



Original Article

Spectrophotometric Determination of Sulfanilamide and Effect of pH on its Absorption Spectrum

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We report the facile Spectrophotometric method for the determination of sulphaniilamide and the effect of pH on its UV-Absorption Spectrum. Sulfanilamide is a first class of benzene sulphonamide showing the marked changes in the UV absorption pattern due to its functional sulphur and amine groups. It shows altered concentration dependent ionization and absorption in both acidic and basic pH when measured at 455 nm. Its reaction with Potassium 1, 2-Naphthoquinone-4-sulphonate, m which provide gain of sensitivity (Detection limit 0.657 and Quantification limit 0 .641 ($\mu\text{g.mL}^{-1}$) without the need of additional step of extraction or heating. The method involve mild reaction conditions and gives precise results. Its usefulness as classic and drug determination in pharmaceutical formulations was demonstrated, suggesting its use as an attractive alternate to many other previously reported methods for analysis of sulfanilamide.

Keywords: Sulphanilamide, UV-Spectrophotometric Determination, Effect of pH, k-NQS, Absorption Spectrum.

1. INTRODUCTION

In 1908, Gelmo first synthesized and introduced a new compound, Para-aminobenzene-sulfonamide, or sulfanilamide. There followed a period of quiescence during which, buried in the German dye industry, the great possibilities of sulfanilamide remained unrecognized for more than a quarter of a century¹. Sulphanilamide appears as a White or yellowish-white crystals or fine powder is a medicinal compound used to guard against certain bacterial infections². It is frequently used in the form of a topical

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cream or powder to treat surface infections, as well as a pill for internal infections. It falls into the category of sulfonamide antibacterial drugs; Common infections treated by sulfanilamide include urinary tract infections, vaginal infections, strep throat, and some staph infections, depending on the type of infection, either a cream or a pill will be prescribed⁴. The administration of sulphaniamide reduced the incidence of complications and diminished the tendency to recurrence a series of 270 cases of erysipelas was treated under controlled conditions.

Sulfanilamide (SNA) is a sulfonamide antibacterial and chemically name is 4-aminobenzenesulfonamide with molecular formula C₆H₈N₂O₂S and molecular weight of 172.205 g.mol⁻¹; the basic structure of the drug, is shown in Scheme³.

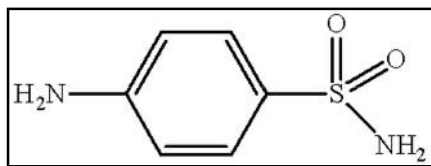


Fig 1: Molecular Structure of Sulphanilamide

Several publications have described the ultraviolet absorption spectra of sulfanilamide derivative but very little has been done toward identification and characterization of the individual absorption bands occurring in these spectra.³⁻⁶ with sulfanilamide, a strong resonance is possible between the amino and the sulfonamide- group. As a result, sulfanilamide has a considerably higher extinction coefficient than aniline and, besides, the character of the spectrum is quite different from that of these other compounds. Sulfanilamide and p-aminobenzoic acid have a higher extinction coefficient in basic than in sodium chloride or water solution. This indicates that the main resonance form makes a greater contribution in the ion than in the undissociated molecule⁶. In alkaline solutions the absorption spectra of sulfanilamide, N1-acetylsulfanilamide, and 'the N'-derivatives of both exhibit single maxima in the ultraviolet. With all four compounds the position of this maximum shifts toward longer wave lengths with little change in intensity as the PH of the solution is reduced to 3.0-3.5. This is interpreted as being the result of the change from the anion to the undissociated molecule.¹ Most cyclic compounds in water solutions give absorption bands in the ultraviolet. While studying this absorption for a mixture of amino acids we observed that the absorption band shifted towards longer wavelengths when the mixture was made alkaline and therefore.⁷ That the ultraviolet absorption maxima of some sulfonamides differ in both position and intensity, depending upon whether the compounds are in alkaline or acid solution, has been shown in several recent publications.⁶⁻⁸

Following an extensive study of the ultraviolet absorption of sulfanilamide derivatives with such a characterization has

now been diminished. The procedure consisted first in the determination of the absorption bands of the simple derivatives over a wide pH range. By considering of the wave length and reaction to pH of these bands, the corresponding maxima were located in the more complicated derivatives. Where necessary, identity was confirmed by observation of suitably constructed analogous compounds. Acidic ionization (in alkaline solution) results in a moves toward shorter wave length with little change in intensity, while basic dissociation (in acid solution) decreases the absorption almost to disappearance of the band.⁸ In the previous date no quantitative data was made available, however, as to the amount of color given by potassium 1,2-naphthoquinone-4-sulfonate with various aliphatic and aromatic amines, although these compounds frequently accompany the amino acids in biological media. This information developed of importance in connection with studies involving the concentration of certain nitrogenous constituents in blood and urine, the present investigation was assumed.⁹ That sulfanilamide has a higher extinction coefficient in base than in water is likewise evidence for a greater contribution from the form with separation of charge in the ion, compared with the undissociated molecule. In another paper this fact is significant in correlating structure and bacteriostatic activity of the sulfonamides.¹⁰

2. MATERIALS AND METHOD

Experimental Section:

Instrument: Systronic-2203 double beam UV-visible spectrophotometer possessing a fixed slit width (1.8 nm) with quartz cells of 1.0 cm path length with a PC Connection were used for all measurements.

