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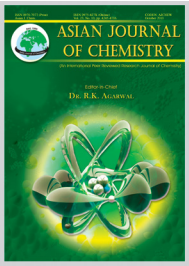

Influence of pH and Temperature on Stability of Sulfamethoxazole Alone and in Combination with Trimethoprim (Co Trimoxazole)

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The present research was under taken to assess the stability of medicinal agent, sulfamethoxazole (pure and in combination with trimethoprim) to the prevailing physical factors like temperature and pH. A pure sulfamethoxazole and a commercially available brand of co-trimoxazole were included in this study for comparison. The stability study was carried out by storing sulphamethoxazole and co-trimoxazole at four different temperature *i.e.*, 25, 37, 50 and 60 ºC and different pH *i.e.*, 3, 4, 5, 6, 7, 8, 9 and 10. Drug contents were determined at 0, 6, 24, 48 and 72 h intervals by non-aqueous acid-base titration method that was developed in our laboratory. The percentage of drug remaining was plotted against time for each temperature and pH. The degradation processes were assumed to follow pseudo-first order kinetics and rate constant (k) were calculated. The heat of activation (ΔHa) at particular pH was calculated by Arrhenius plot. It was observed that sulfamethoxazole in combination with trimethoprim (co-trimexazole) is more stable at higher temperature and lower pH. Degradation pattern of co-trimoxazole is steeper than sulphmethoxazole, but its overall stability pattern is better than sulphamethoxazole alone.

Key Words: pH, Temperature, Stability, Degradation, Sulfamethoxazole, Co-Trimoxazole.

INTRODUCTION

Stability study of an active substance or finished product provides evidence on how the quality of drug product varies with time. It is influenced by a variety of environmental factors such as temperature, humidity and light. Stability testing provides information about degradation mechanism, potential degradation products, possible degradation pathways of the drug as well as interaction between the drug and excipients in the drug product[1,2].

The stability study of sulfamethoxazole and trimethoprim examines the stability of both components of the antibacterial combination, co-trimoxazole. Although this drug have been in practice for the last two or three decades being an antibacterial agent it is still widely prescribed in developing countries like Pakistan. About 52 companies are marketing it in Pakistan, which reflects its enormous prescription.

Our climatic factors such as temperature, storage conditions, transportation environment, exposure to sunlight, packing material can affect the stability of active ingredients in various formulations[3]. Because the distribution environment is highly variable, product must be distributed in a manner that ensures product quality will not be adversely affected. The effect of possible temperature and humidity fluctuations, outside of labeled storage conditions, during transportation of drug products can be evaluated on the basis of the stability analysis of the drug[4].

To determine the stability of sulfamethoxazole pure and co-trimoxazole (sulfamethoxazole and trimethoprim) under Pakistani climatic and geographical conditions, active ingredient was determined to observe the fate of drug.

The most satisfactory method for expressing the influence of temperature on reaction velocity is the quantitative relation proposed by Arrhenius:

$$K = \frac{Se^{-Ha}}{RT}$$

This equation can be written as:

$$\log K = -\left(\frac{\Delta Ha}{2.303R}\right)\left(\frac{1}{T}\right) + \log S \qquad (1)$$

where log S can be considered a constant.

From eqn. 1, a plot of log K *versus* 1/T yields a slope equal to -ΔHa/2.303R from which the value for the heat of activation can be calculated. This type of graph is called Arrhenius plot. The heat of activation (ΔHa) represents the

energy the reacting molecules must acquire to undergo reaction. The higher the value for the heat of activation, the more the stability is temperature-dependence[4].

To determine the influence of pH on the degradation reaction, the decomposition is measured at different hydrogen ion concentrations. The pH of optimum stability can be determined by plotting the logarithm of the rate constant *versus* pH, this form of data display is called pH-rate profile.

The point of inflection of such a plot represents the pH of optimum stability. Knowledge of this point is extremely useful in the development of a stable dosage forms. Studies of this type can be performed at elevated temperatures[4].

EXPERIMENTAL

Sulfamethoxazole pure powder supplied by Hamaz Laboratories (Pvt.) Ltd., Multan (99.46), Septran suspension was obtained from Glaxo Wellcome Pakistan Ltd. Karachi. Sodium hydroxide, hydrochloric acid, sodium nitrite, sodium hydrogen phosphate, ethanol and soluble starch were obtained from E. Merck, Germany. Potassium iodide was provided by Peking, China. Potassium bromide was obtained from Matsunaga, Japan. Potassium biphthalate was supplied by Sigma, USA.

Preparation of buffer solutions: 0.2 M solutions of potassium hydrogen phthalate, potassium dihydrogen phosphate, sodium hydroxide and hydrochloric acid were prepared. Buffer solutions of pH 3-10 were prepared from above solutions[5]. The assay was performed according to procedure described in British pharmacopoeia[6].

Dissolved 200 mg of sulfamethoxazole (pure powder) in 15 mL of ethanol (96 %) and added 50 mL of 2 M hydrochloric acid, added 3 g of potassium bromide, cooled in ice and titrated slowly with 0.1 M sodium nitrite *versus*, stirring constantly and determined the end point by using starch as external indicator (each mL of 0.1 M sodium nitrite *versus* is equivalent to 25.33 mg of $C_{10}H_{11}N_3O_3S$).

Repeated the same procedure by dissolving the 5 mL of co-trimoxazole (Septran susp.) in 15 mL of distilled water, as each 5 mL contains 200 mg of sulfamethoxazole and 40 mg of trimethoprim.

