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Use of *p*-benzoquinone for the spectrophotometric determination of certain sulphonamides

ABDEL-MABOUD I. MOHAMED,* HASSAN F. ASKAL and GAMAL A. SALEH

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

Abstract: A simple spectrophotometric method for the determination of 15 sulphonamides in bulk and in dosage forms is described. The method is based on the interaction of *p*-benzoquinone with sulphonamides in 0.1 M hydrochloric acid. The resulting chromophore is measured at 500 nm. The effects of different variables on colour development were established. Beer's law was obeyed in a concentration range of 10–50 μg ml^{-1}. Results from the analysis of different sulphonamide tablets and ophthalmic solutions marketed locally were in good agreement with that of a reference method. Correlations between $A^{1\%}_{1cm}$ and certain physical parameters such as pK_a values, characteristic volume *Vx*, and molecular connectivity indices ^1X and $^1X^v$ were determined by linear regression equations. A poor correlation was found between $A^{1\%}_{1cm}$ and bulkiness parameters but a highly significant negative correlation was obtained with apparent pK_a values.

Keywords: *Sulphonamides; 1,4-benzoquinone; trimethoprim co-formulations; spectrophotometry; pharmaceuticals.*

Introduction

Pharmacopoeial methods [1, 2] for the determination of most sulphonamide drugs and their dosage forms usually involve titrimetric procedures with sodium nitrite. Other analytical methods for the determination of sulphonamides include titrimetry [3–5], polarography [6], chromatography [7] and spectrophotometry [8–15].

Interactions of amine compounds with several *p*-benzoquinones have been used as the basis of the spectrophotometric determination of many amine-containing medicinal compounds [16–19].

This work describes the determination of 15 sulphonamides in bulk and in pharmaceutical preparations. The proposed method involves the use of *p*-benzoquinone as the chromogenic reagent.

Experimental

Apparatus

Measurements were made with a Uvidec-320 spectrophotometer (JASCO, Tokyo, Japan) with a matched pair of 1-cm glass cells.

Materials

Pharmaceutical grade sulphonamides were obtained as gifts from Bayer (Leverkusen, FRG), Gilac, Ciba–Geigy (Basel, Switzerland) and Nordmark (Stockholm, Sweden) and were used as working standards without further treatment. *p*-Benzoquinone was prepared and purified according to a conventional method [20]. All other reagents and solvents were of analytical grade. Commercial dosage forms were purchased from local sources.

p-*Benzoquinone solution*. A 2% *p*-benzoquinone solution in ethanol was freshly prepared daily.

Standard sulphonamide solutions. Stock solutions of all sulphonamides were prepared at a concentration of 1 mg ml^{-1} in ethanol. Further dilutions with ethanol were made to give drug concentrations of 100–500 μg ml^{-1}.

Procedure

A 1.00 ml volume of sulphonamide solution (100–500 μg ml^{-1}) was transferred by pipette into a 20-ml test-tube containing 1 ml of *p*-benzoquinone solution and 1 ml of 0.1 M hydrochloric acid. The mixture was placed for about 10 min in a thermostatically controlled water-bath at 90°C. The solution was cooled, transferred quantitatively to a 10-ml standard flask and diluted to volume with water. The absorbance was measured at 500 nm in 1-cm cells against a reagent blank prepared in a similar way.

* Author to whom correspondence should be addressed.

Analysis of pharmaceutical preparations. Tablets. Twenty tablets were weighed accurately and powdered. An amount corresponding to 50 mg of sulphonamide was transferred to a 100-ml volumetric flask and diluted with 70 ml of ethanol. The suspension was thoroughly stirred for 10 min, warmed in a water-bath if necessary, cooled, diluted to 100 ml with ethanol and filtered. The first portion of the filtrate was rejected and 1.00 ml of the subsequent filtrate was used for reaction as described in the procedure.

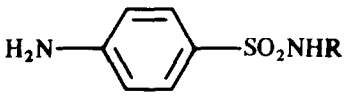
Eye drops. Five millilitres of the eye-drops equivalent to 1 g of sulphacetamide sodium was transferred to a 100-ml volumetric flask and diluted to volume with ethanol. A 5-ml portion of this solution was diluted to 100 ml with ethanol in a volumetric flask to obtain a sample concentration of 500 μg ml^{-1}. A 1.00 ml volume of this solution was used for the assay as described in the procedure.