Material and Reagent: All reagents were of analytical grade. Sulfanilamide and Borax were purchased from SD Finechem Ltd., Boisar, Maharashtra.

Methods:

- **Preparation of Stock Solution (Alkaline):** Prepared by dissolving 0.1g of Sulphanilamide in 100mL of 0.1M sodium hydroxide solution.
- **Preparation of Stock Solution (Acidic):** Prepared by dissolving 0.1g of Sulphanilamide in 100mL of 0.1M hydrochloric acid solution.
- **Preparation of Blank solution:** Standard blank solution of Sulphanilamide was prepared by dissolving accurately weighed 0.1g of Sulphanilamide in 100 mL of Distilled water.
- **NS stock solution:** Dissolve 0.200 g of potassium 1,2-naphthoquinone-4-sulphonate in 50 mL of water and dilute to 100 mL with water.

Preparation of standard stock solutions: Solution of 1000 µg.ml⁻¹ Sulfanilamide was prepared by dissolving accurate weighted 0.100 g of pure drug in 10 mL of 0.4 M HCl and further diluted to the mark in volumetric flask 100 mL with distilled water and stored in a cool (< 25 °C) and dark place, working solution were prepared fresh daily by subsequent

dilutions. Potassium 1,2-Naphthoquinone-4-sulphonate (kQS) solution 0.5% (m/v) in distilled water was prepared fresh daily. Sodium hydroxide 0.1 M prepared by dissolving 0.4 gm. of pure substance in 100 ml distilled water, Borax solution 0.1 M prepared by dissolving 0.1902 g in 25.0 mL double distilled water and diluting to the mark in a 50 mL volumetric flask.

Preparation of sulfanilamide and Interfering sample: To 0.050 g of the bulk drug, 0.005 g of interfering substance mixture glucose were added. Further, 0.0125 g of the resulted mixture was dissolved in 10 mL of 0.1 M HCl and diluted to the mark with distilled water in volumetric flask 100 mL in the same manner as used for the preparation standard drug to obtain $100 \mu\text{g} \cdot \text{mL}^{-1}$.

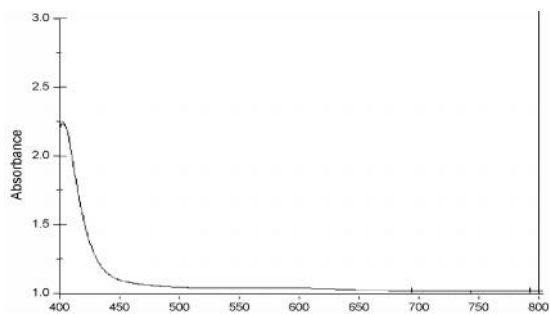


Fig 2: UV-Spectrum of Sulphanilamide in Water

3. RESULT AND DISCUSSION

Part: A) Effect of pH on Sulphanilamide UV-Spectrum:

In derivatives capable of acidic and basic dissociation, the behavior at different pH values has been illustrated in the curves for sulfanilamide (Fig. 1, top). Acidic ionization (in alkaline solution) results in a shift toward shorter wave length with little change in intensity, while basic dissociation (in acid solution) decreases the absorption almost to disappearance of the band. The wave length of the band may shift to some extent with substitution.

From these observations, it can be stated that the principal features of sulfanilamide absorption consist of a single band of high intensity in the neighborhood of 260 nm in neutral solution, which shifts to shorter wave length with alkalinity and decreases in intensity with acidity. Simple substitution has but little effect on the wave length of the band, and very little on the intensity, except in cases where the substitution causes changes in the basic ionization properties of the molecule.

Since the acetylated amino group is no longer capable of forming a salt in dilute acid solution, the absorption due to the sulfanilamide portion should be unaffected. That this is the case is shown in Fig 3. When sulfanilamide is placed in variable pH solution (Figure 4; A-B) both the resonance between the amino group and the ring, and that between the amino group and the sulfonamide group, is blocked. Hence, here again the spectrum reverts to that of benzene-sulfanilamide. In Fig. 2 the data for sulfanilamide are plotted.

The solid line, showing the % dissociation *versus* pH, was calculated from the Henderson-Hasselbalch equation using the value $pK_a = 10.48$ which was found by calculation.

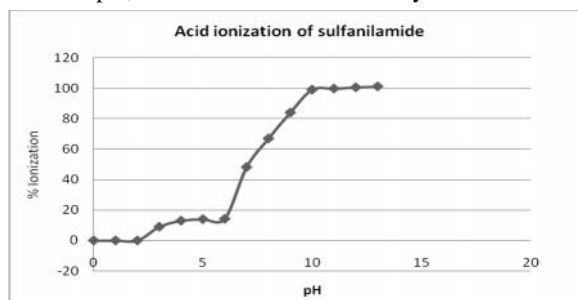


Fig 3: Acid ionization of sulfanilamide: solid line indicates the ionization.

