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Short Communication

Visible spectrophotometric determination of sulphonamides

F. A. MOHAMED, A. I. MOHAMED and S. R. EL-SHABOURI

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt

Abstract: A simple and rapid colorimetric method for the determination of 10 sulphonamides as single entities and sulphamethoxazole in combination with trimethoprim without prior separation was developed. The method is based on the reaction of sulphonamide with phenothiazine and *N*-bromosuccinimide at pH 6 to produce a blue coloured product after acidification. The chromogen for all the sulphonamides was measured at 605 nm. The effect of several variables on colour development (concentration of phenothiazine and *N*-bromosuccinimide, time, pH) were established. Beer's Law was obeyed for all the drugs. The method was successfully applied to the analysis of single component sulphonamide tablets and ophthalmic solutions, with average results of labelled claim of 96.7 ± 0.95 to 100.67 ± 1.2. A good correlation was observed between molar absorptivities and pK_a values of the sulphonamides (r = 0.9005).

Keywords: *Spectrophotometry; sulphonamides; N-bromosuccinimide; phenothiazine; pharmaceuticals.*

Introduction

Several methods have been reported for the determination of sulphonamides in common use. These methods include non-aqueous titration [1, 2], nitite [3, 4], bromometric [5], amperometric [6], complexometric [7] and argentimetric [8] titration, polarography [9], chromatography [10–12] and spectrophotometry [13–17].

Recently in this laboratory, primary aromatic amines and *N*-bromosuccinimide (NBS) were found to react with phenothiazine in acidic solution to produce a blue colour. This reaction has been used for the detection [18] and determination [19] of some phenothiazine drugs. In the present work, the same reaction has been used for the quantitative determination of some sulphonamide drugs using phenothiazine and *N*-bromosuccinimide as reagents.

Experimental

Apparatus

A Zeiss spectrophotometer PM2DL (Zeiss, Oberkochen, West Germany) and a Unicam Sp 1750 UV-VIS spectrophotometer (Pye-Unicam Cambridge, U.K.) were used.

Materials

All the sulphonamide drugs were donated by Bayer, Gilac, CIBA, Geigy and Nordmark and were used without further purification. *p*-Toluene sulphonamide was prepared according to a reported procedure [20]. Pharmaceutical preparations were obtained from the local market. All reagents and solvents were of analytical grade. Deionized water was used throughout the work.

Standard solutions

Stock solutions of all the sulphonamide drugs were prepared at a concentration of 1 mg ml^{-1} in methanol. Further dilutions with methanol were made to give drug concentrations of 10–200 $\mu g\ ml^{-1}$.

Buffer solution

Clark and Lubs [21] buffer solutions (pH 5.8–8.0 and pH 4–6) were prepared for studying the pH-dependence of the colour intensity.

Procedure

To obtain the spectra and the relevant calibration curves, an accurately measured 0.5 ml volume of sulphonamide drug solution (4–400 $\mu g\ ml^{-1}$) was mixed with 1 ml of phenothiazine solution (0.04% w/v phenothiazine in methanol) in a 10-ml volumetric flask. One millilitre of buffer solution pH 6, 1 ml of NBS solution (0.05% w/v NBS in deionized water) and 1 ml of 0.2 M hydrochloric acid were added, then the solution was diluted to 10 ml with methanol. The absorbance was measured at 605 nm against a reagent blank prepared similarly.

Application to pharmaceutical preparations

Tablets. Ten tablets were weighed accurately and powdered in a mortar. An amount corresponding to 50 mg of sulphonamide drug was transferred to a 50-ml volumetric flask and diluted with 30 ml of methanol. The suspension was thoroughly stirred and warmed in a water-bath if necessary, cooled, diluted to 50 ml with methanol and filtered. The first portion of the filtrate was rejected, and 5 ml of the subsequent filtrate were diluted to 50 ml with methanol. An accurately measured 0.5-ml volume of this solution was used for reaction as described in the procedure.

Determination of sulphacetamide sodium in sulphacetamide sodium eye drops

Five millilitres of the eye-drops, corresponding to 1 g of sulphacetamide sodium, were transferred to a 100-ml volumetric flask then diluted to volume with methanol. Five millilitres of the solution were diluted to 50 ml with methanol in a volumetric flask and 5 ml of this solution were further diluted to 50 ml with methanol. An accurately measured 0.5-ml volume of this solution was used for the assay as described in the procedure.