Calculations

Characteristic volume Vx (cm^3 mol^{-1}). The characteristic volume for the molecule was calculated according to McGowan *et al.* [21].

Molecular connectivity indices, ^1X *and* $^1X^v$. ^1X describes the volume of the bonding atom; $^1X^v$ describes the volume and electronegativity of the bonding atom. The indices were calculated for the side chain-R (Table 1) according to Kier *et al.* [22, 23].

Table 1
The sulphonamides studied, their $A^{1\%}_{1cm}$, pK_a and the bulkiness parameters *Vx*, ^1X and $^1X^v$



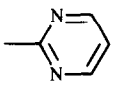
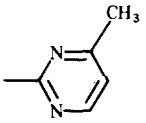
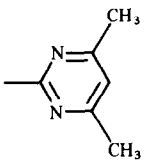
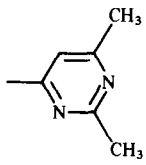
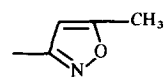
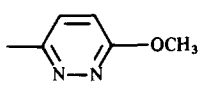
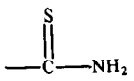
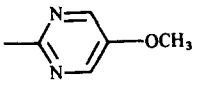
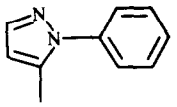
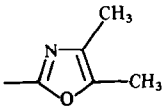
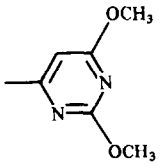
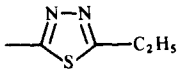
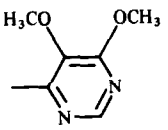
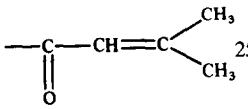
Drug	R	$A^{1\%}_{1cm}$	pK_{a_1}*	pK_{a_2}	*Vx* (cm mol^{-1})	^1X for −R	$^1X^v$ for −R
Sulphacetamide	$-COCH_3$	220	1.78	5.38*	118.75	1.1547	0.7041
Sulphadiazine		202	2.00	6.48*	172.25	2.8165	1.6303
Sulphamerazine		179	2.26	7.06*	186.34	3.2104	2.0510
Sulphadimidine		142	2.26	7.4†	200.40	3.6042	2.4718
Sulphasomidine		137	2.36	7.5*	200.40	3.6042	2.4718
Sulphamethoxazole		217	1.76	5.8*	136.17	2.7104	1.1877
Sulphamethoxypyridazine		182	2.06	7.0*	192.21	3.7484	2.1703

Table 1
(Continued)

Drug	R	$A^{1\%}_{1cm}$	pK_{a_1}*	pK_{a_2}	Vx (cm mol^{-1})	^1X for −R	$^1X^v$ for −R
Sulphathiourea		216	—	4.8‡	155.80	1.1547	0.8033
Sulphamethoxydiazine		172	2.00	7.0‡	192.21	3.7484	2.1533
Sulphaphenazole		217	1.90	5.9‡	223.24	5.2994	3.4381
Sulphamoxole		149	—	7.4‡	186.53	3.1210	2.1054
Sulphadimethoxine		205	2.02	6.1‡	212.17	4.6802	2.6965
Sulphaethidole		235	1.93	5.6*	192.90	3.2483	2.6336
Sulphadoxine		202	2.02§	6.1‡	212.17	4.7139	2.6883
Sulphadicramide		253	—	5.4‡	194.10	2.5486	1.7815

*Ref. 25.
†Ref. 26.
‡Ref. 27.
§Ref. 28.

Results and Discussion

The method involves the reaction of sulphonamides with *p*-benzoquinone in mild acidic conditions to produce red products with maximum absorption at 325 and 500 nm (Fig. 1). Measurement of absorbance was made at 500 nm as all the sulpha drugs exhibited higher absorptivities at this wave-length and the reagent blank had negligible absorbance when measured against pure solvent.

Optimization of variables

The factors affecting colour development, reproducibility, sensitivity and conformity with Beer's law were investigated with sulphacetamide sodium as a model compound since the other sulphonamides behave similarly.

