuticals. CPE relies on the phase separation behavior of non-ionic surfactants near their cloud point temperature, allowing selective preconcentration of analytes with minimal solvent use [21].

In this study, we report a novel and reliable spectrophotometric method for the determination of sulfamethoxazole based on the formation of an ion-pair association complex with nickel(II) ions in a hydrochloric acid medium, followed by CPE utilizing non-ionic surfactants Triton X-114 and Tween 80. The method was comprehensively optimized and validated, offering high sensitivity, precision, and operational simplicity. The analytical performance of the proposed method was further assessed and cross-validated using high-performance liquid chromatography (HPLC) as a reference technique. This approach not only demonstrates strong potential for application in pharmaceutical quality control but also provides a promising platform for environmental monitoring of sulfonamide residues.

Experimental part

Reagents and Standards

All reagents were of analytical grade and used without further purification. A 1% (v/v) solution of Triton X-114 was prepared using reagent-grade surfactant (MACKLIN). Sulfamethoxazole (SMX, purity 98%,

MACKLIN) stock solution at a concentration of 100 μg mL^{-1} was prepared by dissolving 0.010 g of the compound in a small volume of deionized water, followed by dilution to 100 mL with the same solvent. Standard solutions were obtained by serial dilution. Nickel(II) standard solution (1 mg mL^{-1}) was prepared by dissolving 0.400 g of NiCl2·6H_2O (Merck, purity 99.8%) in 100 mL of deionized water.

Instrumentation

The analytical procedures were conducted using the following instrumentation: an electrostatic water bath (Germany), a high-performance liquid chromatography system equipped with a C18 reversed-phase column (Skam, 2012, Germany), a UV-Visible double-beam spectrophotometer (Biochrom Libra S60, UK), and an ultrasonic cleaner (Fuyang, China).

Preparation of pharmaceutical samples

Tablet formulations

Ten sulfamethoxazole-containing tablets were finely ground and homogenized. The average tablet weight was determined gravimetrically. An amount of powder equivalent to 500 mg of the active pharmaceutical ingredient was accurately weighed and transferred into a 100 mL volumetric flask. Approximately 10 mL of 0.4 M hydrochloric acid was added, and the mixture was vigorously shaken for 5 minutes to ensure dissolution. The volume was then adjusted to the mark with deionized water to obtain a 100 μg mL^{-1} SMX solution. The resulting mixture was filtered through Whatman No. 41 filter paper, discarding the initial portion of the filtrate. The filtrate was subsequently diluted with deionized water to obtain the desired working solutions.

Syrup formulations

A commercial syrup formulation containing 200 mg of sulfamethoxazole and 40 mg of trimethoprim per 5 mL was used. A 0.25 mL aliquot of the syrup was accurately transferred into a 100 mL volumetric flask, mixed with 10 mL of 0.4 M HCl, and allowed to stand for 5 minutes. The mixture was then diluted to volume with deionized water to obtain a 100 μg mL^{-1} SMX solution. The solution was filtered through Whatman No. 41 filter paper, and the initial portion of the filtrate was discarded. Fresh working solutions were prepared by appropriate dilution with deionized water and analyzed following the validated procedure described in [22].

General procedure for CPE of Ni(II)

Aliquots of 10 mL aqueous solutions containing 100 μg mL^{-1} of Ni(II) and 100 μg mL^{-1} of SMX were prepared in acidic medium (0.4 M HCl). Surfactant-mediated cloud point extraction was conducted by adding 1% (v/v) Triton X-114, or a mixture of Triton X-114 and Tween 80, followed by incubation in an electrostatic water bath at 75°C for 15 minutes. After phase separation, the surfactant-rich phase was isolated and solubilized in 3 mL of ethanol. The absorbance was measured at the corresponding maximum wavelength (λ_{max}) using ethanol as the blank.

Results and Discussion

Spectral characteristics of the ion-pair complex

The formation of the ion-association complex between sulfamethoxazole (SMX) and the tetrachloronickelate(II) anion ($[NiCl_4]^{2-}$) was investigated by UV-Visible spectrophotometry within the wavelength range of 200–500 nm. The absorbance spectra revealed a characteristic absorption maximum (λmax) at 277 nm for the system employing Triton X-114 alone (Fig. S1), whereas the binary surfactant system Triton X-114 + Tween 80 exhibited a bathochromic shift with λ_{max} at 291 nm (Fig. S2), indicating enhanced micellar solubilization and potential modification in the microenvironment of the chromophore.

Optimization of experimental parameters

Hydrochloric acid concentration

The extraction efficiency was evaluated over a range of HCl concentrations (0.1–1.0 M) using both individual (Triton X-114) and mixed surfactants (Triton X-114 + Tween 80). The absorbance reached a maximum at 0.5 M HCl for both systems (Fig. 1a), suggesting optimal protonation conditions for SMX and efficient formation of the anionic complex $[NiCl_4]^{2-}$ under acidic conditions, which is essential for subsequent ion-pairing and micellar extraction [23].

