Computational Data Analysis of Cloud Point Extraction Method for the Determination of Sulfamethoxazole in Pure and Pharmaceutical

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Abstract: A spectrophotometric method for the determination of sulfamethoxazole (SMZ) was developed using an azo coupling reaction combined with Cloud Point Extraction (CPE) in the presence of the nonionic surfactant Triton X-114. The previously developed azo coupling method demonstrated acceptable accuracy but suffered from limited sensitivity and interference from excipients that affected selectivity. In the present investigation, CPE was employed as a preconcentration step to overcome these limitations, allowing extraction of the colored azo product into the surfactant-rich phase. The method was optimized regarding the surfactant type and concentration, equilibrium temperature, and incubation time. The results showed significant improvement in sensitivity, precision, and accuracy, with a detection wavelength of 489 nm, recoveries around 101.8%, and low relative standard deviations. This technique was successfully applied to pharmaceutical preparations of SMZ without requiring additional complex steps, demonstrating superior sensitivity, selectivity, and environmental compatibility compared with the azo method alone. Thus, CPE provides a simple, cost-effective, and green analytical approach for the determination of sulfamethoxazole in both pure and pharmaceutical forms.

1 INTRODUCTION

Sulfamethoxazole (SMZ) is one of the major members of the sulfonamide family of antibacterial agents. Its chemical name is 4-amino-N-(5-methyl-3-isoxazolyl)benzene sulfonamide, with a molecular weight of 253.28 g/mol and molecular formula $C_{10}H_{11}N_3O_3S$ [1], [2]. The structure of sulfamethoxazole, consisting of an isoxazole ring and a benzene nucleus attached to a sulfonamide group, is illustrated in Figure 1. These structural features are responsible for its distinct pharmacological properties [1]. It is these factors that make the drug have its unique pharmacological properties [3]. Sulfamethoxazole is widely used in the treatment of several bacterial infections, including respiratory, urinary tract, middle ear, and gastrointestinal infections [4]. It is most commonly combined with trimethoprim to form Co-trimoxazole, a drug with broad applications in treating nocardiosis, toxoplasmosis, and Pneumocystis pneumonia [5]. However, bacterial resistance has limited its solo use, making the combination therapy the current standard approach [6]. Although clinically valuable, sulfamethoxazole may cause skin rashes, nausea, and vomiting. From an analytical standpoint, SMZ has attracted considerable attention in pharmaceutical and chemical research, and has been determined by numerous analytical techniques such as voltammetry, HPTLC, HPLC, and UV–Vis spectrophotometry [7], [8]. These methods have disadvantages although they are accurate and dependable [9]. Some of the challenges would be high costs, the use of sophisticated equipment, and the common use of dangerous organic solvents [10]. This has led to the increased demand of simpler, more cost-effective and ecologically friendly alternative methods of analysis [11]. One of the most important changes in this area has been the invention of modern sample preparation techniques [12]. Although the development of analytical equipment has occurred rapidly, the analytical equipment is still often the least precise and repeatable in many cases in the analytical chain, with sample preparation often being the weakest component [13]. One of the most important

innovations in this regard is the Cloud Point Extraction (CPE) technique, a simple, rapid, and green method for sample preconcentration [14]. CPE relies on the micellar behavior of nonionic surfactants in aqueous media [15]. At a certain temperature, known as the Cloud Point Temperature (CPT), the solution separates into two phases: a dilute aqueous phase and a surfactant-rich phase containing the majority of the analyte-solubilized micelles [16]. This process allows effective extraction and concentration of trace analytes prior to analysis [17], [18]. The azo coupling method developed earlier was used in the present study with Cloud Point Extraction (CPE) alongside the application of Triton X-114 as a nonionic surfactant [19], [20]. In this study, the previously developed azo coupling method was combined with CPE using Triton X-114 to achieve higher sensitivity, minimize matrix interference, and ensure environmental compatibility. This work is a development and continuation of the authors' previous study on sulfamethoxazole analysis.

