Spectrophotometric Determination of Sulfamethoxazole Based on Charge-Transfer Complexation with Sodium Nitroprusside

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Abstract

A simple, accurate and precise spectrophotometric method has been developed for the analysis of sulfamethoxazole (SMZ) in pure form and pharmaceutical preparation. The method involves a direct charge transfer complexation of sulfamethoxazole (SMZ) with sodium nitroprusside (SNP) in alkaline medium and the presence of hydroxyl amine hydrochloride. Variables affecting the formation of the formed orange colored complex were optimized following two approaches univariate and central composite experimental design (CCD) multivariate. Under optimum recommended conditions, the formed complex exhibits λmax at 512 nm and the method conforms Beer's law for SMZ concentration in the range of 5.0-150.0 (µg.mL^{-1}) with molar absorptivity 1.139×10^{3} L.mol^{-1}.cm^{-1}, and r = 0.9997. Analysis of SMZ pharmaceutical dosages shows a good agreement with the real amounts.

Keywords: spectrophotometric determination; sulfamethoxazole; sodium nitroprusside; charge-transfer complexation.

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Introduction

Sulfamethoxazole, 4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (M. wt. 253.279 gm. mol^{-1}) [1]. Sulfamethoxazole an antibacterial drug which approved for treatment of the oversensitive forms of Hemophilic influenza, Staphylococcus aurous, Streptococcus, Escherichia coli, and for oral anaerobes. It also represents therapeutic approach to the treatment of the urinary tract infections as an alternative to amoxicillin-based antibiotics. The literature contains various methods for the determination of sulfamethoxazole in its pharmaceutical formulations. This involves spectrophotometric [2-7], HPLC [8-11], HPTLC [12], Capillary zone electrophoresis [13], Micellar electrokinetic chromatography [14], derivative ratio spectrometry [15], flow injection sensor [16], sulfamethoxazole-imprinted polymer [17], spectrofluorometry [18], fluorescence spectrophotometric [19] and NMR [20] methods. The structural formula of SMZ is shown in Scheme (1).

In this work a simple and sensitive visible spectrophotometric method was developed for quantitative determination of sulfamethoxazole in its pure form and in pharmaceutical formulation. The suggested method is based on the formation of charge transfer molecular complex of drug with sodium nitroprusside in alkaline medium in the presence of hydroxylamine hydrochloride [21], [22].

Experimental

Apparatus

A CECLL UV / VIS double beam spectrophotometer (model CE 7200. UK (7000 series) with 1 cm quartz cells, a Sartorius-BL 210 scientific balance(Germany), a heater with magnetic starrier (IKA- Combimag Rct.), a lab tech water bath (Korea) were used for this study. The experimental design and the coefficients of the response surface equation were determined by statistical 12 (Stat. Soft. Inc., release 2013) software.

Reagents

All reagents and chemicals used of analytical grade. Sulfamethoxazole powders were provided from the State Company for Drug Industries and Medical Appliances Samara- Iraq (SDI) in pure form (99.99%).

Reagents solutions

- Sodium Carbonate monohydrate (6% w/v): 6g of $Na_2CO_3.H_2O$ was dissolved in 100 mL of distilled water.
- Sodium Nitroprusside 0.2% (w/v): 0.2g of SNP was dissolved and diluted to the mark with distilled water in a 100 mL volumetric flask.
- Hydroxylamine hydrochloride 0.4% (w/v): 0.4g of $NH_2OH.HCl$ was dissolved in 100 mL of distilled water.

Preparation of standard stock solution (1000 µg.mL^{-1})

An accurately weight 0.1 g of SMZ was dissolved in 5 mL of 0.1M $Na_2CO_3.H_2O$ and diluted in 100mL-volumetric flask with distilled. Other dilute solutions were freshly prepared by subsequent dilution with distilled water as required.

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Preparation of sample stock solution

Different commercial SMZ pharmaceutical dosages (i.e. tablets and syrup) were analyzed to assay their contents of the drug. 10 tablets were powdered and an amount of the powder equivalent to 0.1 gm sulfamethoxazole dissolved in 5 mL of 0.1 M $Na_2CO_3.H_2O$ solution in 100 mL volumetric flask. The mixture was shaken, and left for 5 min. before dilution with distilled water to the mark. Any undissolved materials were filtered-out via Whatman No.41 filter paper. Five milliliters of the syrup sample solution mixed with 5mL of 0.1M $Na_2CO_3.H_2O$ solution in 100mL-volumetric flask. The flask was shaken and left to stand for five minutes before diluting it's contained with distilled water to the mark. Any undissolved materials were filtered-out via Whatman No.41 filter paper. All the samples were further diluted to achieve the concentration of SMX in the working range.

