



SPECTROPHOTOMETRIC STUDY OF THE INTERACTION BETWEEN HEXACYANOFERRATE (II) ION AND SULPHAMETHOXAZOLE CATALYZED BY MERCURY(II) ION

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ABSTRACT

Potassium hexacyanoferrate when mixed with sulphamethoxazole at pH 4 in 20% dimethyl formamide (DMF) in the presence of mercury (II) forms an intense reddish brown coloured species. The maximum colour development occurs at 30 mins after mixing. The colour formation is spectrophotometrically monitored under the established optimum experimental conditions. The optimum conditions established are: pH 4, 20% DMF solvent, 520 nm as wavelength of maximum absorbance, 3-4 fold excess of hexacyanoferrate (II) to sulphamethoxazole, 1×10^{-5} M concentration of mercury (II). The colour reaction is successfully investigated for the spectrophotometric determination of sulphamethoxazole in the range 0.5 mg/mL-1.6 mg/mL. The proposed method is suitably applied for the estimation of the drug in Septron tablets.

Key words: Spectrophotometric, Dimethyl formamide (DMF), Potassium hexacyanoferrate ($K_4Fe(CN)_6$), Sulphamethoxazole (SMX).

INTRODUCTION

Sulpha drugs are extensively used in the treatment of many bacterial diseases¹⁻³. Several methods involving diazotization and coupling⁴⁻⁶ or Schiff's base formation⁷⁻⁸ are reported for the spectrophotometric determination of sulpha drugs⁹⁻²⁰.

It was reported in the literature that ligand exchange reactions involving K_4Fe

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(CN)₆ can be used for the determination of amino compounds and nitro compounds²¹⁻²⁵. However no attention was paid to the determination of sulphamethoxazole (SMX) spectrophotometrically by the ligand exchange reaction between K₄Fe (CN)₆ and SMX catalysed by mercury (II). The results are communicated in this paper. Potassium when mixed with SMX at pH 4 in 20% DMF in presence of Hg (II) form an intense reddish brown coloured species. The intensity of this colour is proportional to the amount of SMX at 520 nm under the optimum experimental conditions established in this investigation. This observation is successfully applied for the analysis of SMX in pharmaceutical preparations.

EXPERIMENTAL

Stock solutions of K₄Fe (CN)₆ (0.1 M) and Hg (II) (5×10^{-2} M) are prepared by dissolving the requisite amount of the salts in double distilled water. The Hg (II) solution is standardized using standard procedure²⁴. A solution of 2×10^{-2} M SMX is prepared in DMF and in methanol. A sodium Acetate acetic acid and buffer solution of pH 4 is used in the studies. All substances used are of AR grade required dilutions of the solutions are made as and when necessary.

Preparation of tablet solutions

10 tablets are taken and powdered well. Each tablet of 500 mg contains 400 mg of SMX and 100 mg of trimethoprim. 100 mg of the powdered tablet is made upto the mark using DMF solvent in a 10 mL standard flask. 3.2 mL of the solution when diluted in the DMF to 10 mL gives a stock solution of the tablet of concentration 0.01 M.

Apparatus

A Systronics Spectrophotometer Model-106 with wavelength range 300-900 nm is used for measuring absorbances. A toluene Hg thermostat is used for maintaining the temperature of the reaction mixture constant.

Procedure

4 mL of buffer solution of pH 4, 1 mL of K₄Fe (CN)₆ solution (0.1 M), 2 mL of DMF (20%) solvent and 1ml of mercury (II) solution (1×10^{-4} M) are taken in each of the 10 mL standard flask. The Flasks are kept in the thermostat. Known aliquots of standard SMX solution are added to the flasks. The solution in each flask is made upto 10 mL with double distilled water and immediately a stopwatch is started. The absorbance of each solution is recorded at 520 nm after 20 minutes against the buffer solution. The results show

that a linear curve is obtained between the amount of SMX and absorbance. This suggests that suitability of the method for the spectrophotometric determination of SMX in the range 0.5 mg/mL to 1.6 mg/mL.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the individual constituent solutions of the reaction mixture as well as that of the reaction mixture are recorded and are shown in Fig. 1. The Spectra showed that the reaction mixture exhibit maximum absorbance at a wavelength of 520 nm. Therefore the wavelength of 520 nm is fixed for further studies.