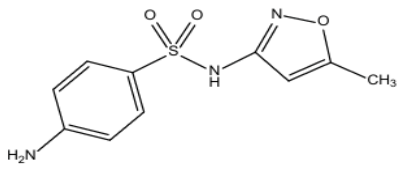

Figure 1: Structure of sulfomethoxazole (SMZ).

2 EXPERIMENTAL

2.1 Materials and Reagents

All chemicals used were of analytical grade, and all experiments were performed using double-distilled water. Pure and pharmaceutical forms of sulfamethoxazole (SMZ) were obtained from certified sources. A stock solution of SMZ (1000 µg/ml) was prepared by dissolving 0.100 g of pure drug in distilled water and diluting to 100 ml.

Solutions of β-naphthol (1000 µg/ml) were prepared in ethanol and completed with distilled water to 100 ml. Aqueous solutions of sodium nitrite (1% w/v) and sulfamic acid (1% w/v) were freshly prepared. Phosphoric acid (0.5 M) and potassium hydroxide (0.5 M) were also prepared by appropriate dilution of concentrated reagents. The nonionic surfactant Triton X-114 was used to prepare a 10% v/v CPE solution in distilled water.

2.2 Apparatus

A Shimadzu UV–Vis spectrophotometer (λmax = 489 nm, 1 cm quartz cells) was used for all absorbance measurements. A thermostated water bath (50°C), centrifuge (4000 rpm), analytical balance (±0.1 mg), and standard laboratory glassware (10 ml volumetric flasks, pipettes, and beakers) were employed.

2.3 Preparation of Standard Solutions

A working standard solution (100 µg/ml) was prepared by dilution of the stock solution. From this, calibration standards ranging from 1–12 µg/ml were freshly prepared using distilled water before each analysis.

2.4 Azo Coupling Reaction

In the previously reported method, the azo coupling reaction is based on a diazotization–coupling process, suitable for compounds containing aromatic amine groups, such as sulfamethoxazole. Sulfamethoxazole was reacted with sodium nitrite in an acidic medium at a low temperature to form a diazonium salt. The excess nitrite was removed by adding sulfamic acid, and the resulting diazonium salt reacted, in a basic medium (KOH), with β-naphthol to produce a stable orange azo dye. The absorbance of this dye was measured spectrophotometrically at 489 nm, following Beer–Lambert's law. The method achieved a recovery rate of approximately 100%, with high molar absorptivity and sensitivity, and a detection limit of about 0.35 µg/ml. This simple, fast, and reliable technique was successfully employed for the analysis of sulfamethoxazole in both pure and pharmaceutical forms, with minimal interference from common excipients [21].

2.5 Cloud Point Extraction Procedure

The CPE procedure was carried out as follows: To a 10 mL volumetric flask, add 1 mL of the azo dye solution, 10 mL of 10% Triton X-114 solution, and distilled water up to the mark. The solution was transferred into a test tube and centrifuged at 4000 rpm for 20 minutes, followed by incubation in a water bath at 60°C for 20 minutes. Afterward, the tube was placed in an ice bath for 1 minute to promote micelle phase separation. The surfactant-rich phase was then separated manually and dissolved in 1 ml ethanol, after which the absorbance was measured at 489 nm using a UV–Vis spectrophotometer [22].

3 RESULTS AND DISCUSSION

3.1 Effect Type of Surfactant with Sulfamethoxazole

The surfactant plays a crucial role in the cloud point extraction (CPE) process since the efficiency of extraction depends on micelle formation. In this experiment, 1 ml of each surfactant (Tween 20, Triton X-100, Tween 80, SDS, Triton X-114, and CTAP) was added (Corrected grammatical error "were added" → "was added," per A19) to 10 ml of distilled water containing 1 ml SMZ, 0.5 mL H_3PO_4, 0.5 ml $NaNO_2$ (1%), 0.7 ml KOH, 0.6 ml β-naphthol, and 0.1 ml H_3NSO_3. After centrifugation at 4000 rpm for 15 minutes, the separated phase was dissolved in ethanol, and absorbance was measured at λmax = 489 nm. The highest absorbance value (0.384) was observed using Triton X-114, indicating its superiority as the extraction surfactant. The lowest value (0.124) was obtained using CTAP, as shown in Table 1 and Figure 2.

