Indirect Potentiometric Titration of Sulphamethoxazole in the Presence of Trimethoprim in Co-trimazole Tablets Using Copper Based Mercury Film Electrode

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A simple and rapid indirect potentiometric titration of sulphamethoxazole in the presence of trimethoprim contained in co-trimazole tablets is described. The method is based on the formation of a complex of sulphamethoxazole with a known excess of silver ions and the titration of unreacted silver ion potentiometrically using an inexpensive lab-made copper based mercury film electrode (CBMFE). The titration conditions have been optimized for the determination of 1.0—10.0 mg of sulphamethoxazole in pure and dosage forms. The precision and accuracy of the method have been assessed by the application of lack of fit test and other statistical methods. Overall mean recovery and relative standard deviations obtained were 99.88% and 1.32% ($n=7$) respectively. No interference was caused by other excipients present in pharmaceutical dosage forms. The application of this method for sulphamethoxazole assay in the presence of trimethoprim in tablets was validated by the comparison of results obtained by the proposed method with that of the British Pharmacopoeia (BP) method using *F*- and *t*-statistical tests of significance.

Key words sulphamethoxazole; pharmaceutical preparation; indirect potentiometric titration; copper based mercury film electrode

Sulphamethoxazole [4-amino-*N*-(5-methyl-3-isoxazolyl)-benzene sulphonamide] is widely used in the prevention and cure of bacterial infections. Sulphamethoxazole is mostly marketed in combination with trimethoprim as a co-trimazole dosage form. Assay of sulphamethoxazole in the presence of trimethoprim has drawn considerable attention as evidenced by a number of methods reported, such as spectrophotometry,[1—4] capillary electrophoresis,[5] fluorimetry,[6] calorimetry,[7] nuclear magnetic resonance spectrometry[8] and chromatographic methods.[9] Sulphamethoxazole has also been determined by fourier transform infrared spectrometry,[10] atomic absorption spectrometry,[11] voltammetry,[12] oscillographic chronopotentiometry,[13] and visual titrimetry.[14—16] Some of these methods have shortcomings, like poor selectivity and accuracy, or they require expensive equipment. A number of potentiometric methods have been reported, because of their ease of application, simplicity, and speed of analysis.[17—23] Most potentiometric methods use a sulphamethoxazole-selective electrode or other ion-selective electrodes which are either expensive or not readily available in the market, or involve difficult methods of fabrication.

Fabrication and application of lab-made inexpensive copper based mercury film electrode (CBMFE) for the potentiometric determination of ascorbic acid and isoniazid have already been reported.[24,25] In the present communication, a simple and rapid method of indirect titration of sulphamethoxazole, using CBMFE as an indicator electrode, is reported. United States Pharmacopoeia,[26] European Pharmacopoeia,[27] American Pharmaceutical Association Foundation,[28] Indian Pharmacopoeia,[29] and British Pharmacopoeia[30] recommend direct titration of sulphamethoxazole with sodium nitrite in an acidic media at a low temperature. However, this method is not selective, and titrant stability is affected by acidity, temperature, dissolved oxygen and atmospheric carbon dioxide. The proposed method is based on treating sulphamethoxazole with a known excess of silver nitrate and titrating the unreacted silver nitrate against ammonium thiocyanate using CBMFE as an indicator electrode. The proposed method is found to be precise, accurate and sensitive.

Experimental

CBMFE was prepared using a commercially available plastic sleeved copper wire as described in our earlier communications.[24,25] About a 1.0 cm portion of one end of a wire, 1.0 mm in diameter, was polished with emery paper and cleaned with concentrated HNO_3, after epoxy seal was applied at the junction of the plastic sleeve. The polished wire was coated with mercury by dipping it in mercuric nitrate solution (0.02 M) for 10 min. The electrode surface was wiped with filter paper and then rinsed with water.

The titration cell was equipped with CBMFE as an indicator electrode and a double junction calomel electrode containing sodium nitrate solution (0.1 M) in the outer tube. A digital pH/mV meter (precision ± 1 mV) was used to measure the potentials. A mini magnetic stirrer was used to stir the solution.

Reagents All reagents were of analytical grade and deionised distilled water was used to prepare solutions.

Stock Solution of Sulphamethoxazole: This solution, containing 1.0—2.0 mg per milliliter, was prepared by dissolving the appropriate amount of sulphamethoxazole in a minimum quantity of 0.1 M NaOH, diluting to a constant volume and standardizing by biamperometric titration against sodium nitrite.[30]

Silver Nitrate Solution (0.1 M): This was prepared by dissolving 4.247 g of silver nitrate in 250 ml of water and stored in an amber colored bottle. It was potentiometrically standardized against sodium chloride.[31]

Ammonium Thiocyanate (0.1 M): 3.8 g of ammonium thiocyanate was dissolved in 500 ml of water and standardized potentiometrically by titrating with $AgNO_3$.[32]

Triethanolamine–Nitrate Buffer (pH 8.2): The pH of 0.2 M triethanolamine was adjusted to 8.2 by the addition of 0.05 M nitric acid.