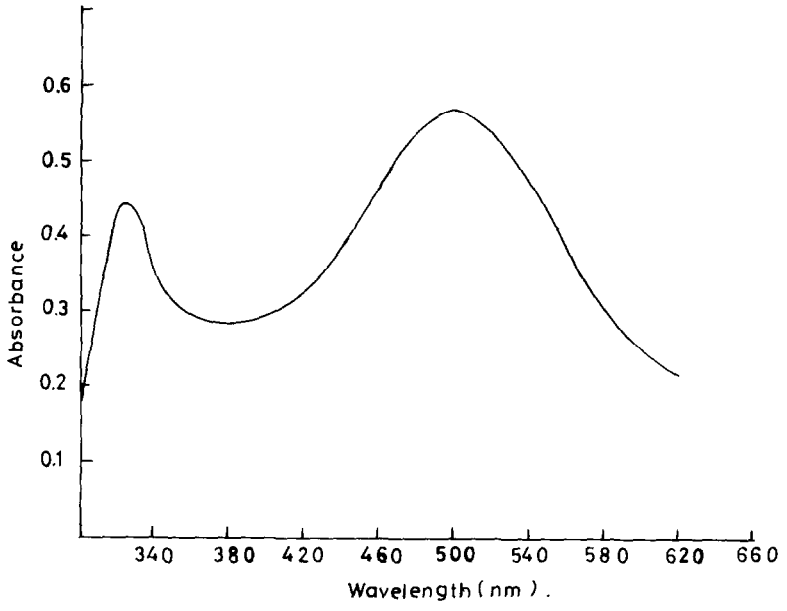


Figure 1
Absorption spectrum of sulphacetamide sodium (30 μg ml^{-1}) reaction product with *p*-benzoquinone in 0.1 M HCl.

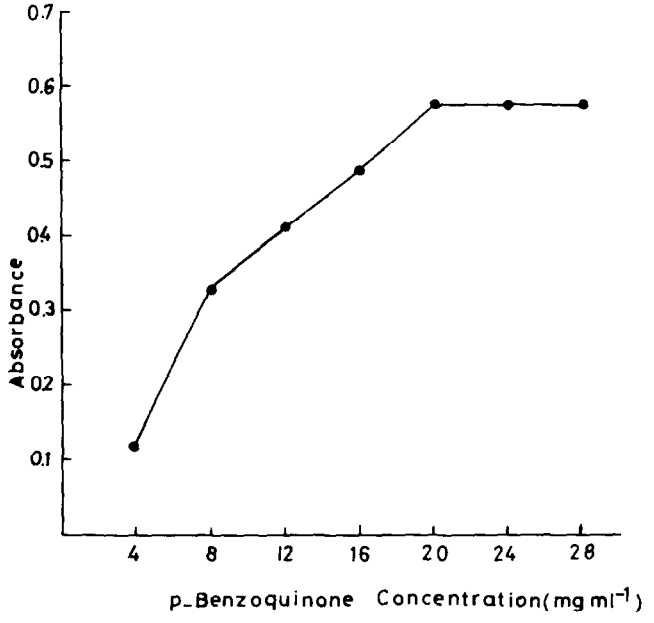


Figure 2
Effect of *p*-benzoquinone concentration on the absorbance of sulphacetamide sodium (30 μg ml^{-1}) reaction product.

For a fixed concentration of sulphacetamide sodium, 1 ml of *p*-benzoquinone solution was found to be sufficient to give maximum colour intensity. Higher reagent concentrations did not affect colour intensity (Fig. 2).

Studies on the effect of pH showed that the reaction is pH-dependent. In neutral or alkaline media, intense red colours were produced in both the test and blank solutions which may give discrepant results due to the high blank values at 500 nm. Maximum colour intensity was obtained at pH $\leq$ 3.0. One millilitre of 0.1 M hydrochloric acid was used throughout this work.

Figure 3 shows that at 25°C the colour was developed very slowly. At 100°C maximum absorption was reached after 5 min but there was a marked increase in the blank value after a further 5 min.

At 90°C maximum absorption readings were reached after about 7 min and remained unchanged for a further 20 min. In addition, the blank readings remained unchanged. Therefore heating at 90°C for 10 min is recommended.

The solvents studied were water, methanol, ethanol, iso-propanol, acetonitrile, acetone and dioxane. Water was found to be the solvent of choice.