Table 1: Effect of surfactant type on sulfamethoxazole absorbance.

Addition	Absorbance = 489 nm
Tween 20	0.275
Triton X-100	0.302
Tween 80	0.295
SDS	0.329
Triton X-114	0.384
CTAP	0.124

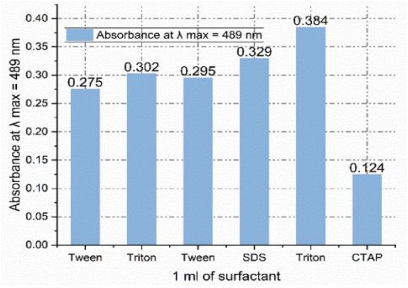

Figure 1: Type of surfactant's effect on absorption.

3.2 Effect of Triton X-114 Volume

To determine the optimal surfactant volume, solutions containing different amounts of Triton X-114 (0.2–2.0 ml) were prepared under identical conditions. The absorbance increased with the surfactant volume up to 1.2 ml (0.397), after which a slight decrease occurred due to dilution of the micellar phase. Therefore, 1.2 ml was chosen as the optimum surfactant volume for further experiments, as shown in Table 2 and Figure 3.

Table 2: Effect of the volume of Triton X-114 on the absorbance.

Volume of Triton X-114	Abs of SMZ = 489 nm
0.2	0.220
0.4	0.258
0.6	0.304
0.8	0.345
1.0	0.381
1.2	0.397
1.4	0.386
1.6	0.365
1.8	0.351
2	0.348

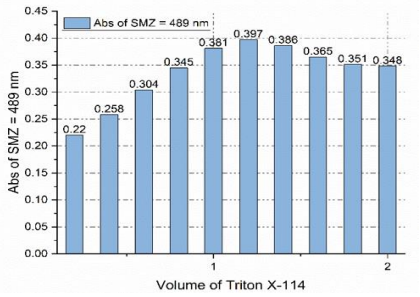

Figure 3: Triton X-114 volume's effect on absorption.

3.3 Effect of Equilibrium Temperature

The effect of temperature on CPE efficiency was studied in the range of 35–65°C. Each solution contained 1 ml SMZ, 0.5 mL H_3PO_4, 0.5 mL $NaNO_2$ (1%), 0.7 ml KOH, 0.6 ml β-naphthol, 0.1 mL H_3NSO_3, and 1.4 ml Triton X-114 (10% v/v). After incubation for 20 minutes and centrifugation, the absorbance of the surfactant-rich phase was measured at 489 nm. The optimum absorbance was obtained between 45–50°C, with a maximum of 0.452 at 45°C, but higher temperatures caused a slight decrease, likely due to partial decomposition of the azo dye. Hence, 50°C was selected as the optimal equilibrium temperature for subsequent experiments, as shown in Table 3 and Figure 4.

Table 3: Equilibrium temperature's effect on absorbance.

Temperature	Abs of SMZ = 489 nm
35	0.401
40	0.422
45	0.452
50	0.437
55	0.426
60	0.368
65	0.321

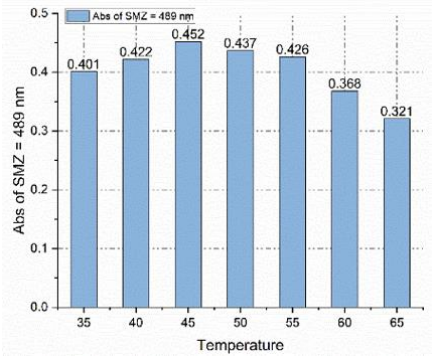

Figure 4: Equilibrium temperature's effect on absorption.

3.4 Effect of Incubation Time

The sulfamethoxazole solution [1 ml SMZ, 0.5 ml H 3 PO 4, 0.5 ml NaNO2 (1%), 0.8 ml KOH, 0.6 ml β-naphthol, 0.1 ml H 3 NSO3, and 1.4 ml Triton X-114 (10% v/v)] was left to incubate under different time durations before centrifugation. Different incubation times (5–35 minutes) were tested under the same experimental conditions. As shown in Table 4 and Figure 5, the absorbance increased gradually with incubation time and reached its highest value (0.455) at 25 minutes. However, considering analytical efficiency, 20 minutes was selected as the optimal incubation time, offering excellent precision and reproducibility.