Procedure An aliquot containing 1.0—10.0 mg of sulphamethoxazole was taken and the pH was adjusted to 8.2 by the addition of NaOH (0.1 M)/HNO_3 (0.1 M). One ml of triethanolamine–nitrate buffer (pH 8.2) was added followed by the addition of 1.0—5.0 ml of silver nitrate (0.1 M). A white precipitate of silver–sulphamethoxazole complex appeared shortly thereafter. The solution was diluted to 50 ml with water. The unreacted silver nitrate was titrated against 0.01—0.02 M NH_4SCN, using CBMFE as an indicator electrode. After each titration, the surface of the electrode was gently wiped with filter paper and rinsed with water. A freshly prepared CBMFE was used for 4—5 titrations. Then, the electrode was renovated by freshly coating it with mercury after cleaning the surface as described.

Procedure for Sulphamethoxazole Assay in Tablets Co-trimazole

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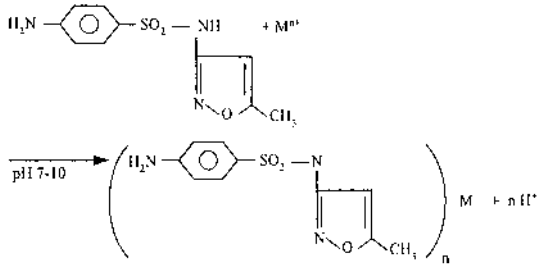
tablets containing 400 mg of sulphamethoxazole and 80 mg of trimethoprim as the active ingredient were taken for analysis. Twenty tablets containing sulphamethoxazole and trimethoprim were weighed and pulverised. An appropriate amount of the pulverised sample, equivalent to 500 mg of sulphamethoxazole, was stirred well with 60 ml of 0.5 M NaOH for half-an hour. The solution was quantitatively filtered into a 250 ml volumetric flask and the combined filterate and washings were diluted to the mark with water. Five milliliters of this solution was taken for analysis.

Results and Discussion

The acidic nature of the sulphonamide group ($–SO_2NH–$) of sulphamethoxazole helps it to form an insoluble complex with most of the metal ions such as Ag^+, Cu^{2+}, Hg^{2+} *etc*. The complex formation takes place through the displacement of a hydrogen atom of the sulphonamide group by these metal ions. For deprotonation of sulphamethoxazole, a slightly alkaline medium is needed.



where M is Ag^+, Cu^{2+}, Hg^{2+} *etc*.

In the present work, potentiotitrimetric determination of sulphamethoxazole, based on the complex formation of the drug with various metal ions, was investigated, using CBMFE as an indicator electrode. Although sulphamethoxazole forms an insoluble complex with various metal ions, the requirements for a successful potentiometric titration limits the number of metals as reagents for potentiometric titration. The attempts to titrate sulphamethoxazole directly with Hg^{2+}, Cu^{2+} and Ag^+ did not give satisfactory results, probably due to a slow reaction of the metal ion with the drug. It is clear that reactions suitable for use of titrimetric procedures must be stoichiometric and fast if a titration is to be carried out smoothly and quickly. In general, ionic reactions do proceed rapidly and present few problems. On the other hand, reactions involving covalent bond formations or rupture are frequently much slower, and a variety of practical procedures is used to overcome this difficulty. The most obvious ways of driving a reaction to quick completion are either to heat the solution, to use a catalyst, or to add an excess of the reagent. In the last case, a back titration of the excess reagent will be used to locate the stoichiometric point for the primary reaction. Further, the addition of a known excess of Hg^{2+} to sulphamethoxazole for precipitation and the back titration of surplus Hg^{2+} with EDTA also did not give satisfactory results, as the location of the end point was difficult to find due to the dissolution of the complex in the excess EDTA. The addition of a known excess of $AgNO_3$ to sulphamethoxazole for quantitative precipitation and back titration of surplus Ag^+ with NH_4SCN, without filtering the precipitate, gave reproducible results.