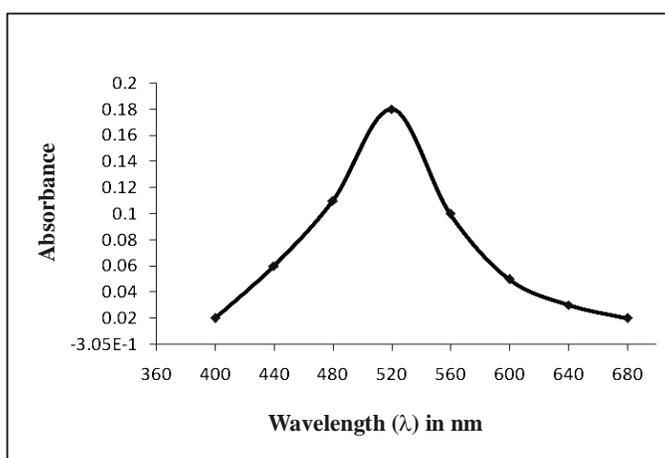


Fig. 1: Spectrum of $K_4Fe(CN)_6 + SMX + DMF + Hg(II)$ coloured species

Effect of pH

The absorbance of the reaction mixtures prepared in the buffer solution of the different pH values is recorded at 520 nm against the buffer solution and is shown in Table 1. The data indicates maximum absorbance at pH 4. The effect of the volume of the buffer solution of pH 4 shows that 4 mL of the buffer solution is enough to get maximum absorbance. Thus 4 mL of the buffer mixture of pH 4 is chosen for further studies.

Effect of Solvent

The results obtained in the studies on the effect of DMF percentage in the reaction mixture indicates that 20% DMF solvent is required to have maximum absorbance. Hence 20% of DMF solvent is fixed for further studies. The data is shown in Table 2.

Table 1: Effect of pH on the absorbance at 520 nm

4 mL Buffer solution of pH 1-6, 2 mL of conductivity water

1 mL of DMF solvent, 1 mL of Hg (II) (5×10^{-5} M)1 mL of $K_4Fe(CN)_6$ (0.1 M), 1 mL of SMX (2×10^{-2} M)

Total Test solution mixture = 10 mL

pH	Absorbance	
	20 min.	30 min
1	0.02	0.05
2	0.08	0.12
3	0.11	0.16
4	0.18	0.23
5	0.06	0.08
6	0.02	0.03

Table 2: Effect of volume percentage of DMF solvent on absorbance at 520 nm

4 mL Buffer Solution of pH 4, (3-x) mL of conductivity water

X mL of DMF solvent, 1 mL of Hg (II) (5×10^{-5} M)1 mL of $K_4Fe(CN)_6$ (0.1 M), 1 mL of SMX (2×10^{-2} M)

Total volume of test solution = 10 mL

%DMF (v/v)	Absorbance	
	20 min	30 min
10	0.09	0.14
20	0.18	0.23
30	0.17	0.21
40	0.12	0.15

Effect of $K_4Fe(CN)_6$

From the results in Table 3, it is clear that a minimum of 3 to 4 fold excess of $K_4Fe(CN)_6$ to SMX is essential to have maximum colour development. Further studies are therefore carried out with 3 fold excess of $K_4Fe(CN)_6$.

Table 3: Effect of $K_4Fe(CN)_6$ concentration on the absorbance at 520 nm

4 mL Buffer Solution of pH 4, (3-x) mL of conductivity water
 1 mL of DMF solvent 20% (v/v), 1 mL of Hg (II) (5×10^{-5} M)
 X mL of $K_4Fe(CN)_6$ (2.5×10^{-2} M), 1 mL of SMX (2×10^{-2} M)
 Total volume of Test solution = 10 mL

$K_4Fe(CN)_6 \times 10^3$ M	Absorbance	
	20 min.	30 min.
0.5	0.04	0.05
1.0	0.06	0.09
1.5	0.09	0.12
2.0	0.11	0.16
3.0	0.13	0.18
4.0	0.15	0.20
5.0	0.16	0.21
6.0	0.18	0.23
7.0	0.18	0.23

Effect of mercuric chloride

The effect of Hg (II) concentration on the reaction mixture is studied and the results are presented in the Table 4. It is evidently clear that a concentration of 1×10^{-5} M of Hg (II) is necessary for the full development of the colour.