Table 4: Incubation time effect on absorbance.

Time /min	Abs of SMZ = 489 nm
5	0.314
10	0.360
15	0.387
20	0.429
25	0.455
30	0.441
35	0.436

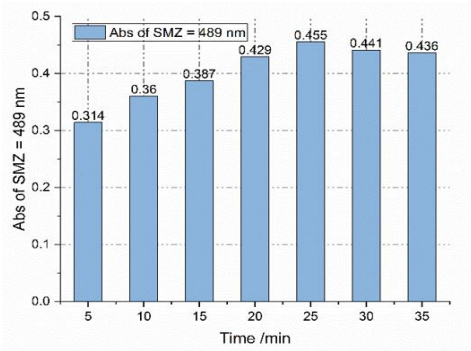

Figure 5: Effect of incubation time on the absorbance.

3.5 Preparation of Calibration Curve in CPE

Calibration curve was constructed for sulfamethoxazole concentrations from 1 to 14 µg/ml under optimized CPE conditions (1.2 ml Triton X-114, 50°C, 20 min incubation). Absorbance was measured at 489 nm after phase separation and dissolution in ethanol.

A linear relationship was observed between absorbance and concentration, confirming that the Beer–Lambert law was obeyed in this range. This validates the suitability of the developed CPE–spectrophotometric method for quantitative determination of sulfamethoxazole. The data is given in Table 5 and the calibration plot is shown in Figure 6.

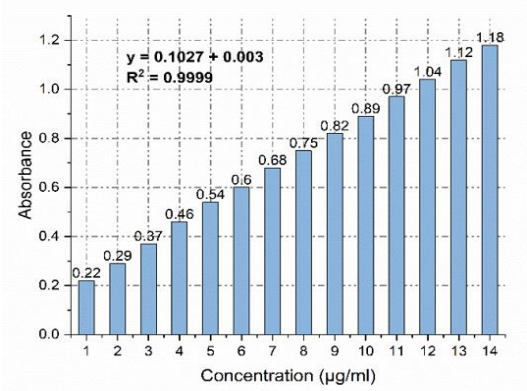

Figure 6: Calibration curve for (SMZ) drug by azo coupling reaction method.

Table 5: Calibration curve data for sulfamethoxazole using the CPE method.

Concentration (µg/ml)	Absorbance
1	0.22
2	0.29
3	0.37
4	0.46
5	0.54
6	0.60
7	0.68
8	0.75
9	0.82
10	0.89
11	0.97
12	1.04
13	1.12
14	1.18

Table 6: Estimated data for the active ingredient sulfamethoxazole in pharmaceuticals.

Amount	12	9	6	3
Found	11.83	8.8294	5.8600	2.8700
R %	98.5833	98.1044	97.6667	95.6667
AvgR %	97.505275			
E_{rel}%	-1.4167	-1.8956	-2.3333	-4.3333
Avg E_{rel}%	-2.494725			
RSD%	0.0010	0.0041	0.0086	0.0309
Avg RSD%	0. 01115			

Table 7: Precision and accuracy data for cloud point estimation of sulfamethoxazole drug.

Amount µgm/ml	*Found	Recovery%	Average %	Accuracy level	Errel %	Average Errel %	RSD %
12	12.1258	101.0483	101.8554	± 0.03178	1.0483	1.85542	0.0013
9	9.1860	102.0667		± 0.12059	2.0667		0.0064
6	6.0549	100.9067		± 0.10673	0.9067		0.0086
3	3.1020	103.400		± 0.19731	3.4000		0.0309
Tilt accuracy level	0.1027			± 0.1388936			

Table 8: Comparison between azo coupling and cloud point extraction methods in the spectrophotometric determination of sulfamethoxazole.