It is evident that the experimental points fit the theoretical curve well, particularly through the middle range where it is nearly linear.¹

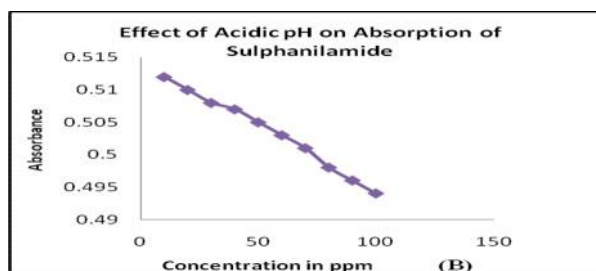
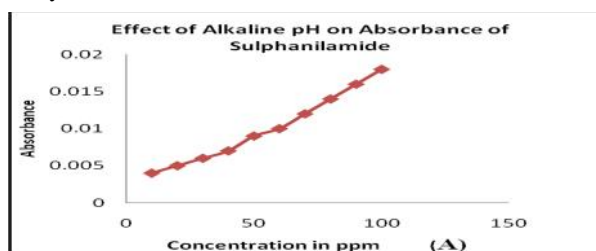


Fig 4: Effect of Different pH Conditions on Sulphanilamide; Acidic (A) and Basic (B) pH at Various Concentrations

Part B): Determination of Sulphanilamide by using k-NQS:

When the solution of sulfanilamide was mixed with kQS in alkaline medium at room temperature, strong coloration was developed, showing a broad band in the region of 400-800 nm). It was observed that the product is orange colored exhibiting (λ_{max}) at 455 nm, against reagent blank (Figure 1-B), and the λ_{max} of derivative chromogenic reagent (potassium 1,2-naphthoquinone-4-sulfonic) is at 485 nm. Which indicates the formation as sulfanilamide possesses amino groups, it involves in yielding colored produced by nucleophilic displacement of the sulfonic acid group of 1,2-naphthoquinone-4-sulfonic acid in alkaline conditions (Fig. 5-B). The intensity of this band increased with increased concentration of sulfanilamide. Although a certain degree of alkalinity was found necessary, an excess frequently resulted in a marked reduction for color produced in the reaction.

When the naphthoquinone-sulfonic acid reagent was added to a faintly alkaline solution of a compound containing a

reactive amino group a deep blackish brown color rapidly developed, especially in the presence of sodium carbonate. Upon acidification the color of the solutions generally became reddish brown.

Table 1: Quantitative parameters for the sulfanilamide with k-NQS

Parameter	Univariate conditions
max (nm)	455
Color	Orange
Linearity range ($\mu\text{g.mL}^{-1}$)	5.0-35.0
Regression equation	
Calibration sensitivity ($\text{mL.}\mu\text{g}^{-1}$)	0.0404
Correlation coefficient (r) %	0.9998
Molar absorptivity ($\text{L. mol}^{-1}.\text{cm}^{-1}$)	$=6.9478 \times 10^4$
Detection limit ($\mu\text{g.mL}^{-1}$)	0.657
Quantification limit ($\mu\text{g.mL}^{-1}$)	1.641

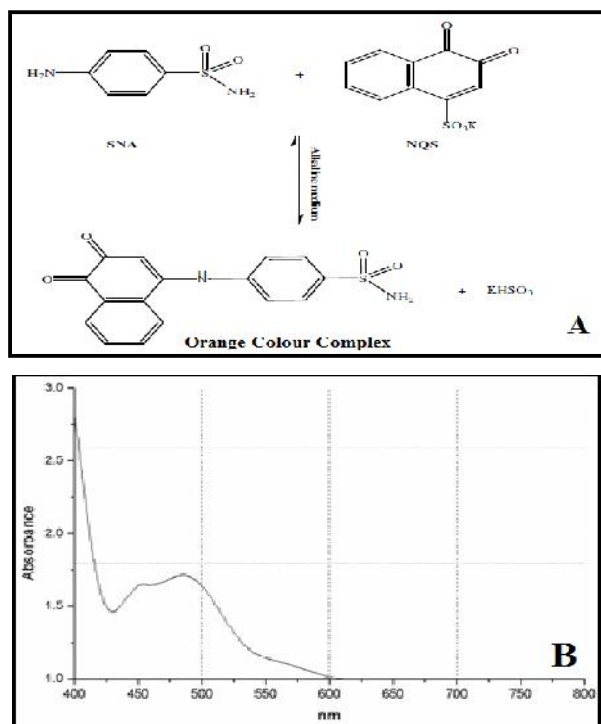


Fig 5: The proposed mechanism of the reaction between sulfanilamide and potassium 1, 2-naphthoquinone-4-sulfonate in (A) and UV-Spectra of Sulphanilamide and with kQS (B).

4. CONCLUSION

To our knowledge, No researches has carried out a thorough investigation of these changes in relation to pH, although data from such studies are essential before one can apply the ultraviolet method to the qualitative and quantitative analyses of these drugs.

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