Effect of temperature

Temperature was found to be a critical factor influencing cloud point formation (CPF) and ion-pair extraction efficiency [24]. Studies conducted within the range of 70–90 °C revealed that optimal extraction was achieved at 75 °C for Triton X-114 and 80 °C for the mixed surfactant system (Fig. 1b). These temperatures ensure effective micelle formation and promote the partitioning of the hydrophobic ion pair complex into the surfactant-rich phase.

Effect of heating time

The influence of incubation time on the efficiency of cloud point extraction was evaluated by varying the heating duration at optimal temperatures. A plateau in absorbance was observed at 15 minutes for both surfactant systems (Fig. 1c), indicating that this duration is sufficient to attain thermodynamic equilibrium and complete phase separation [25].

Effect of surfactant volume

The surfactant volume was systematically varied to identify the minimal concentration required to achieve maximal extraction [26]. The optimal volume for both Triton X-114 and the mixed system was determined to be 0.5 mL (Fig. 1d), which provided sufficient micellar content for efficient entrapment of the ion-association complex without dilution of the surfactant-rich phase.

Effect of Ni(II) concentration

The concentration of Ni(II) plays a pivotal role in the stoichiometry and efficiency of complex formation [27]. Varying the concentration in the aqueous phase revealed an optimum at 100 μg per 10 mL solution for both surfactant systems (Fig. 3S), beyond which no significant enhancement in absorbance was observed, suggesting saturation of complexation sites.

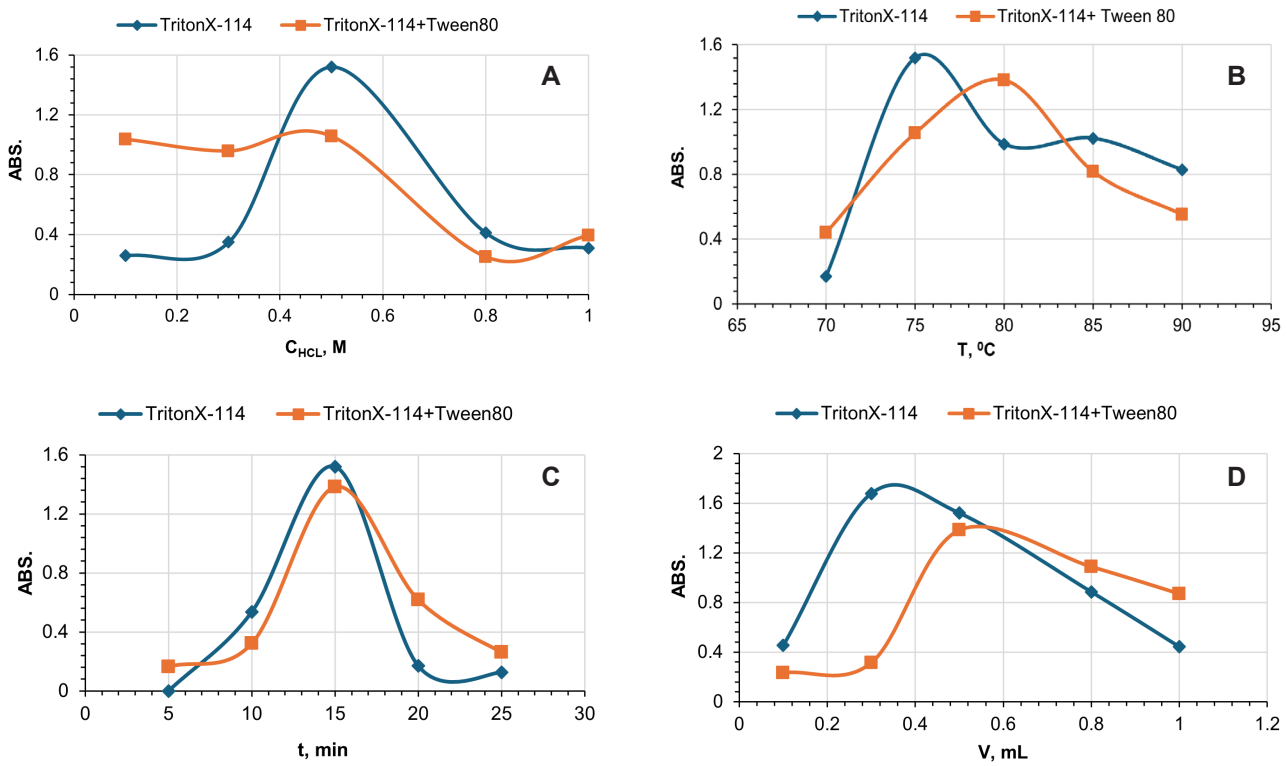

Figure 1. Effect of a) HCl concentration, b) temperature, c) heating time, d) surfactant volume on the ion pair complex formation.