The determination of 1.0—10.0 mg of sulphamethoxazole was investigated by the back titration of surplus Ag^+ with NH_4SCN. The various parameters involved in the formation of a silver–sulphamethoxazole complex and the back titration of unreacted Ag^+ have been optimized. The exact stoichiometry of the silver–sulphamethoxazole complex reported by Hassan-Eldesowki is 1 : 1.[11] In order to fix the optimum pH for the analysis, the precipitations and titrations with 5.0 mg of sulphamethoxazole were carried out at various pH levels with appropriate buffers. The titration and precipitation in the pH range 4—6 with acetate buffered solution, and in the pH range 6.8 to 8.7 with triethanolamine–nitrate buffer, gave reproducible results. The complex was formed readily in the pH range 7.0—8.7 due to deprotonation of the acidic $–SO_2–NH–$ group and high solubility of sulphamethoxazole in this pH range. On the other hand, the complex formations were slow in the pH range 4—7 and it was necessary to warm the solution for 2—3 min at 50 °C for the completion of precipitation. One ml of triethanolamine–nitrate buffer (0.2 M) was sufficient to maintain pH in the range 6.8—8.7 and to prevent hydrolysis of surplus Ag^+ remaining after treating 1.0 to 10.0 mg of sulphamethoxazole with 1.0 to 5.0 ml of 0.01 M $AgNO_3$.

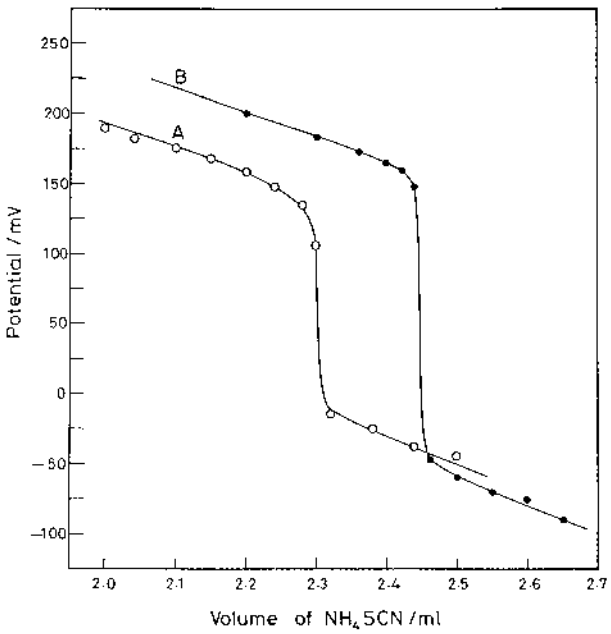


Fig. 1. Indirect Potentiometric Titration of Sulphamethoxazole

Curve A; 4.10 mg of sulphamethoxazole +4.0 ml of 0.01 M $AgNO_3$; back titration of surplus $AgNO_3$ with 0.01065 M NH_4SCN. Curve B; 7.17 mg of sulphamethoxazole +4.0 ml of 0.02 M $AgNO_3$; back titration of surplus $AgNO_3$ with 0.0212 M NH_4SCN.

During the titration, the stable equilibrium potential was established instantly by CBMFE with unreacted Ag^+ of concentration $<10^{-3}$ M. The titration curves for determination of sulphamethoxazole (4.10, 7.17 mg), when unreacted Ag^+ was titrated with 0.01 and 0.02 M NH_4SCN, are given in Fig. 1. A potential break of 200 mV was observed for the addition of 0.02 ml of 0.02 M NH_4SCN. The end point was located graphically from the first derivative of the titration curve.

Precision and Accuracy In order to assess the precision and accuracy of the proposed method, seven replicate determinations of sulphamethoxazole at eight concentration levels in the range 1.0—10.0 mg were carried out and results obtained are given in Table 1. The overall relative standard deviation for 56 determinations was 1.32% whereas mean standard analytical error was 0.0226. The overall mean recovery was 99.88%. The amount taken for analysis was compared with the mean of seven replicate determinations at each con-

Table 1. Results of Seven Replicate Analyses of Sulphamethoxazole and Statistical Treatment of Data