General procedures

Under conditions obtained by univariate optimization

To a series of 10mL calibrated flasks containing (50-1500) µg of SMZ, one milliliter of 0.2 % (w/v) SNP solution and one milliliter of 0.4% (w/v) hydroxylamine hydrochloride were added respectively. The solutions were shaken thoroughly; then 0.2mL of 6% (w/v) $Na_2CO_3.H_2O$ solution was added to each. After letting the mixtures to stand for seven minutes at 25 °C in the dark, the volumes were brought to the mark with distilled water, mixed well and values of absorbance at 512.0 nm were measured versus the blank.

Under conditions obtained by multivariate CCD conditions

To a series of 10mL-calibrated flasks containing (50-1500) µg of SMZ, 2.3mL of 0.2% (w/v) SNP solution and 1.8mL of 0.4% (w/v) hydroxylamine hydrochloride were added respectively. The solutions were shaken thoroughly; then 0.6mL of 6% (w/v) Na_2CO_3 solution was added to each. After letting the mixtures to stand for seven minutes at 25 °C in the dark, the volumes were brought to the mark with distilled water, mixed well and values of absorbance at 512.0 nm were measured versus the blank.

Results and Discussion Absorption

Spectrum and reaction scheme

When SMZ is reacted with SNP according to the recommended experimental conditions Scheme (2), the absorption spectrum of the formed colored complex compound showed a maximum absorption at 512.0 nm against reagent blank solution. While, the blank has no significant absorbance in this region, as it is shown in Figure (1).

Optimization of reaction conditions

Two different approaches i.e. one-factor-a time univariate, and CCD multivariate, were followed to establish optimum reaction conditions.

Univariate method

The effect of the amounts of SNP, Hydroxylamine hydrochloride, Na_2CO_3, and reaction time on the formation of SMZ-SNP complex were studied. The results show that 1.0 mL of 0.2% (w/v) sodium nitroprusside was needed to give best results (Figure 2a). The effect of volume of 0.4% (w/v) of $NH_2OH.HCl$ solution was examined, 1.0 mL of this solution gave the maximum intensity Figure (2b).

The results on Figure 2c shows that 0.2 mL of 6% $Na_2CO_3.H_2O$ was sufficient for attaining the maximum and constant absorption intensities. Under these conditions the optimum time

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and temperature for the complex-formation reaction was found to be seven minutes at 25°C, Tables (1) and (2).

Design of experiment method

A design of experiments (DOE) was used to determine the relationship between three experimental factors (namely the amounts of reagent, $NH_2OH.HCl$, and $Na_2CO_3.H_2O$) affecting the studied reaction yield. in central composite design, around the central point (which is assumed to be 0) for each of the studied factors, the design is assumed to be symmetric. The studied variables with levels are given in Table (3).

According to CCD, a cube could represent a three factors system (k=3), and its axes correspond to the three factors. The design consists of a number of 20 experiments (2^k factorial points = 8, $2 \times k$ axial points = 6, and six center points). Table (4) shows the values of the studied experimental variables and their corresponding measured absorbance at 512 nm.

Tables (5) illustrates the analysis of variance of the experimental results in Table (4).
Table (5) show that at 95% confidence level ($p < 0.05$), the overall effect of the linear terms in the model is significant while the other terms i.e. the interaction terms are not. Moreover, Table (5) shows the standard error of coefficients of the second order polynomial model and t and p values.
Moreover, according to the obtained second order polynomial model, three dimensional plots of the response surfaces for any two pairs of the studied variables against the response were constructed to illustrate the relationship between them and the response at the critical level (optimum value) of other factors, Figure (3).

Calibration curves and analytical data

To evaluate the proficiency of the recommended method for determination of sulfamethoxazole, calibration curves were constructed by plotting the absorbance values for a series of solutions containing varying concentrations of SMZ under the optimal conditions measured against the corresponding reagent blank at 512 mn.
The graphs obtained under conditions established via univariate optimization and multivariate CCD method, Figures (3) and (4) respectively, were linear in the range of SMZ concentration (5.0 -150.0) $\mu g.mL^{-1}$ evaluated by linear regression. Some of optical and statistical parameters of the SMZ-SPN complex and the constructed calibration plots are summarized in Table (6).
In both procedures, the values of correlation coefficient (r) for the regression equation were high indicating the linearity of the plotted curves. Detection and quantification limits were calculated according to ICH guide. The high values of the molar absorptivities and the low values of detection limits indicate that both procedures are sensitive.