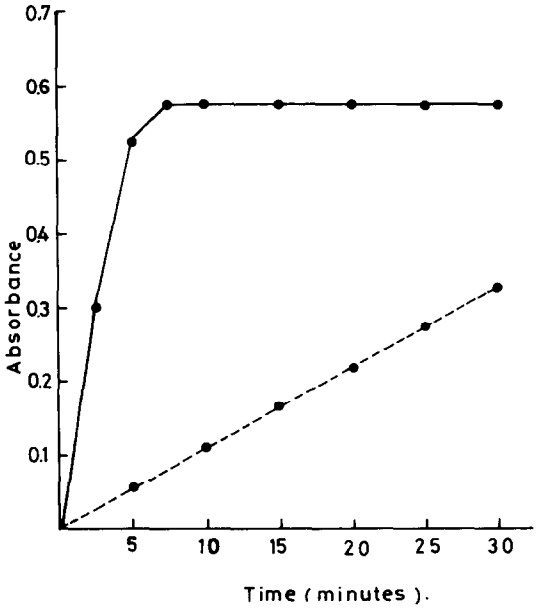


Figure 3
Effect of time and temperature on the absorbance of sulphacetamide sodium (30 μg ml^{-1}) reaction product. - - - -, 25°C; ——, 90°C.

Under the optimum conditions Beer's law was obeyed over a very wide range of concentrations of all the sulphonamide drugs studied. Table 2 shows the linear calibration ranges and regression parameters for the proposed method.

Analysis of pharmaceutical preparations

The recommended procedure was applied to the determination of some sulphonamides in various dosage forms without prior separation. The results given by the proposed method and by the Bratton–Marshall [8] method were compared statistically and found not to differ significantly (Table 3). Recovery experiments were carried out for each drug in its respective formulation. The good recoveries indicate the absence of interference from the frequently encountered excipients or additives.

Analysis of sulphamethoxazole or sulphamoxole in admixture with trimethoprim

The assay of trimethoprim and either sulphamethoxazole or sulphamoxole in commercial formulations sometimes requires prior separation of each drug [2]. Under the specified reaction conditions, trimethoprim does not react with *p*-benzoquinone reagent; this allows the analysis of sulphamethoxazole or sulphamoxole in admixtures with trimethoprim. To check the selectivity, admixtures of trimethoprim with either sulphamethoxazole or sulphamoxole in the ratio 1:5, the ratio usually found in official formulations, were prepared and analysed by the proposed

Table 2
Statistical data and absorption characteristics of the sulphonamides

Drug	$\varepsilon_{max} \times 10^{-3}$ at 500 nm	Linear calibration range (μg ml^{-1})	Intercept	Slope	Correlation coefficient (r)
Sulphacetamide	5.20	5–70	0.0416	0.0178	0.9984
Sulphadiazine	5.05	5–80	0.0598	0.0142	0.9960
Sulphamerazine	4.73	5–80	0.0329	0.0146	0.9986
Sulphadimidine	3.96	7–100	0.0208	0.0121	0.9937
Sulphasomidine	3.81	8–80	−0.0177	0.0155	0.9947
Sulphamethoxazole	5.50	5–70	0.0520	0.0165	0.9903
Sulphamethoxypyridazine	5.70	5–80	0.0433	0.0139	0.9991
Sulphathiourea	4.99	5–60	0.0222	0.0194	0.9964
Sulphamethoxydiazine	4.82	5–90	0.0434	0.01284	0.9943
Sulphaphenazole	6.82	5–60	0.0149	0.0202	0.9961
Sulphamoxole	3.98	7–80	0.0031	0.0146	0.9969
Sulphadimethoxine	6.36	5–50	−0.0279	0.0233	0.9952
Sulphaethidole	6.67	5–50	−0.0054	0.0240	0.9990
Sulphadoxine	6.27	5–60	0.0129	0.0189	0.9969
Sulphadicramide	7.07	5–60	0.0326	0.022	0.9941

Table 3
Assay of dosage forms using the proposed method and the Bratton–Marshall method [8]

Drug and formulation	Claimed amount	% found ± SD (n = 5) Proposed method	Bratton–Marshall method
Sulphadiazine			
Sulphadiazine tablets	0.5 g/tablet	95.26 ± 2.0	95.89 ± 1.8
Sulphaphenazol			
Neosulpha tablets	0.5 g/tablet	101.39 ± 0.8	102.88 ± 1.0
Sulphacetamide sodium			
eye drops	20% w/v solution	95.84 ± 2.6	96.38 ± 1.1
Sulphamethoxydiazine			
Longactine tablets	0.5 g/tablet	103.76 ± 1.3	104.60 ± 1.2
Sulphathiourea			
Bendonal tablets	0.5 g/tablet	96.69 ± 0.4	97.10 ± 0.7

