New Application of Chromotropic Acid for the Determination of Some Sulphonamides in their Pharmaceutical Preparations

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Abstract

The formation of the azo-dye using chromotropic acid as a coupling agent was applied to the determination of five sulphonamides. The spectrophotometric studies as well as microanalysis of the studied sulphonamides- chromotropic acid azo dyes revealed the existence of the 1:1 coupling product. The absorbance of the formed azo dye is measured at 510 nm, and has a large molar absorptivity ($\varepsilon = 2.87 - 3.29 \times 10^4$ 1 mol^{-1}cm^{-1}). Beer's law was obeyed over the concentration range 0.5-9.0 µg ml^{-1}. The assay results of pharmaceutical formulations showed good accuracy and precision over the concentration range used.
The proposed method avoids time-consumption, extraction process and temperature control.

Keywords: Sulphonamides; Chromotropic acid; Azo dyes; Spectrophotometry.

Introduction

Sulphonamides are widely used in the treatment of urinary tract infections, burn therapy, conjunctivitis and chloroquine resistant malaria. Several procedures have been proposed for the identification and determination of sulphonamide compounds[1-4]. Methods of analysis are based upon the properties conferred upon the molecule by the presence of the primary aromatic amino group or the acidic hydrogen in the –SO$_2$- NH- group in the molecule[5-8]. Current methods for the determination of sulphonamides include titrimetric[9,10], ultraviolet and visible spectrophotometric[11,12] and chromatographic[13] techniques.

The objective of the investigation reported in this paper - was to present a new spectrophotometric method for the determination of some sulphonamides in some pharmaceutical preparations, based on the diazotization of the sulphonamides with sodium nitrite in hydrochloric acid, the diazonium salt was coupled with chromotropic acid.

Experimental
Reagents

All reagents were of analytical-reagent grade, water used in this investigation was always twice distilled water.
Caution: All the transfers of solutions should be made with an automatic pipette

Standard nitrite solution (1%): A 1.0 g amount of sodium nitrite is dissolved in water and diluted to 100 ml with water in a calibrated flask.

Chromotropic acid (CA) reagent 10^{-2} M: Dissolve 0.4 g of chromotropic acid (4,5-dihydroxynaphthalene-2,7 disulphonic acid disodium salt dihydrate) in water and diluted to 100 ml. The solution was stored in a brown bottle in a refrigerator.

Standard sulphonamide solutions 10^{-3} M: Sulphonamides obtained from Elnasr Pharmaceutical Chemicals (Cairo, Egypt), were used to prepare the stock solution by dissolving an appropriate amount of drug in 10 ml of 0.3 M HCl then diluted to 100 ml with water. Working standard solutions containing 50 µgml^{-1} for each sulpha were prepared daily by accurate dilution.

Recommended Procedure

Prepare a calibration curve by transferring volumes containing 25-250 µg of standard sulphonamide solution to 25-ml standard measuring flasks, followed by 0.8 ml of hydrochloric acid (0.5 M) and 0.5 ml of 1% sodium nitrite solutions. Swirl and allow to stand for 5 min. Add 0.8 ml chromotropic acid (CA), 0.8 ml of sodium carbonate (6%) solution, swirl again, dilute to the mark and allow to stand for 15 min. Measure the absorbance against a reagent blank at 510 nm. Plot the absorbance against µg of sulphonamide per ml.

Procedures for Pharmaceutical Preparations

Tablets: An accurately weighed amount of powdered tablets, equivalent to 100 mg of the sulphonamide was transferred to 100 ml beaker, stirred with 10 ml of 0.3 M HCl for 5 min, filter the mixture into 100- ml calibrated measuring flask, wash the residue several times with water and dilute to volume with water. Use 2.5 ml of this solution and dilute to 50 ml with water and proceed as given in the general procedure.

Cream: Dissolve accurately 1.429 g of the cream equivalent to 10 mg sulphadiazine in 5 ml of 0.3 M HCl, shake for 5 min, filter the mixture to get rid of the precipitated AgCl, wash the residue then dilute the volume with water to 100 ml. 50 µgml^{-1} solution was prepared then apply general procedure.

Ampoules: A volume of sulphonamide sodium solution, equivalent to 10 mg of sulphadimidine was diluted with 5 ml of 0.3 M HCl and made up to 100 ml with water in a calibrated flask, 50 µg ml^{-1} was prepared and then subjected to analysis by the recommended procedure.