Accuracy and precision

The accuracy of the method was established by analyzing five replicate of the pure drug at three concentration levels and the precision was examined by determining the coefficient of variation (C.V. %) on the same solutions of drug sample. Results in Table (7) show low values of C.V % (in the range of 0.137%-0.435%) and for the relative error percent (which did not exceed ±0.8%) for both procedures, indicate high accuracy and precision of the proposed method.

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Interference study

To assess the analytical potential of the proposed method, the effect of some common excipients; sucrose, vanillin, glucose, lactose, and starch which often accompany drug, were examined by carrying out the determination of 50.0 $\mu g.mL^{-1}$ of SMZ in the existences of mentioned compounds. The results presented in Table (8) indicate that no significant interferences were found from any of the studied excipients in determination of SMZ except vanillin that completely interferes.

Application of the method

For examining the suitability of the recommended method for SMZ determination in real samples, it was applied to determine the drug in some pharmaceutical preparations. the collected results of the analyses shown in Table 9, indicate that the recovery percent values are ranged between (96.80-100.26) and those for C.V.% does not exceed 1.136 which illustrates that the proposed method is satisfactory.

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Table (1) : Effect of reaction time on the color development in the estimation of (50.0 µg.mL^{-1}) SMZ.

Time (min.)	Absorbance
3	0.185
5	0.211
6	0.225
7	0.243
8	0.233
10	0.218
15	0.201

Table (2) : Effect of temperature on the color development in the determination of (50.0 µg.mL^{-1}) SMZ.

Temperature °C	Absorbance
20	0.213
25	0.243
30	0.207
35	0.165
40	0.168
45	0.161
50	0.146

Table (3) : The levels of the experimental variables.

Factors	Levels		
	-1	0	+1
Vol. of 0.2% SNP solution(mL)	0.5	2.0	3.5
Vol 0.4% $NH_2OH.HCl$ solution (mL)	0.5	1.5	2.5
Vol. of 6% Na_2CO_3 solution (mL)	0.1	0.6	1.0

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Table (4) : The experimental conditions and their response based on CCD using of (50 µg.mL^{-1}) SMZ solution.

No	Vol. of 0.2% SNP sol.(mL)	Vol. of 0.4% NH_2OH sol. (mL)	Vol. of 6% Na_2CO_3 sol. (mL)	Abs
1	2.0	1.5	0.6	0.132
2	3.5	2.5	1.1	0.092
3	0.5	2.5	0.1	0.000
4	0.5	1.5	0.6	0.034
5	2.0	1.5	0.6	0.135
6	0.5	0.5	1.1	0.108
7	2.0	2.5	0.6	0.200
8	2.0	1.5	0.6	0.139
9	2.0	1.5	0.6	0.135
10	2.0	1.5	0.6	0.133
11	2.0	0.5	0.6	0.076
12	2.0	1.5	1.1	0.110
13	3.5	0.5	1.1	0.000
14	3.5	0.5	0.1	0.181
15	2.0	1.5	0.1	0.000
16	0.5	0.5	0.1	0.199
17	0.5	2.5	1.1	0.020
18	3.5	2.5	0.1	0.018
19	3.5	1.5	0.6	0.122
20	2.0	1.5	0.6	0.133

Table (5) : The coefficients of the nonlinear polynomial model and p values.

Variable	Regression coefficient	Standard error of coefficient	t-value	P
Constant	0.23160	0.09502	2.43732	0.03501
SNP vol.	0.03187	0.07159	0.44512	0.66572
(SNP vol.)2	-0.01295	0.01622	-0.79823	0.44328
NH_2OH vol.	-0.20689	0.11761	-1.75919	0.10905
$(NH_2OH$ vol.$)_2$	0.03086	0.03650	0.84555	0.41757
Na_2CO_3 vol.	0.11141	0.19885	0.56025	0.58764
$(Na_2CO_3$ vol.$)_2$	-0.20855	0.14600	-1.42835	0.18367
SNP vol.× NH_2OH vol.	0.01800	0.01427	1.26164	0.23571
SNP vol.× Na_2CO_3 vol.	-0.00600	0.02853	-0.21027	0.83768
NH_2OH vol.× Na_2CO_3 vol.	0.09150	0.04280	2.13779	0.05825

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Table (6) : Some optical characteristics and statistical data for the determination of SMZ via the recommended procedures.