Table 4
Analysis of sulphamethoxazole and sulphamoxole in presence of trimethoprim

Preparation	Claimed amount	% found ± SD (n = 5) Proposed method	Bratton–Marshall method
Sulphamethoxazole	400 mg		
and trimethoprim synthetic admixture	80 mg	101.57 ± 0.8	101.40 ± 1.0
Septazole tablets	400 mg/tablet	99.21 ± 2.0	98.86 ± 1.9
Sulphamoxole and trimethoprim	400 mg	100.05 ± 1.8	100.00 ± 1.7
synthetic mixture	80 mg		
Entrim tablets	400 mg/tablet	99.67 ± 1.1	101.20 ± 1.3

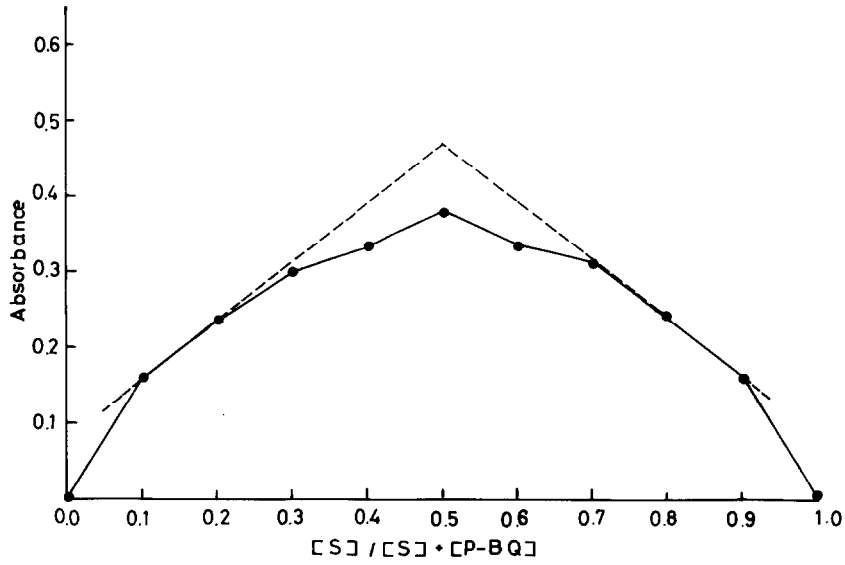


Figure 4
Job's continuous variation plot for sulphacetamide sodium (S) and *p*-benzoquinone (p-BQ) (0.025 M).

method. Excellent recoveries of each sulpha drug from the binary mixtures and tablets were obtained (Table 4) and this confirms the absence of interference of trimethoprim with the procedure.

Tests to correlate the sensitivity of the assay, expressed as $A^{1\%}_{1cm}$ values of sulphonamides analysed by the proposed method, and the pK_{a_1} and pK_{a_2} values by linear regression analysis gave a good correlation

$$A^{1\%}_{1cm} = 496.579 - 149.119\ pK_{a_1} \quad (1)$$
$$(n = 12,\ r = -0.8916)$$

$$A^{1\%}_{1cm} = 433.874 - 37.869\ pK_{a_2} \quad (2)$$
$$(n = 15,\ r = -0.9621).$$

This means that the $A^{1\%}_{1cm}$ values of these drugs are essentially negatively affected by their degree of ionization. In contrast, correlation tests of $A^{1\%}_{1cm}$ with bulkiness parameters, de-

