Research Article

# Diazotization Coupling Reaction for Micro Spectrophotometric Determination of Sulfamerazine in Pharmaceutical Preparations

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**Received:** 19-03-2017; **Revised:** 08-05-2017; **Accepted:** 23-05-2017.

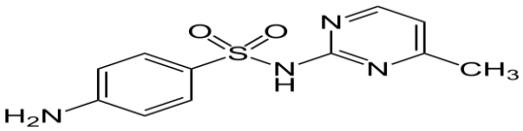
**ABSTRACT**

New, simple and sensitive specteophotometric method has been developed for the determination of sulfamerazine. The method is based on the diazitization coupling reaction of sulfamerazine with resorcinol in alkaline medium were studied. The reaction gave an intense water soluble yellow product that has a maximum absorption at 422 nm and Ɛmax of 2.97 × $10^4$ L.$mole^{-1}$.$cm^{-1}$. A linear correlation (1-8µg per 25ml 0.4 – 3.2 ppm) was found between absorbance at λmax and concentration. The results obtained are both precise (RSD was better than 0.8) and accurate (relative error was better than – 0.11). The coloured product was found to be 1:1 sulfamerazine: resorcinol. The stability constant and the rate constant of reaction under optimized conditions at room temperature were 0.25 x$10^5$ L.$mole^{-1}$ and 2.25x$10^{-2}$ $min^{-1}$ . The proposed method was successfully applied to the determination of sulfamerazine in synthetic samples of pharmaceutical formulation.

**Keywords:** Diazotization coupling reaction, Sulfamerazine, Resorcinol, Spectrophotometric determination.

## INTRODUCTION

Sulfamerazine contains 99.0 per cent and not more than the equivalent of 101.0 per cent of 4-amino-N-(4-methyl-2-pyrimidinyl) benzenesulphonamide $C_{11}H_{12}N_4O_2S$, whereas it's chemical structure:



Sulfamerazine is an antibiotic drug used for the medication of many infectious diseases of animal, more used in veterinary medicine and human[1] . It is also enter the human by food series out of milk and meat products. In addition to this these clinical applications, Sulfamerazine is frequently applied as a compound for the locating of the N- acetylation activity in human[2]. Different of analytical methods have been reported for the biological and determination of Sulfamerazine such as polargraphic behavior[3], gas chromatography[4], high performance liquid chromatography[5,6], effect of Sulfamerazine on hepatic mixed function[7], photochemical degradation[8] , ultrasonic irradiation[9], corrosion inhibition characteristics on mild steel immersed in HCl solution[10] and direct spectrophotometric determination[11] , spectrophotometric method is yet still an outshine technique due to economy and simplicity. The present study describe a simple and sensitive method based on diazotization of Sulfamerazine and coupling with resorcinol in alkaline medium, to assay this drug in bulk samples and in different of pharmaceutical preparations .

## MATERIALS AND METHOD

### Apparatus

All chemicals used in this work were of analytical grade. Spectrophotometric measurement was made with Shimadzu UV – Visible – 1700 double beam spectrophotometer using 1. 00 cm glass cells. The pH measurements were performed with a HANNA pH meter H19811-5 Instrument. All kinetic measurements were made on Single beam UV- Visible T60spectrophotometer.

### Reagents

***Sulfamerazine stock solution (100 µg.$ml^{-1}$)***

A 0.020 g amount of pure Sulfamerazine (SIGMA) was dissolve in 20 ml of ethanol then complete to 200 ml in a volumetric flask with ethanol. working standard of thymol solutions were prepared by simple dilution of the appropriate of the compound in ethanol completing the volume in a volumetric flask.

***Sodium nitrite solution (1% w/v)***

1 g of sodium nitrite was dissolved in 25 ml of distilled water then complete to 100 ml in a volumetric flask**.**

***Hydrochloric acid solution (1M)***

This solution was prepared by diluting suitable amount of 11.64 M HCl (BDH) with distilled water in 200 ml volumetric flask**.**

***Sodium hydroxide solution (5%)***

5 g of sodium hydroxide was dissolved in 25 ml of distilled water then complete to 100 ml in a volumetric flask.

***Foreign materials solutions (100 µg mL$^{-1}$)***

These solutions were prepared by dissolving an amount of the compound in distilled water completing the volume in a volumetric flask.

## RESULT AND DISCUSSION

The result of this investigation indicated that the reaction of diazotization of Sulfamerazine and coupling with resorcinol yield highly soluble coloured product which can be utilized as a suitable assay procedure for for Sulfamerazine. This yellow coloured product has a maximum absorption at 42 nm, the blank at this wavelength shows zero absorbance (Fig 1, 2), was adopted in all subsequent experiments.