Parameter	Univariate	CCD
λmax (nm)	512.0	
Color	Orange	
Linearity range (µg.mL^{-1})	5.0-150.0	
Regression equation	y = 0.0045 [SMZ ug.mL^{-1}] + 0.0052	y = 0.0049 [SMZ ug . mL^{-1}] + 0.0001
Calibration sensitivity (mL.µg^{-1})	0.0045	0.0049
Correlation coefficient (r)	0.9997	0.9993
Correlation of linearity (R^2)	0.9995	0.9988
Molar absorptivity (L.mol^{-1}.cm^{-1})	ε =1.1399×10^3	ε = 1.2412 x 10^3
Sandell's sensitivity (µg.cm^{-2})	0.222	0.204
Detection limit (µg.mL^{-1})	0.76	0.69
Quantification limit (µg.mL^{-1})	2.533	2.326

Table (7) : Accuracy and precision for the estimation of SMZ by the recommended method.

Method	Conc. of SMZ (µg.mL^{-1})		*Relative Error %	C.V. %
	Taken	Found*		
For univariate	30	29.79	-0.700	0.183
	70	70.51	0.729	0.391
	120	119.36	-0.533	0.435
For CCD	30	30.105	0.350	0.137
	70	70.321	0.459	0.265
	120	120.112	0.093	0.282

*Average of five determinations.

Table (8): Percent recovery for (50.0 µg.mL^{-1}) of sulfamethoxazole in the presence of 200 µg.mL^{-1} of excipients.

Excipients	Taken SMZ concentration (µg.mL^{-1})	Sulfamethoxazole Conc. Taken (50.0 µg.mL^{-1})	
		Conc. Found* (µg.mL^{-1})	Recovery* %
Sucrose	50	50.11	100.22
Vanillin		Dark color with precipitate	
Glucose		50.25	100.50
Lactose		49.41	98.82
Starch		49.83	99.66

*Average of three determinations.

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Table (9) : Application of the recommended method for the determination of SMZ in pharmaceutical preparations.

Sample	Conc. taken ($\mu g.mL^{-1}$)	*Conc. found ($\mu g.mL^{-1}$)	Recovery %	C.V %
TRIMOL/tablet Julphar, U.A.E	50.00	49.95	99.90	1.101
Trimoks syrup ATABAY, Turkey	50.00	50.13	100.26	1.097
Methoprim tablet (SDI-Iraq)	50.00	48.4	96.80	1.136

*Average of three determinations.

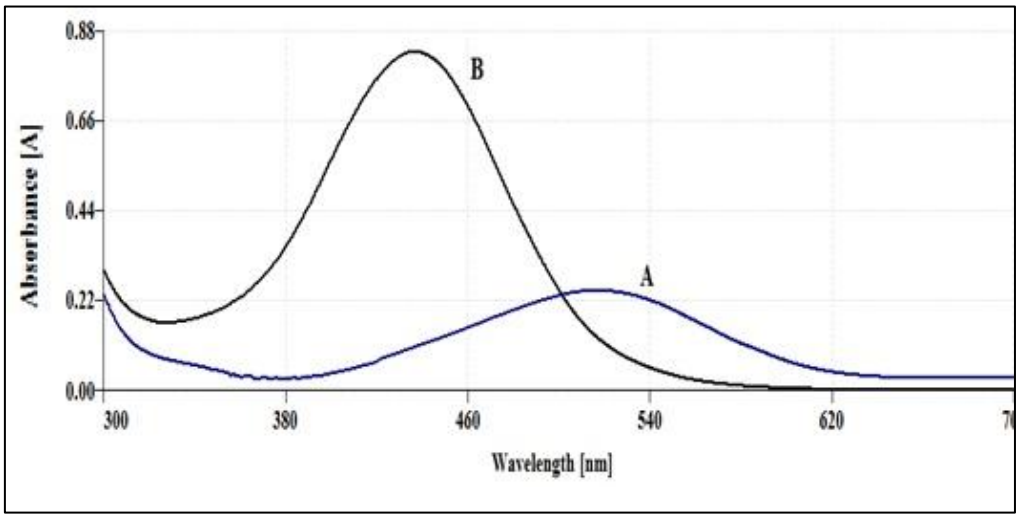

Figure (1): Absorption spectra of (A) reaction product of (50.0 $\mu g.mL^{-1}$) SMZ against reagent blank, (B) reagent blank against distilled water, under the optimum conditions.