Apparatus

A Shimadzu 1601 double –beam spectrophotometer with quartz cells of 1-cm optical path length was used.

Results and discussion

It has been found that the chromotropic acid azo dyes of sulphonamides absorb maximally at 510 nm (Fig.1). The bluish red colour produced from this reaction is thus suggested for the

colorimetric determination of these drugs. Attempting to obtain the most intense and stable colour, the variables of the experimental conditions were critically investigated as follows:
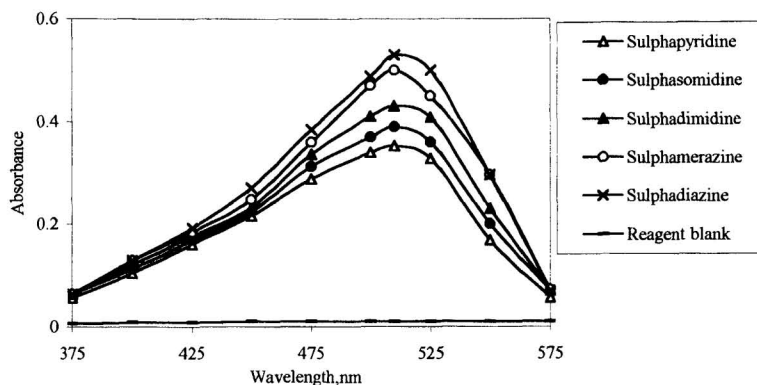

Fig.1. Absorption spectra of sulphonamideazo-CA dyes

Effect of coupling agent concentration:

When various concentrations of CA solution were added to a fixed concentration of the sulphonamide, 0.8 ml of 10^{-2}M CA solution was enough to develop the colour to its full intensity. There was no change in the absorbance at higher concentrations as shown in Fig.(2).
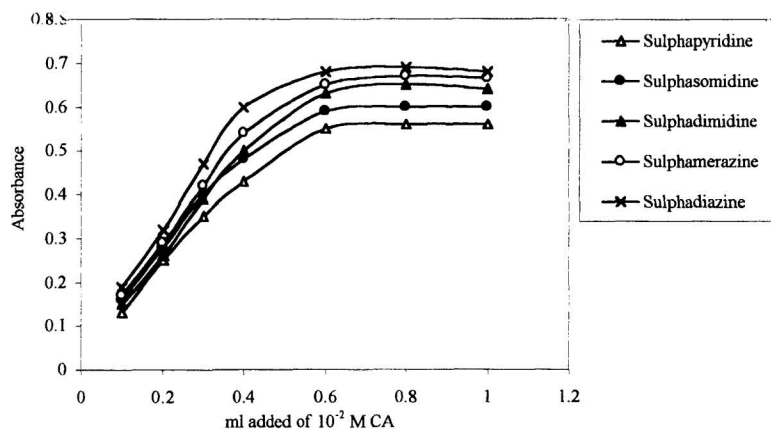

Fig.2. Effect of chromotropic acid cocentration on the determination of sulphonamides, [sulpha] = 2x10-5, λ = 510 nm.

Effect of pH:

On investigating the influence of pH on the formation of sulphonamideazo-CA dye, it is found that the colour of the compound in solution is evidently influenced by the pH. The intensity of the coloured species increase with decrease of $[H^+]$ until a maximum value is reached at pH 6.8-7.4.

Fig.(3) shows that the absorbance of the dye tends to increase with increasing the concentration of sodium carbonate solution until it attains a plateau at 0.5-1.0 ml of 10% Na_2CO_3, corresponding to pH 6.8-7.4.