Parameter	Azo Coupling	Cloud Point Extraction
Principle	Diazotization with β-Naphthol to form a colored product measured spectrophotometrically	Extraction at cloud point using Triton X-114 followed by spectrophotometric measurement
Linear range (µg/ml)	1 – 12	1 – 14
λmax (nm)	489	489
Molar absorptivity (L/mol·cm)	39,534	Higher
Limit of detection (LOD, µg/ml)	0.35	Lower
Limit of quantitation (LOQ, µg/ml)	0.12	Lower
Recovery (%)	100.38	101.85
Precision (RSD%)	0.0010	0.0013
Interferences	Minimal	Dependent on surfactant condition
Experimental conditions	Simpler (40°C, water, 30 min)	Slightly more complex (50°C, centrifugation)
Advantage	Simple and accurate	Higher recovery, suitable for complex matrices

3.6 Accuracy and Precision Test

To evaluate accuracy and precision, solutions of different concentrations (3, 6, 9, and 12 µg/ml) were analyzed under optimal CPE conditions.

The results showed high recovery values (95.6–98.5%) and very low relative standard deviations (RSD < 0.04%), demonstrating the method's excellent reliability for pharmaceutical analysis. All experiments were performed in triplicate, and the average recovery was 97.50%, confirming the reproducibility of the proposed method. As shown in Table 6 showing the data and findings that estimate the drug sulfamethoxazole in pharmaceutical preparations.

3.7 Application

The developed method was applied to pharmaceutical formulations of sulfamethoxazole at concentrations of 3, 6, 9, and 12 µg/ml. The mean recovery rate was 101.85%, indicating high accuracy and good reproducibility. These findings demonstrate that the proposed CPE technique is reliable, precise, and applicable for real pharmaceutical samples without complex pretreatment, as shown in Table 7 and 8.

4 CONCLUSIONS

The study successfully developed a cost-effective and environmentally friendly spectrophotometric method for the quantitative determination of

sulfamethoxazole (SMZ) using a combination of azo coupling and Cloud Point Extraction (CPE) with Triton X-114. This combined approach enhanced sensitivity, selectivity, and recovery while minimizing the interference of excipients often encountered in traditional spectrophotometric methods.

The optimized conditions - including surfactant volume, equilibrium temperature, and incubation time - yielded high recovery rates with excellent precision and accuracy. Therefore, the proposed method represents a green and sustainable alternative to conventional extraction and analytical procedures. It is suitable for both pure and pharmaceutical forms of sulfamethoxazole, providing a simple, reproducible, and eco-friendly analytical technique.

REFERENCES

[1] M. Thani, S. Dadoosh, A. Fahad, A. Abdullah, and Y. Fahad, "Synthesis of new azo compound and its application for spectrophotometric determination of sulfamethoxazole and extraction using cloud point extraction," International Journal of Drug Delivery Technology, vol. 12, no. 3, pp. 1311-1318, 2022.

[2] N. S. Mohammed, N. Theia'a, and P. Abdul-Jabar, "Development method for spectrophotometric analysis of sulfamethoxazole using vanilline reagent," Asian Journal of Applied Chemistry Research, vol. 6, no. 5, pp. 41-49, 2020.

[3] Y. Chu, C. Zhang, R. Wang, X. Chen, N. Ren, and S.-H. Ho, "Biotransformation of sulfamethoxazole by microalgae: removal efficiency, pathways, and mechanisms," Water Research, vol. 221, p. 118834, 2022.

[4] D. K. Chellappan, et al., "Exploring the role of antibiotics and steroids in managing respiratory diseases," Journal of Biochemical and Molecular Toxicology, vol. 36, no. 10, p. e23174, 2022.

[5] D. Jamil, R. S. AlSayed, and N. K. Abood, "New spectrophotometric determination sulfamethoxazole drug via various analytical methods in pharmaceutical formulation," Journal of Kufa for Chemical Sciences, vol. 3, no. 1, pp. 197-210, 2023.

[6] J. G. McCutcheon, A. Lin, and J. J. Dennis, "Characterization of Stenotrophomonas maltophilia phage AXL1 as a member of the genus Pamexvirus encoding resistance to trimethoprim–sulfamethoxazole," Scientific Reports, vol. 12, no. 1, p. 10299, 2022.