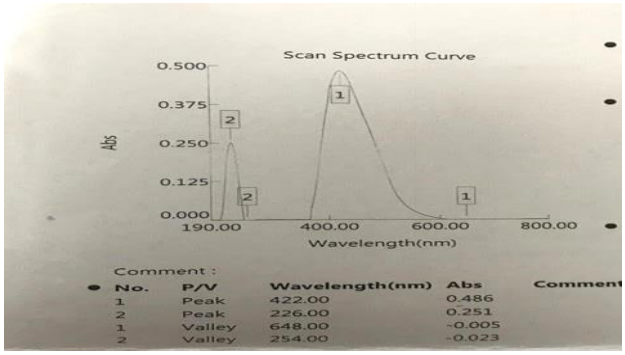



**Figure 1:** Absorption spectra of azo dye [1ml of drug 100µg/ml + 0.5ml of acid 1M+ 2ml of 1% $NaNO_2$+ 1.5ml of resorcinol +2ml NaOH]versus reagent blank and blank versus distilled water .

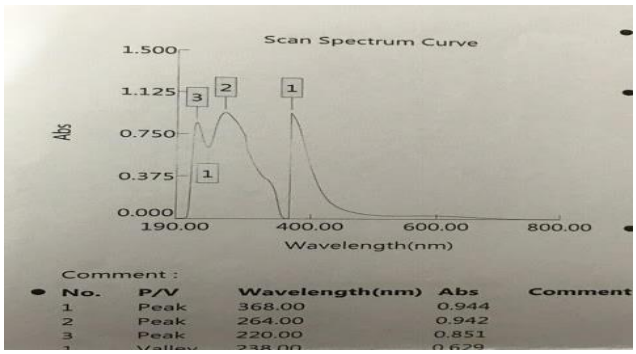



**Figure 2:** Absorption spectra of reagent blank and blank versus distilled water

The experimental conditions for the determination of Sulfamerazine were achieved. Diazonium reaction occurred in an acidic medium and coupling with resorcinol in alkaline medium[12, 13]. The influence of various reaction variable on the colour development was tested to establish the most favorable conditions and these are:

### Effect of acidity on diazotization

To establish the optimum conditions (stability of the dye resulting from the reaction of Sulfamerazine with the reagent intensity of the dye formed, minimum blank value and relatively rapid reaction rate), the effect of different acids were studied. Only $CH_3COOH$ with concentration 1M was found to be Optimum. The HCl, $HClO_4$ and $H_2SO_4$ results in low sensitivity of the diazotization of Sulfamerazine and was not stable. The effect of the amount of $CH_3COOH$ used was also investigated and 0.5 ml volume seems to be optimum for an intense azo dye absorbance (Fig 3).

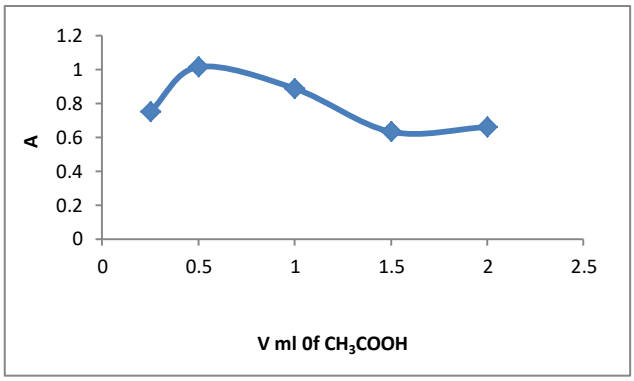



**Figure 3:** Effect of type of acid on diazotization reaction Effect of Sodium nitrite concentration to obtain the optimum results, the amount of NaNO2 was studied. Various concentration of $NaNO_2$ was added to fix of Sulfamerazine 10mM and HCl 1M, 2 ml of 1% $NaNO_2$ was used in all subsequent experiments. The excess of sodium nitrite could be removed by addition 1:1 mole ratio of Sulfamerazine to 1% $NaNO_2$Fig .4.

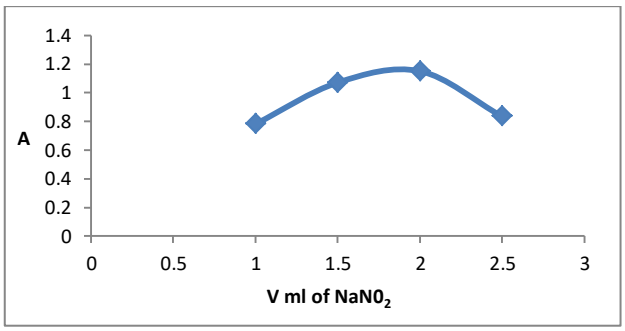



**Figure 4:** Effect of sodium nitrite of acid on diazotization reaction

### Effect of resorcinol of concentration

When various concentration of resorcinol reagent was added to affixed concentration of dye , 1.5 ml of 1 mM Sulfamerazine solution was sufficient to develop the colour to its full intensity and gave minimum blank value , above 3 ml , the absorbance of the blank value was increased causing a decrease in the absorbance of the sample. Therefore, 1.5 ml of 1 mM of resorcinol was used in all subsequent experiments Fig5.