The acidity necessary for full colour development was also studied and it was found that the colour started to develop at an acidity of about 0.6 to 0.8 ml of 0.5 M HCl. There was a decrease in absorbance at higher concentrations.
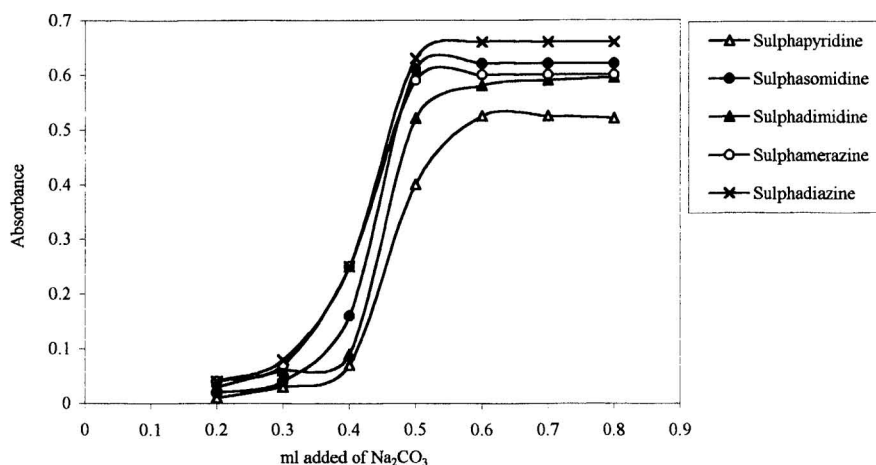

Fig.3. Effect of sodium carbonate concentration on the determination of
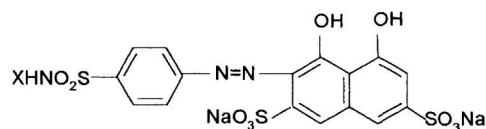sulphonamides,
[sulpha] = 2x10-5, λ = 510 nm.

Effect of temperature and stability of the dye:

The effect of temperature on the colour intensity was studied. It was found that the same absorbance was obtained when the colour was developed either at 20 or 25°C. Therefore, it is recommended that the reaction is carried out at room temperature.
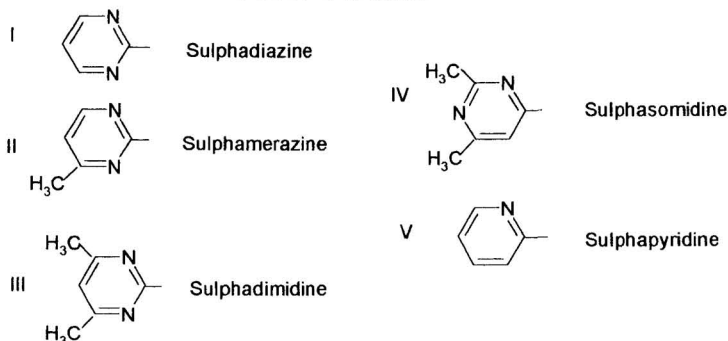
Under the conditions described above, the reaction mixture has to stand for 25 min to allow full azo dye formation, after which the colour remains stable for at least 10 h.

Structure of the dyes:

The stoichiometry of the reaction between CA and the diazonium slat derived from sulphonamides was investigated using the molar ratio[14] and continuous variation[15] methods. The results showed that the dyes have the composition 1:1 (sulphonamide : CA) Indicating mono azo dye. Therefore, the structure of the dye can be written as follows:

Where X is as follows

I   Sulphadiazine

II   Sulphamerazine

III Sulphadimidine

IV   Sulphasomidine

V   Sulphapyridine

The conditional formation constants of the dyes in aqueous solution under the experimental conditions (log K_f) are within the range 11.09-11.46.

Analytical Parameters:

Under the experimental conditions described, standard calibration curves for diazotized sulphonamides with CA were constructed by plotting absorbance versus concentration of sulphonamides. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table (1).

Table(1)   Analytical parameters for some sulphonamideazo- Chromotropic acid dyes

Parameters	Sulpha				
	Diazine	Merazine	Dimidine	Somidine	Pyridine
λ_{max} (nm)	510	510	510	510	510
Beer's law limits (μg ml^{-1})	0.5-7.5	0.5-8.5	0.5-8.5	0.5-9.0	0.5-9.0
Molar absorptivity (l mol^{-1} cm^{-1})	3.15×10^4	3.20×10^4	3.29×10^4	2.96×10^4	2.87×10^4
Sandell sensitivity (μg cm^{-2})	7.95×10^{-3}	8.39×10^{-3}	8.42×10^{-3}	8.83×10^{-3}	9.23×10^{-3}
Slope (specific absorptivity)	0.123	0.125	0.120	0.110	0.111
Intercepte	0.009	-0.032	0.002	0.006	- 0.007
Corrélation coefficient (r)	0.9999	0.9988	0.9998	0.9986	0.9983
Relative standard déviation (RSD% n=7)	0.93	1.19	0.42	1.34	1.42
Ringbom optimum concentration range (μg ml^{-1})	1.41-7.08	1.58-7.08	1.58-7.08	1.58-6.30	1.43-6.31
LOD (μg ml^{-1})	0.15	0.19	0.07	0.24	0.13

The molar absorptivity, the Sandell sensitivity and the linear regression equation for each sulpha are listed also in the same Table. The correlation coefficients were 0.9981-0.9999; indicating good linearity. For more accurate results, Rihgbom' plots[16] for optimum concentration ranges were obtained.