Results and Discussion

Ten sulphonamides with different substituents, viz., sulphathiazole, sulphamoxole, sulphacetamide, sulphadiazine, sulphasomidine, sulphamethazine, sulphamethoxydiazine, phenazasulphamethoxypyridine, sulphaphenazole and sulphamethoxazole were found to react with phenothiazine and *N*-bromosuccinimide (NBS) to produce a blue

colour upon acidification. The absorption spectra of the reaction products of all the drugs exhibit two wavelengths of maximum absorption (λ_{max}) at 440 and 605 nm with different absorptivities. Figure 1 shows the absorption spectra of sulphacetamide, sulphamethoxazole and sulphadiazine as examples. Measurements of absorbance was made at 605 nm as all the sulpha drugs exhibited higher absorptivities at this wavelength.

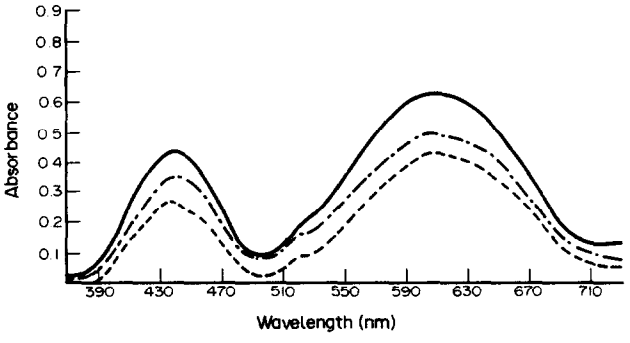


Figure 1

Optimization of variables

The effect of variation of the concentration of phenothiazine was studied. The highest and most reproducible absorbances were obtained by using 1 ml of 0.4 mg ml^{-1} phenothiazine solution in methanol (Fig. 2).

The concentration of *N*-bromosuccinimide was found to affect markedly the colour intensity. Figure 3 illustrates the effect of NBS concentration on the absorbance of the reaction products of sulphacetamide. The optimum concentration of NBS was found to be given by 1 ml of 0.5 mg ml^{-1} solution.

To investigate the effect of pH, the reaction was carried out in acid, alkali and buffer solutions. It was found that the addition of acids, such as hydrochloric acid, before the reagents produced high blank values. This could be attributed to the oxidation of phenothiazine which occurs in acidic medium. No colour was produced upon the addition of alkali. The influence of buffer on the colour development was also studied. One millilitre of phosphate buffer (pH 5.7–8.0) was added to the sample solution and the reaction was carried out as described in the experimental section. Maximum colour intensity was obtained at pH 6 (Table 1). Phthalate buffer of pH 6 [21] also gave the same absorbance indicating that the buffer constituents do not affect the colour development.

The reaction of sulphonamides with phenothiazine and NBS reagents has been shown to be dependent on the order of the addition of reagents to the sample solution. It was found that the use of the following order; sample solution, buffer, phenothiazine solution and NBS solution produced a pink colour (λ_{max} 520 nm). Addition of alkali such as 0.1 M sodium hydroxide to the final reaction mixture produced no effect on the intensity of absorption or position of the λ_{max}, whereas addition of acid gave a blue colour (λ_{max} 605 nm) accompanied by a marked increase in the intensity of absorption and complete decolourization of the blank. Table 2 shows the effect of the addition of 1 ml of various concentrations of hydrochloric acid. The highest and most reproducible colour intensities were obtained by using 1 ml of 0.2 M acid. Therefore, this concentration was used throughout the work. Other acids, such as sulphuric acid and nitric acid, gave similar results and acetic acid produced lower values.

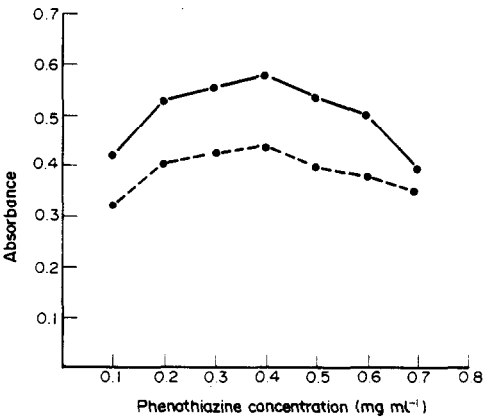


Figure 2

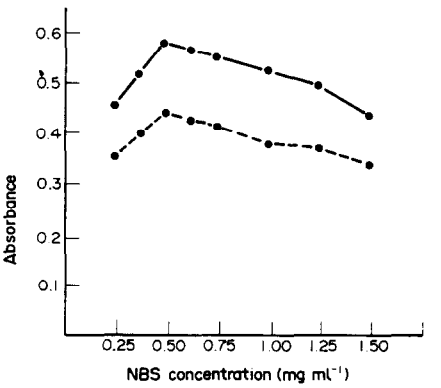


Figure 3

Table 1
Effect of pH on the absorbance of the reaction product of sulphacetamide* and phenothiazine-NBS reagents