### Effect of Sodium hydroxide concentration

It was found experimentally that the colored product was found only in alkaline medium. Different bases were examined these include sodium bicarbonate , potassium hydroxide, ammonia and sodium hydroxide only sodium hydroxide was found optimum since it gives a high sensitivity, minimum blank value and high stability of the colored product . Other bases were examined and found

unsatisfactory. The dye formation reached maximum with about 2 ml of 5% NaOH solution Fig.6.

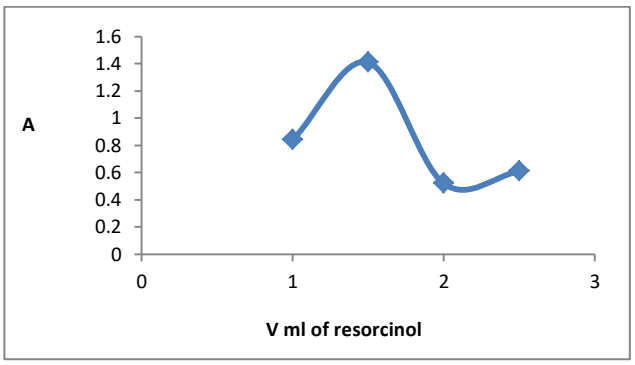



**Figure 5:** Effect of reagent resorcinol on diazotization reaction

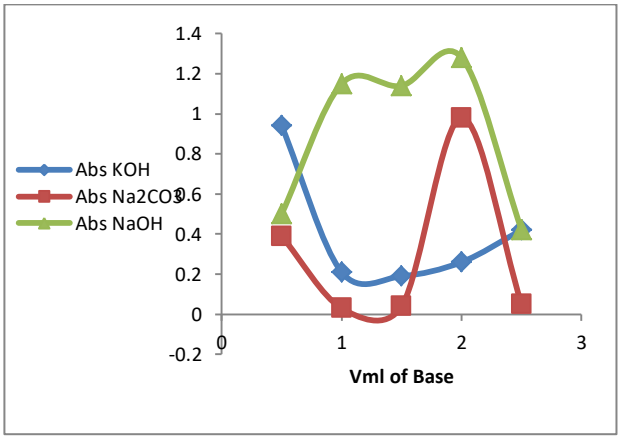



**Figure 6:** Effect of the concentration of base on the coupling reaction

**Development time and stability period**

The colour intensity reached maximum after resorcinol solution had been reacted with dizonium salt of Sulfamerazine in alkaline medium for more than 4 hrs. The colour obtained was stable for at least 4 hrs and this stability period was sufficient to allow several measurements to be performed sequentially.

**Calibration graph**

Employing the condition described under procedure a linear calibration graph for Sulfamerazine is obtained which shows that Beers law is obeyed over the concentration range of 1-8 µg per 25 ml 0.4-3.2 ppm with a correlation coefficient 0.9835 and intercept 0.0235. The molar absorptivity of the coloured product with reference to Sulfamerazine was $2.97 \times 10^4$ $Lmol^{-1}cm^{-1}$.

**Structure of the dye**

The stoichiometry of the reaction between diazotized Sulfamerazine and resorcinol was investigated using the continuous variation method [14] .The results obtained Fig.7 show a 1:1 diazotization coupling formed between Sulfamerazine and resorcinol at 424 nm.