[7] J. Musial, D. T. Mlynarczyk, and B. J. Stanisz, "Photocatalytic degradation of sulfamethoxazole using TiO2-based materials–Perspectives for the development of a sustainable water treatment technology," Science of The Total Environment, vol. 856, p. 159122, 2023.

[8] M. K. Gupta, et al., "A comparative review on high-performance liquid chromatography (HPLC), ultra performance liquid chromatography (UPLC) & high-performance thin layer chromatography (HPTLC) with current updates," Current Issues in Pharmacy and Medical Sciences, vol. 35, no. 4, pp. 224-228, 2022.

[9] W. Parys, M. Dołowy, and A. Pyka-Pająk, "Significance of chromatographic techniques in pharmaceutical analysis," Processes, vol. 10, no. 1, p. 172, 2022.

[10] A. Radhi, et al., "Instrumental techniques of pharmaceuticals analysis: a review," Middle European Scientific Bulletin, vol. 45, no. 1, pp. 21-37, 2024.

[11] H. A. Khalil, A. F. El-Yazbi, D. A. Hamdy, and T. S. Belal, "Application of HPTLC, spectrofluorimetry and differential pulse voltammetry for determination of the antifungal drug posaconazole in suspension dosage form," Annales Pharmaceutiques Françaises, vol. 77, no. 5, pp. 382-393, 2019.

[12] S. Sharma, N. Singh, A. D. Ankalgi, A. Rana, and M. S. Ashawat, "Modern trends in analytical techniques for method development and validation of pharmaceuticals: a review," Journal of Drug Delivery & Therapeutics, vol. 11, 2021.

[13] H. S. AlSalem, et al., "High performance thin layer chromatography (HPTLC) analysis of anti-asthmatic combination therapy in pharmaceutical formulation: assessment of the method's greenness and blueness," Pharmaceuticals, vol. 17, no. 8, p. 1002, 2024.

[14] M. A. Bezerra, et al., "Recent developments in the application of cloud point extraction as procedure for speciation of trace elements," Applied Spectroscopy Reviews, vol. 57, no. 4, pp. 338-352, 2022.

[15] E. A. Azooz, R. K. Ridha, and H. A. Abdulridha, "The fundamentals and recent applications of micellar system extraction for nanoparticles and bioactive molecules: a review," Nano Biomed. Eng., vol. 13, no. 3, pp. 264-278, 2021.

[16] N. M. Muslim, B. K. Hussain, N. M. Abdulhussein, and E. A. Azooz, "Determination of selenium in black tea leaves using the air-assisted cloud point extraction method: evaluation of the method's environmental performance," Analytical and Bioanalytical Chemistry Research, vol. 11, no. 1, pp. 11-22, 2024.

[17] S. Bharti, "Recent advances in heavy metal removal from wastewater using nanomaterials and cloud point extraction: a comprehensive review," International Journal of Environmental Science and Technology, vol. 22, no. 6, pp. 5057-5084, 2025.

[18] X.-W. Wei, et al., "A review on pretreatment and analysis methods of polyether antibiotics in complex samples," Critical Reviews in Analytical Chemistry, vol. 54, no. 8, pp. 3453-3477, 2024.

[19] K. Oukebdane, R. Semmoud, and M. A. Didi, "Cloud point extraction of Telon Orange anionic azo-dye from aqueous sulphate solutions using Aliquat 336 ionic liquid/Tween 40 as extracting system: factorial design optimization methodology," Desalination and Water Treatment, vol. 247, pp. 272-280, 2022.

[20] E. Ghasemi and M. Kaykhaii, “Application of a novel micro-cloud point extraction for preconcentration and spectrophotometric determination of azo dyes,” Journal of the Brazilian Chemical Society, vol. 27, pp. 1521-1526, 2016.

[21] N. A. H. Alwan and I. S. Mohammed, “Spectrophotometric determination of sulfamethazole drug by using azo coupling reaction,” Iraqi Journal for Applied Science, vol. 1, no. 1, pp. 88-101, 2024.

[22] A. F. Hasan and I. S. Mohammed, “Spectrophotometric determination and cloud point extraction of sulfadiazine in pure and pharmaceutical form,” Biochemical & Cellular Archives, vol. 21, no. 1, 2021.