Stoichiometry of the ion-pair complex

Job's method of continuous variation and slope ratio analysis were employed to elucidate the molar ratio of SMX to Ni(II) in the extracted complex. The slope ratio, calculated from the absorbance versus concentration plots of SMX and Ni(II) (Figs. S4 and S5), yielded a value close to 1:1 (0.93), indicating the formation of a 1:1 ion pair: $[SMX^+][NiCl_4]^-$.

Solvent effect on CP layer solubilization

Following extraction, the surfactant-rich phase was solubilized in various organic solvents differing in polarity and dielectric constants (Table S1). The effect of solvent polarity was assessed by plotting λmax against the dielectric functions φ(D) and F(D) (Equations 1 and 2), as shown in Fig. S6. Results suggest that the dielectric constant does not linearly correlate with spectral shifts, implicating other solvation dynamics in the microenvironment of the complex [28].

$$\varphi(D) = (D - 1) / (D + 2) \quad (1)$$

$$F(D) = 2(D - 1) / (2D + 1) \quad (2)$$

Kinetic study via initial rate method

The kinetics of complex formation was assessed under pseudo-first-order conditions using the initial rate method. Absorbance versus time curves (Fig. S7) were analyzed to calculate the reaction rate (V), which follows the Equations 3 and 4:

$$V = \Delta A / \Delta t = \acute{K}\, C^n \quad (3)$$

$$\log V = \log \Delta A / \Delta t = \log \acute{K} + n \log C \quad (4)$$

Linear regression of log V versus log C (Fig. S8) produced correlation coefficients (R^2) exceeding 0.99, with reaction orders (n) close to 1, confirming a first-order dependence with respect to SMX concentration (Table S2).

Calibration curve

A calibration curve for SMX determination was constructed under optimal CPE-LIE conditions at λ_{max} 277 nm (Triton X-114) and 291 nm (Triton X-114 + Tween 80), showing excellent linearity in the 10–100 ppm range ($R^2 > 0.997$) (Fig. S9). Analytical parameters including molar absorptivity, limits of detection (LOD), and quantification (LOQ) are summarized in Table 1.

Table 1. Analytical parameters of the method.

Parameter	Triton X-114	Triton X-114 + Tween 80
λ_{max} (nm)	277	291
Bear's law obey (ppm)	10-100	10-100
Slope	0.0161	0.0138
Intercept	0.0783	0.0107
R^2	0.9989	0.9972
Molar absorptivity (L mol^{-1} cm^{-1})	14.140×10^3	12.177×10^3
LOD (μg mL^{-1})	13.3	13.4
LOQ (μg mL^{-1})	40.4	40.6

Table 2. Quantification of sulfamethoxazole in pharmaceutical formulations using CPE–LIE and HPLC methods.

Name	Manufacturer	Labeled dose	Measured concentration μg mL^{-1}		Recovery%	
			CPE-LIE method	HPLC method	CPE-LIE method	HPLC method
Septrin (Tablet)	Aspen, Germany	400 mg	394.6	389.4	98.6	97.4
KINDIPRIM (Suspension)	AL-KINDI, Iraq	200 mg / 5mL	198.6	194.2	99.3	97.1

HPLC validation and comparison

To validate the spectrophotometric results, high-performance liquid chromatography (HPLC) was employed using a C18 reversed-phase column, with UV detection at 200 nm and a mobile phase consisting of acetonitrile:water (40:60 v/v) acidified with 0.4% H_2SO_4. Chromatograms for the SMX standard and pharmaceutical formulations are shown in Figs. S10–12. Chromatographic and extraction-based methods showed comparable results in terms of SMX quantification, with recoveries ranging from 97.09% to 99.33% (Table 2), confirming the reliability of the CPE-LIE approach.

Conclusion

In this study, a cloud point extraction (CPE) technique coupled with liquid ion exchange (LIE) was employed for the spectrophotometric determination of sulfamethoxazole (SMX) via complexation with Ni(II) as a chlorocomplex anion ($[NiCl_4]^{2-}$) in acidic medium (HCl). Nonionic surfactants—Triton X-114 and a binary mixture of Triton X-114 and Tween 80—served as extraction media. The maximum absorbance wavelengths of the ion-association complex were observed at 277 nm using Triton X-114 and at 291 nm for the mixed surfactant system. Optimal extraction conditions were established as follows: HCl concentration of 0.5 M, incubation temperature of 75 °C, and heating time of 15 minutes. The ideal surfactant volumes were determined to be 0.3 mL for Triton X-114 and 0.8 mL for the mixed system, while the optimal Ni(II) concentration was 100 μg mL^{-1}.

Kinetic evaluation was performed using the initial rate method, confirming pseudo-first-order reaction behavior with respect to SMX concentration. The method exhibited excellent linearity within the concentration range of 10–100 ppm. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 13.3 μg mL^{-1} and 40.4 μg mL^{-1}, respectively, when using Triton X-114, and 13.4 μg mL^{-1} and 40.6 μg mL^{-1} for the mixed surfactant system.

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