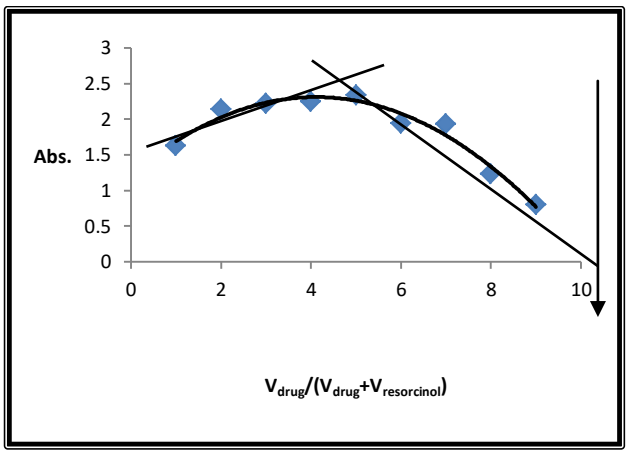
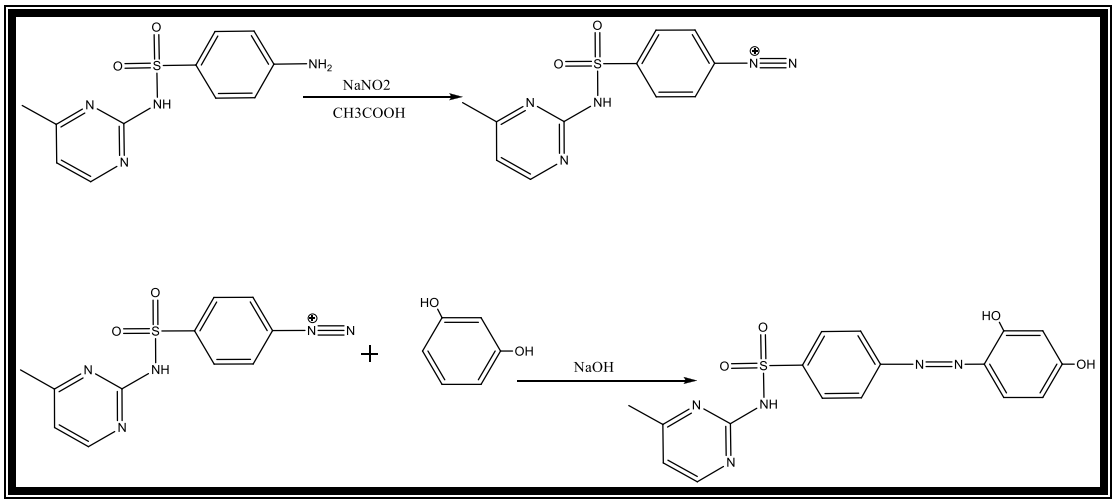



**Figure 7:** Continuous variation of structure of dye formation

Therefore, the formation of the dye probably occurs according to the following equations;




The apparent stability constant was calculated by comparing the absorbance of a solution containing stoichiometric amounts of Sulfamerazine and resorcinol with that of a solution containing a five –fold excess of resorcinol reagent. The average conditional stability constant of the dye in water, under the describe experimental conditions is 0.25 x $10^5$ L mole-1.

### Effect of interferences

In order to assess the possible analytical application of the proposed method, the effect of some foreign ions often accompany this drug in pharmaceutical products were studied by adding different amounts of foreign ions to 2.0 µg.ml-1 of Sulfamerazine can be determined without any interference in the presence of a 5 fold excess of the foreign ions Table. 1.

**Table 2:** Effect of foreign materials

| Interference taken 50 ppm | %Error | Recovery % |
|---|---|---|
| Glucose | - 0.75 | 99.25 |
| Lactose | + 0.1 | 100.1 |
| Sucrose | + 0.05 | 100.05 |
| Starch | - 0.27 | 99.73 |

### Applications

### Accuracy and Precision

To determine the accuracy and precision The propose method ware applied to the determination of Sulfamerazine in synthetic mixture were analyzed by the procedures described above . The results are shown in Table .2 indicate that satisfactory precision and accuracy could be attained with the proposed method.

**Table 3:** Accuracy and precision of the method

| Amount taken of Sulfamerazine p.p.m | E % | *RSD% |
|---|---|---|
| 0.8 | - 0.05 | 0.65 |
| 1.2 | + 0.08 | 0.80 |
| 2.0 | - 0.11 | 0.43 |

*For seven determinations

### Rate of reaction

Rate of reaction were determined spectrophotometrically by measurement of the different in absorbance of the reaction mixture versus time. All experiments were achieved under pseudo-first order conditions by keeping concentrations of two reactants in twenty fold excess over that the third one[15]. The solutions were thermo state at 25 ± 0.1° C and the change in absorbance was measured until the reaction was complete. Rate constant was determined by the first order plot using the equation;

$$Kt = 2.303 \log A_\infty / A_\infty - A_t$$

Where $A_\infty$ is the final absorbance and $A_t$ the absorbance at any time t, after addition of resorcinol and appearance of the color. The validity of this interpretation was checked by plotting log $A_\infty / A_\infty - A_t$ against t, straight line was obtained and the pseudo-first order rate constant is determined from the slope and was found to be$2.25 \times 10^{-2}$ $min^{-1}$ .

## CONCLUSIONS

The present study demonstrates an excellent approach for the development of spectrophotometric method for determination of Sulfamerazine, high selectivity for diazotization coupling reaction of Sulfamerazine is achieved with resorcinol.

**Acknowledgment:** The authors are grateful to the Department of chemistry, Faculty of Sciences, University of Kufa to complete the requirements of research.

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**Source of Support:** Nil, **Conflict of Interest:** None.