Table 4: Effect of Hg (II) concentration on the absorbance at 520 nm

4 mL Buffer Solution of pH 4, (3-x) mL of conductivity water
 1 mL of DMF solvent 20% (v/v), x mL of Hg (II) (5×10^{-5} M)
 1 mL of $K_4Fe(CN)_6$ (6×10^{-2} M), 1 mL of SMX (2×10^{-2} M)
 Total volume of test solution = 10 mL

Hg (II) $\times 10^6$ M	Absorbance	
	20 min.	30 min.
2.0	0.13	0.19

Cont...

Hg (II) x 10 ⁶ M	Absorbance	
	20 min.	30 min.
4.0	0.16	0.21
6.0	0.18	0.23
8.0	0.19	0.24
10.0	0.19	0.24
12.0	0.20	0.25

Calibration curve-obedience of Beer-Lambert's law

A linear curve is obtained between the absorbance and the amount of SMX in the range 0.05 mg/mL to 0.3 mg/mL. This suggests the suitability of the proposed method for the quantitative analytical spectrophotometric estimation of SMX in the range 0.05 mg/mL to 0.3 mg/mL. The data is presented in Table 5 and Fig. 2.

Table 5: Effect of SMX concentration on the absorbance at 520 nm -Obedience of Beer- Lambert's Law-Lalibration Curve

4 mL Buffer Solution of pH 4, 2 mL of conductivity water
 (2-x) mL of DMF solvent 20% (v/v), 1 mL of Hg (II) (1×10^{-4} M)
 1 mL of $K_4Fe(CN)_6$ (6×10^{-2} M), x mL of SMX (1×10^{-2} M)
 Total volume of Test solution = 10 mL

[SMX] x 10 ³ M	Absorbance	
	20 min.	30 min.
0.2	0.03	0.04
0.4	0.07	0.08
0.6	0.10	0.12
0.8	0.13	0.16
1.0	0.17	0.20
1.2	0.20	0.23
1.4	0.22	0.25

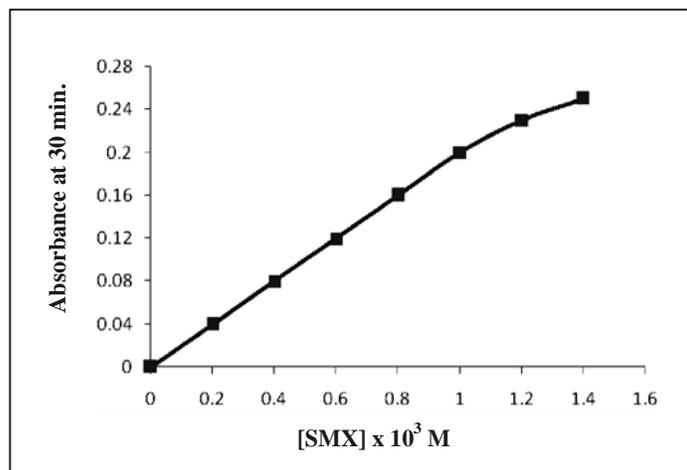


Fig. 2: Calibration curve

Application of the method

The amount of SMX is interpolated from a precalibrated curve constituted with pure SMX following the procedure described earlier. The results are presented in Table 6. The results show that there is good agreement between amount determined by the present method and the amount specified on the tablets.

Table 6: Application of the proposed method for the analysis of the septron tablet

4 mL Buffer solution of pH 4, 2 mL of conductivity water

(2-x) mL of DMF solvent 20% (v/v), 1 mL of Hg (II) (1×10^{-4} M)

1 mL of $K_4Fe(CN)_6$ (6×10^{-2} M), x mL of septron tablet solution

(32 mg powdered tablet dissolved in 10 mL DMF solution)

Total volume of test solution = 10 mL

Volume of Tablet Solution in mL	Absorbance at 30 min.	Amount of drug in mg/mL	
		Interpolated from graph	Specified on the tablet
0.4	0.081	0.104	0.108
0.8	0.17	0.203	0.201
1.2	0.23	0.300	0.306

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