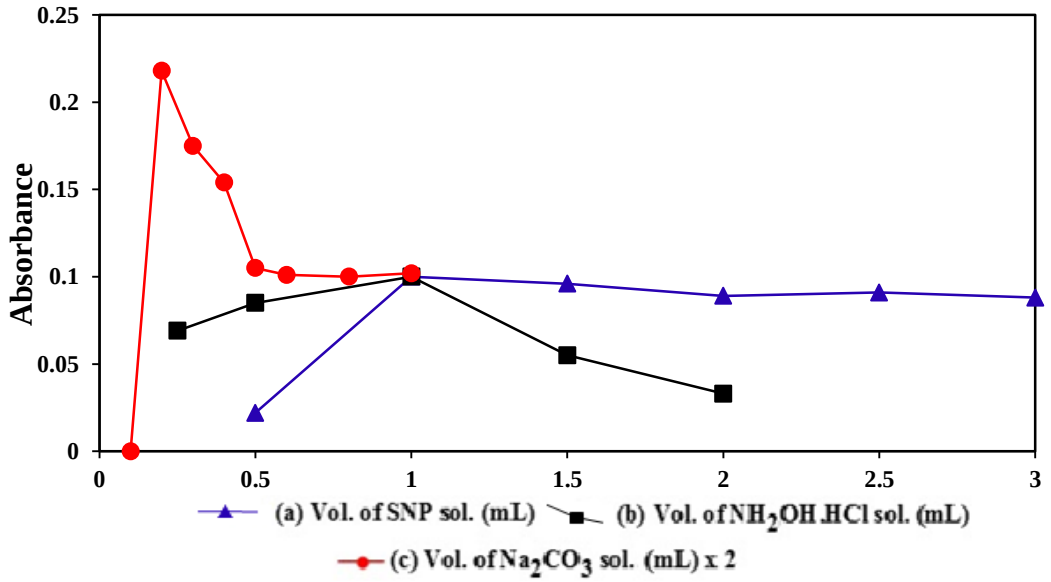

Figure (2): Effect of (a) volume 0.2% (w/v) SNP solution, (b) volume of 0.4% (w/v) $NH_2OH.HCl$ solution, (c) volume of 6% $Na_2CO_3.H_2O$ on the formation of SMZ-SNP complex.

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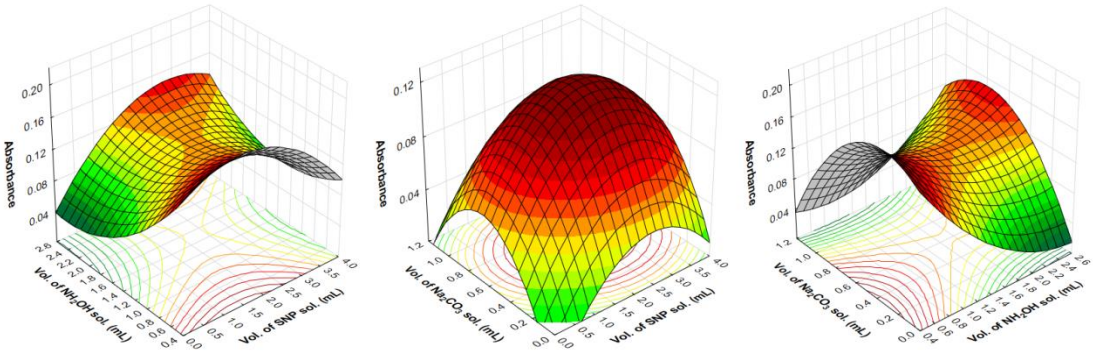

Figure (3): The response surface for the absorbance of sulfanilamide-SNP complex as a function of any pair of variables and keeping third variable constant at its optimum value.