Five replicate determinations at different concentration levels were carried out to test the precision of the method (Table 2). The relative standard deviations were less than 1.5%, indicating reasonable repeatability of the selected method.

Table(2): Tests of precision of the method on pure sulphonamides.

Sulpha	Found ± SD (ug ml^{-1})	Found ± SD (ug ml^{-1})	RSD %	Standard Error	Confidence Limits (P=0.05)
Diazine	2	2.09 ± 0.02	0.813	0.007	2.09 ± 0.02
	4	3.93 ± 0.03	0.611	0.011	3.93 ± 0.03
	6	0.15 ± 0.04	0.618	0.017	6.15 ± 0.05
Dimidine	2	1.98 ± 0.03	1.311	0.012	1.98 ± 0.05
	4	4.07 ± 0.01	0.280	0.005	4.07 ± 0.01
	6	6.07 ± 0.02	0.264	0.009	6.07 ± 0.03
Merazine	2	1.93 ± 0.02	1.119	0.009	1.93 ± 0.03
	4	4.08 ± 0.06	1.470	0.027	4.08 ± 0.07
	6	5.94 ± 0.05	0.842	0.022	5.94 ± 0.06
Pyridine	2	2.04 ± 0.02	0.883	0.008	2.04 ± 0.02
	4	3.91 ± 0.05	1.280	0.022	3.91 ± 0.06
	6	5.96 ± 0.07	1.170	0.030	5.96 ± 0.09
Somidine	2	2.08 ± 0.01	0.208	0.002	2.080 ± 0.01
	4	4.13 ± 0.02	0.426	0.008	4.13 ± 0.02
	6	6.11 ± 0.02	0.393	0.009	6.11 ± 0.03

n=5

The applicability of the method to the determination of sulphonamides in pharmaceutical preparations was examined. The results are presented in Table (3).

Table (3): Determination of the cited drugs in commercial samples

Sample	Label claim	Taken (µg)	% found ± SD[*]	
			Proposed method	USP method[16]
Sulphadiazine tablets[a]	0.5 g / tab.	2	98.0 ± 0.03	99.2 ± 0.03
		4	99.1 ± 0.05	99.3 ± 0.09
		6	101.5 ± 0.90	100.5 ± 0.10
Argiderm cream[b] (sulphadiazine)	0.7 g / 100 g	2	97.4 ± 0.04	98.5 ± 0.10
		4	100.6 ± 0.09	99.9 ± 0.12
		6	99.9 ± 0.07	99.7 ± 0.09
Sulphadimidine sodium vial[c]	33.3 g / 100 ml	2	102.0 ± 0.08	100.0 ± 0.06
		4	98.3 ± 0.10	97.8 ± 0.13
		6	100.9± 0.11	99.5 ± 0.11

[*] Standard deviation (n=5)
[a] Sulphadiazine tablets contain o.5 g per tablet, (El Nasr Co.)
[b] Argiderm cream each 100 g contain 1 g silver sulphadiazine, (Amoun Pharmaceutical Industries Co. (APIC) S.A.E. El Salam City, Cairo, Egypt).

^eSulphadimidine sodium 33.3 % (vial) for veterinary use, (The Alexandria Co. for Pharmaceutical and chemical industries, Alexandria, Egypt).

No significant interference is noticed from the excipients commonly used in pharmaceutical preparations. The results obtained are in good agreement with those obtained by the BP and USP[9,10] methods. The proposed method is simple accurate as well as sensitive.

Conclusion

A simple and sensitive spectrophotometric method for the trace determination of some sulphonamides in aqueous solution has been devised and can be easily used for the routine analysis of drug alone and in the pharmaceutical preparations, and neither temperature control nor solvent extraction is required.

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