pH	Absorbance† at 605 nm
5.7	0.501
6.0	0.580
6.6	0.459
7.0	0.356
8.0	0.096

*Final sulphacetamide concentration 5 μg ml^{-1}.
†Average of 4 determinations.

The colour was produced at room temperature and consequently there was no need to heat the reaction mixture. The reaction time was determined by following the development of colour at 5, 10, 15 . . . 60 min. Maximum absorption was obtained immediately. No change of absorbance was observed within 1 h, and only a 10% decrease in absorbance was observed after 6 h, indicating the high stability of the chromogen.

Table 2
Effect of different concentrations of hydrochloric acid

Concentration of HCl molarity	Absorbance at 605 nm
0.1	0.563
0.2	0.580
0.5	0.564
1.0	0.560
2.0	0.543
3.0	0.523
10.0	0.441

Dilution of the blue coloured reaction products of all the sulphonamide drugs by different solvents produced no effect on the position of the λ_{max} values while the intensity of absorption was only slightly affected. Table 3 shows that acetonitrile, methanol or ethanol can be used as diluting solvents. Methanol was used for dilution throughout this work.

Under these optimum conditions Beer's Law was obeyed over a very wide range of concentrations of all the sulphonamide drugs studied (Table 4).

The sensitivity of the assay, expressed as the molar absorptivity (ϵ values) varied with the sulphonamide and was found to be dependent upon its pK_a value. Statistical analysis of the relationship between pK_a and ϵ by the method of least squares produced a straight line expressed as follows:

$$\epsilon = a + b\ pK_a$$

$$\epsilon = 4.41 \times 10^4 - 2.94 \times 10^3\ pK_a\ (n = 10;\ r = 0.9005).$$

It was concluded from this relationship and the results in Table 4 show that drugs with small pK_a values give high ϵ values and vice versa with the exception of sulphamethoxydiazine.

Analysis of dosage forms

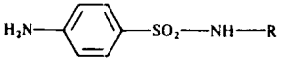
The developed method was applied to the determination of some sulphonamides in various dosage forms without prior separation. Recovery experiments were carried out

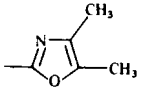
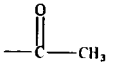
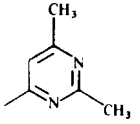
Table 3
Effect of variation of solvent on the absorbance at 605 nm

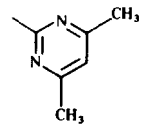
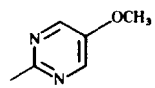
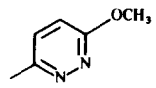
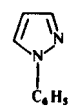
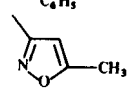
Solvent	Absorbance*
Water	0.542
0.2 M HCl	0.533
Methanol	0.580
Ethanol	0.575
Isopropyl alcohol	0.542
Acetonitrile	0.650
Acetone	0.547
Dimethylsulphoxide	0.500
Dioxane	0.547

*5 $\mu g\ ml^{-1}$ sulphacetamide was used. Average of 3 determinations.

Table 4
Statistical data and absorption characteristics of the sulphonamides



Drug	R	pK_2 [22]	ϵ_{max} at 605 nm	Calibration range $\mu g\ ml^{-1}$	Intercept	Slope	Correlation coefficient
Sulphathiazole		7.25	2.37×10^4	0.5–10	0.0046	0.0853	0.9986
Sulphaxomole		7.4	2.12×10^4	0.4–20	0.0370	0.0670	0.9983
Sulphacetamide		5.4	2.78×10^4	0.2–10	0.0805	0.0997	0.9966
Sulphadiazine		6.52	2.51×10^4	0.3–15	0.0643	0.0804	0.9975
Sulphasomidine		7.4	2.14×10^4	0.4–15	0.0352	0.0695	0.9957