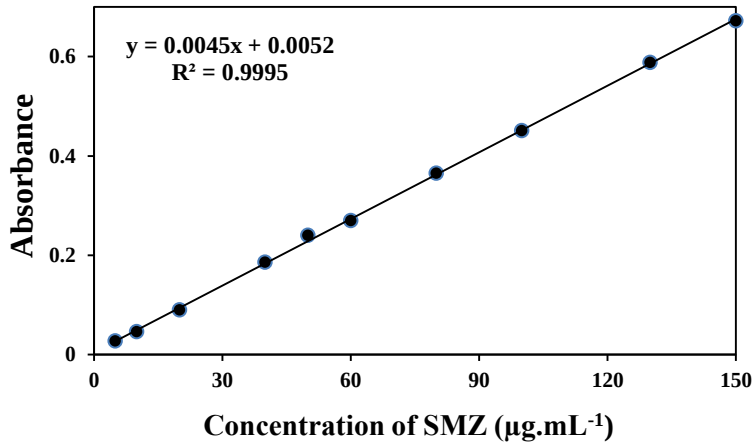

Figure (4): Calibration plot for SMZ under optimal conditions established by univariate optimization.

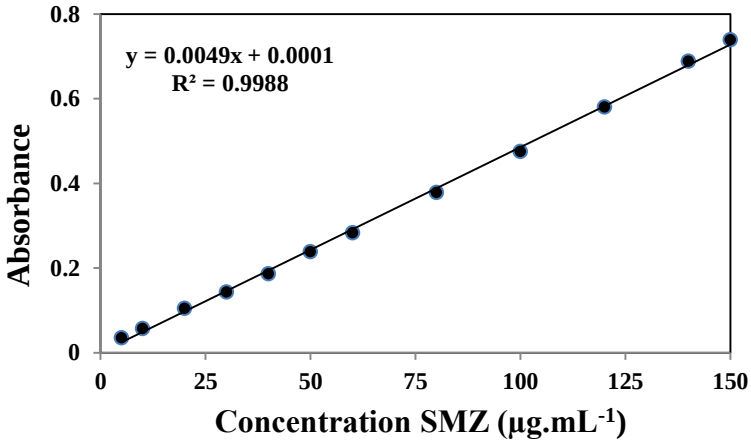

Figure (5): Calibration plot for SMZ under optimal conditions established by multivariate CCD optimization.

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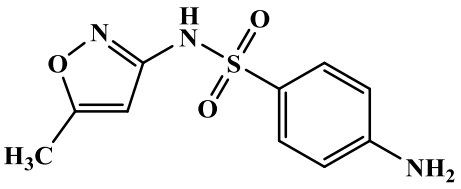
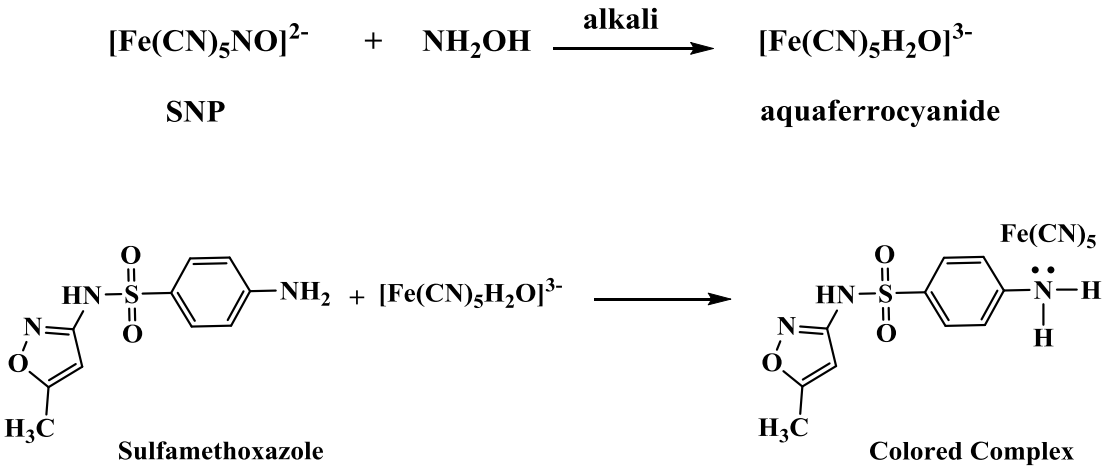


Scheme (1): The structural formula of sulfamethoxazole.



Scheme (2) : The suggested reaction mechanism for charge-transfer reaction between SMZ and sodium nitroprusside.

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