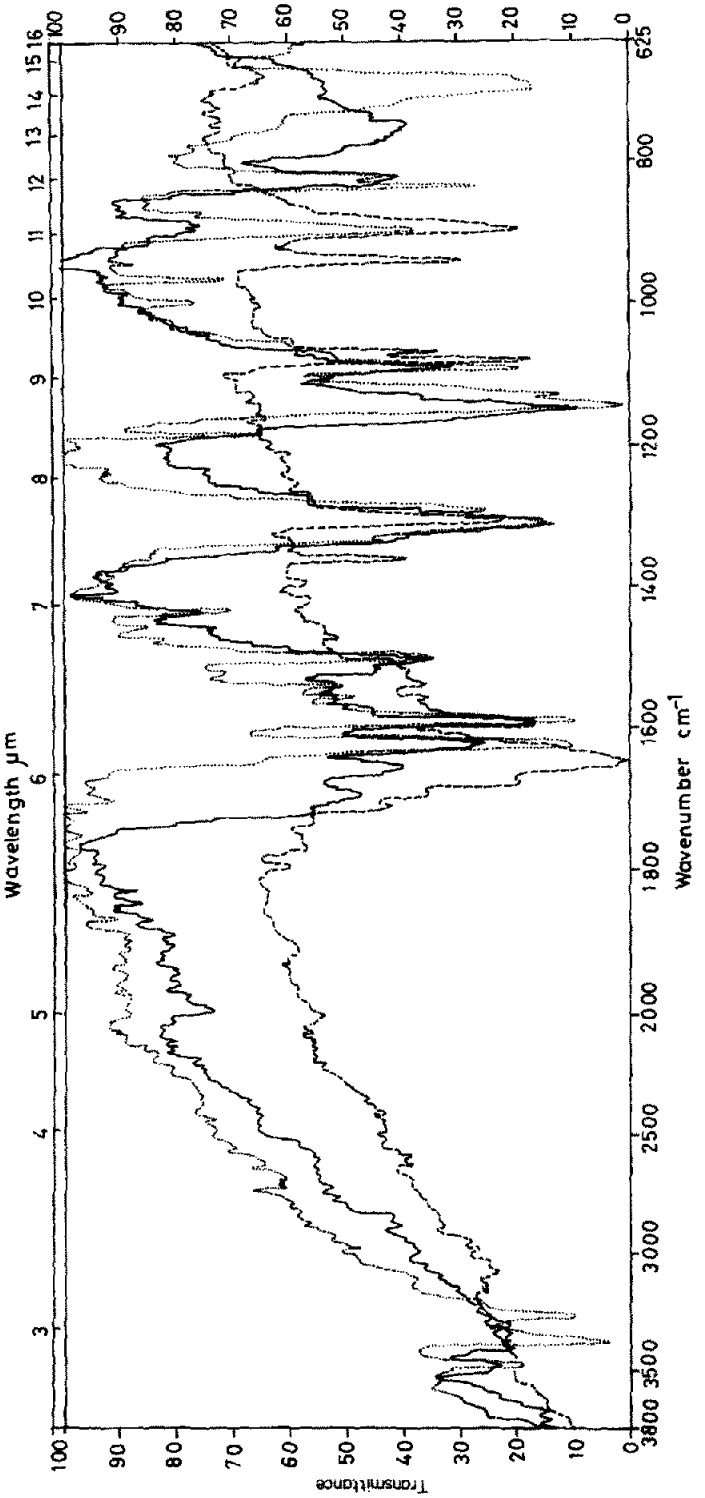
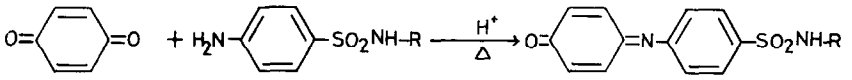


Figure 5
IR spectra of sulphacetamide sodium (· · · ·), *p*-benzoquinone (– – – –) and their reaction product (———).



Scheme 1

fined by the characteristic volumes Vx and molecular connectivity indices ^1X and $^1X^v$ of the side chain-R, yielded the following poorly correlated equations:

$$A^{1\%}_{1\,cm} = 249.266 - 0.292\ Vx \quad (n = 15,\ r = -0.2439) \tag{3}$$

$$A^{1\%}_{1\,cm} = 218.416 - 7.005\ {}^1X \quad (n = 15,\ r = -0.2391) \tag{4}$$

$$A^{1\%}_{1\,cm} = 217.852 - 10.965\ {}^1X^v \quad (n = 15,\ r = -0.2393). \tag{5}$$

These results reflect the small importance of bulkiness parameters as effective factors in the development of the chromogen.

Application of Job's method of continuous variation [24] showed that all the studied compounds reacted with *p*-benzoquinone in the molar ratio 1:1 (Fig. 4). Hence a mono-substituted derivative is the most probable derivative formed under the stated conditions.

There is no positive IR evidence for the formation of —C=N since the —C=O band of benzoquinone overlaps with the —C=N band region; in addition, —C=N functions are present in most of the studied sulpha drugs, but the absence of the characteristic asymmetric and symmetric NH stretching bands is indicated (Fig. 5).

Scheme 1 shows the possible reaction pathway as predicted from literature reports [16–19] and from the results of the present work, where the free primary amine moiety of the sulphonamide condenses with the carbonyl group of *p*-benzoquinone to form the condensation product.

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