Sulphamethazine		7.4	2.13×10^4	0.5–15	0.0038	0.0712	0.9963
Sulphamethoxydiazine		7.0	2.51×10^4	0.5–15	0.0149	0.0808	0.9992
Sulphamethoxypyridazine		7.2	2.45×10^4	0.3–15	0.0178	0.0786	0.9935
Sulphaphenazole		6.09	2.58×10^4	0.5–15	0.0385	0.0699	0.9977
Sulphamethoxazole		6.0	2.67×10^4	0.3–12	0.0604	0.0866	0.9978

for each drug in its respective formulation. The excellent recoveries indicate the absence of interference from frequently encountered excipients or additives such as lactose, gum tragacanth, starch, etc. (Table 5).

Analysis of sulphamethoxazole in mixture with trimethoprim

Trimethoprim is usually combined with sulphamethoxazole in tablet form and the assay of the binary mixture may require prior separation. Under the conditions of the proposed procedure trimethoprim does not react with phenothiazine and NBS reagents. This allows the analysis of sulphamethoxazole in mixture with trimethoprim. To check the selectivity, a mixture of trimethoprim and sulphamethoxazole in the ratio 1:5, which is the ratio usually found in official formulations, was prepared and analyzed by the proposed method. Excellent recoveries of sulphamethoxazole from the binary mixture and tablets were obtained (Table 6) and this confirms the absence of interference of trimethoprim in the procedure.

Table 5
Assay of dosage forms using the proposed method and the Bratton–Marshall method

Drug and formulation	Claimed amount	% Found ± S.D. (n = 6) Proposed method	Bratton–Marshall method [13]
Sulphadiazine Sulphadiazine tablets	0.5 g/tablet	98.36 ± 0.71	97.17 ± 1.05
Sulphaphenazole Neosulpha tablets	0.5 g/tablet	100.79 ± 0.92	102.70 ± 0.98
Sulphamethoxydiazine Longactine tablets	0.5 g/tablet	99.52 ± 1.01	100.12 ± 1.03
Sulphacetamide sodium Sulphacetamide sodium eye drops	20% solution	97.71 ± 0.85	96.38 ± 1.10
Sulphamethoxazole Septazole tablets	0.5 g/tablet	99.33 ± 0.88	98.50 ± 0.91

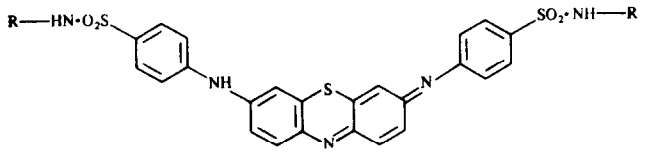
Table 6
Analysis of sulphamethoxazole in the presence of trimethoprim

Preparation	Claimed amount	% Found	S.D. (n = 6)
Synthetic mixture*	400 mg	99.17	0.83
Bactrim tablets	400 mg/tablet	100.89	0.76

*Mixture of sulphamethoxazole and trimethoprim (5:1).

Investigation of the reaction mechanism

A suggestion for the mechanism of the reaction is as follows: phenothiazine is oxidized by NBS to the phenazathionium cation which then undergoes a nucleophilic attack with sulphonamide at the 3- and/or 7-positions to produce a dye similar in structure to methylene blue.



Scheme 1

In order to investigate whether the attack of a sulphonamide is through the $—SO_2NH—$ or $—NH_2$ function, four compounds viz. aniline, sulphanilic acid, sulphanilamide and *p*-toluene sulphonamide, were analyzed by the proposed procedure. It was found that aniline, sulphanilic acid and sulphanilamide produced a red colour (λ_{max} 520 nm) which changes to blue (λ_{max} 605 nm) upon addition of 1 ml of 0.2 M hydrochloric acid with apparent molar absorptivities of 1.2×10^4, 1.9×10^4 and 2.9×10^4, respectively. In contrast, *p*-toluene sulphonamide gave a violet colour (λ_{max} 565 nm, $\epsilon_{max} = 5.5 \times 10^3$) which was not affected by the addition of acids.

From these observations it is concluded that the nucleophilic attack on the phenazathionium cation by sulphonamide is likely to occur through the aromatic primary amine function. The nucelophilicity of this function is affected by the pK_a value of the $—SO_2NH$-R function and this affects the intensity of the colour produced, as discussed above.

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