Sl. No.	Amount taken (μ) (mg)	Amount found (mg)	Mean ($\bar{x}$)±S.D. (s)	% Mean recovery	Std. analytical error ($s/\sqrt{n}$)	Student's-t $\frac{\mu-\bar{x}}{s/\sqrt{n}}$
1	1.02	1.04, 1.04, 1.02, 0.98, 0.98, 1.02, 1.04	1.02±0.0269	99.72	0.0102	0.218
2	2.05	2.09, 2.09, 2.09, 2.07, 2.01, 2.01, 2.07	2.06±0.0363	100.55	0.0137	0.834
3	4.10	4.12, 4.12, 4.06, 3.98, 4.14, 4.14, 4.06	4.09±0.0587	99.72	0.0222	0.514
4	5.12	5.06, 5.06, 5.11, 5.11, 5.16, 5.16, 5.08	5.11±0.0424	99.72	0.0160	0.899
5	6.14	6.07, 6.07, 6.07, 6.12, 6.12, 6.19, 6.19	6.12±0.0537	99.65	0.0203	1.056
6	7.17	7.21, 7.21, 7.21, 7.21, 7.30, 7.20, 7.09	7.20±0.0611	100.48	0.0231	1.486
7	8.19	8.16, 8.06, 8.06, 8.06, 8.16, 8.27, 8.27	8.15±0.0942	99.49	0.0356	1.163
8	10.24	10.26, 10.26, 10.21, 10.05, 10.05, 10.16, 10.32	10.19±0.1058	99.49	0.040	1.322
			Mean	99.88		

The calculated F-value in lack of fit test was 1.046.

Table 2. Comparison of Seven Replicate Determinations of Sulphamethoxazole in Co-trimazole Tablets by the Proposed Method and BP Method and Statistical Treatment of Data

Sl. No.	Brand name	Stated amount per tab (mg)	Amount found by proposed method (mean±S.D.)	Amount found by BP method	$F^{a)}$	$t^{a)}$
1	Septron	400	384.86±3.13	388.29±2.93	1.14	2.12
2	Bactrim	400	391.86±2.67	390.71±2.21	1.46	0.88
3	Ciplin	400	396.57±1.62	395.71±2.06	1.62	0.87
4	Oriprim	400	378.86±2.11	380.29±2.29	1.18	1.21

a) Calculated values of F and t for (6, 6) and 12 degrees of freedom at 5% level of significance.

centration level by Student's t-test. The calculated Student's t-value at each concentration level did not exceed 1.49; this was below the critical value of 2.45 at a 5% level of significance and six degrees of freedom, whereas mean Student t-value for the overall eight concentration levels was 0.943. It indicated the absence of any systematic error associated with the analysis.

Further, a lack of fit test[33,34] was performed to check whether the regression of the amount taken for analysis *vs*. the amount found by seven replications for the data given in Table 1 fit a linear model. The calculated F-value of 1.046, obtained in the lack of fit test, was less than the critical F-value of 2.30, for the 6, 48 degrees of freedom at a 5% level of significance. It indicated that the data fit a linear model with the absence of any systematic errors associated with the analysis.

Interference Study Five replicate analyses of 5.0 mg of sulphamethoxazole with each of 20 mg of usual excipients in the drug formulations such as starch, lactose, magnesium stearate and sucrose were performed, and no interference was observed due to their presence. In order to apply the proposed method for sulphamethoxazole assay in co-trimazole tablets containing trimethoprim in a 4 : 1 ratio, and to check the interference of trimethoprim, five replicate analyses were carried out with 8.0 mg of sulphamethoxazole in the presence of 2.0 to 6.0 mg of trimethoprim. The mean recovery of sulphamethoxazole was more than 98% in the presence of 6.0 mg of trimethoprim, although the end point was reduced with increasing amounts of trimethoprim, probably due to the tendency of trimethoprim to form a weak complex with Ag^+. The end point break for the addition of 0.02 ml of 0.02 M NH_4SCN was 110 mV in the presence of 6.0 mg of trimethoprim.

Validity of the Proposed Method. Determination of Sulphamethoxazole in Co-trimazole Tablets The proposed method was successfully applied for sulphamethoxazole assay in four commercially available co-trimazole tablets. The result of seven replicate determinations of sulphamethoxazole in pharmaceutical dosage forms by the proposed method, as well as by the BP method, are presented in Table 2. The BP method was biamperometric titration of sulphamethoxazole with 0.1 M $NaNO_2$ at a low temperature.[32] The variances of the two methods were compared by a two-tailed F-test. The calculated values of F were less than the critical value of 5.82 for 6, 6 degrees of freedom at a 5% level of significance, for all the pharmaceutical dosage forms analyzed. It indicated that the precision of the two methods did not differ significantly.

The two tailed t-test was applied to compare the amount found by seven replicate analyses by both methods. The calculated t-values for all the samples analyzed were less than the critical t-value of 2.18 at a 5% level of significance and 12 degrees of freedom, indicating that the results of the two methods did not differ significantly.

Conclusion

The proposed method is found to be very simple, precise and sensitive and can be carried out at room temperature. But the BP method is based on diazotization titration which requires maintaining a low temperature. Thus, the proposed method is a practical alternative to the BP method. The proposed potentiometric method can be applied to sulphamethoxazole assay in dosage forms with the advantage of sharp detection of the end point with a large potential break.

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