Experimental protocol: The effects of two factors (temperatue and pH) of sulfamethoxazole and co-trimoxazole containing 200 mg of sulfamethoxazole were investigated. Sulfamethoxazole were dissolved in 15 mL ethanol taken in 200 mL titration flask. Prepared 34 (duplicate) flask by using same procedure. Analyzed one sample flask at '0' time and placed four flasks (covered) at room temperature (25 ºC), four flask were placed on 37 ºC in water bath, the other four were placed in another water bath at 50 ºC and remaining four were kept at 60 ºC in water bath. Each flask was selected from each group after 6, 24, 48 and 72 h and their analysis wad done.

Same procedure was repeated with co-trimoxazole using 5 mL of suspension (Septran) dissolved in 15 mL water. Each sample was performed in duplicate.

A group of 8 titration flasks each measuring 200 mL was prepared by dissolving 200 mg sulfamethoxazole in 15 mL ethanol. 10 mL of buffer solution of pH 3, 4, 5, 6, 7, 8, 9, 10) were added in 8 flasks, respectively. Prepared four groups of 8, 8 flasks using same procedure. One group was maintained at room temperature (25 ºC), second group was kept at 37 ºC in water bath, third group was maintained at 50 ºC and the fourth group was kept in water bath at 60 ºC. One flask from each group was selected for analysis after 6, 24, 48 and 72 h and their analysis were performed repeated the same procedure by dissolving 5 mL co-trimoxazole suspension in 15 mL water. Each sample was performed in duplicate.

RESULTS AND DISCUSSION

The application of certain physicochemical principles in the performance of stability studies has proved to be of considerable advantage in the development of stable dosage forms. When sulfamethoxazole was studied at 25, 37, 50 and 60 ºC at 6, 24, 48 and 72 h intervals, it was found that percentage residual drug at 25, 37 and 50 ºC at 60 ºC after 72 h were 78, 60, 20 and 14 %, respectively (Fig. 1).

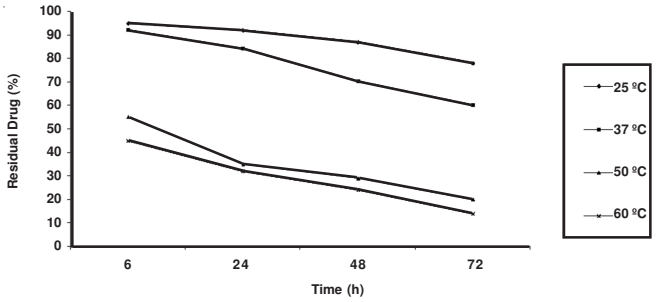

Fig. 1. Percentage residual drug of sulfamethoxazole *versus* time on different temperatures (25, 37, 50 and 60 ºC)

When co-trimoxazole was studied under the same conditions, it was observed that percentage residual drug of sulfamethoxazole in co-trimoxazole was from 83 % at 25 ºC to 25 % at 60 ºC, which shows that trimethoprim has significant rol on the stability of sulfamethoxazole towards temperature (Fig. 2).

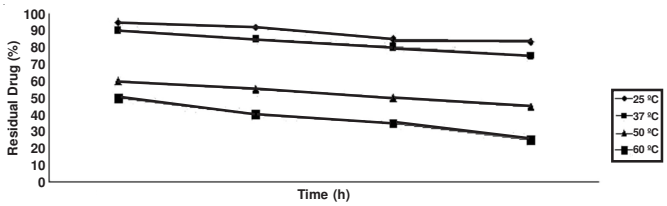

Fig. 2. Percetage residual drug of sulfamethoxazole *versus* time on different temperatures (25, 37, 50 and 60 ºC) in co-trimoxazole

Data obtained from the analysis suggested that structure of sulfamethoxazole and trimethoprim cleaved at high temperatue but effect is more in sulfamethoxazole[7]. When rate constant K was calculated at pH 3 from 25-60 ºC it was observed that rate constant was increased from pH 3-10 at all temperatures (Table-1).

It indicates that not only temperature accelerates the rate constant but pH also increases the rate constant of sulfamethoxazole.

In co-trimoxazole at pH 3 rate constant of sulfamethoxazole increased at all temperatures. From pH 4-7 at 25 ºC rate constant increased but after 37 ºC the rate constant

TABLE-1
OBSERVED PSEUDO-FIRST-ORDER RATE CONSTANT (K h^{-1}) OF SULFAMETHOXAZOLE DEGRADATION AT (25, 37, 50 AND 60 ºC) AT DIFFERENT pH VALUES (3, 4, 5, 6, 7, 8, 9, 10)

pH	Temperature (ºC)			
	25	37	50	60
3	51.42×10^{-3}	70.57×10^{-3}	93.73×10^{-3}	113.73×10^{-3}
4	54.32×10^{-3}	71.89×10^{-3}	97.47×10^{-3}	117.47×10^{-3}
5	60.27×10^{-3}	78.79×10^{-3}	101.31×10^{-3}	121.31×10^{-3}
6	63.33×10^{-3}	82.33×10^{-3}	105.33×10^{-3}	121.33×10^{-3}
7	69.64×10^{-3}	87.44×10^{-3}	109.24×10^{-3}	129.24×10^{-3}
8	79.55×10^{-3}	96.56×10^{-3}	117.57×10^{-3}	136.57×10^{-3}
9	82.97×10^{-3}	100.44×10^{-3}	121.91×10^{-3}	140.91×10^{-3}
10	90.07×10^{-3}	108.49×10^{-3}	130.91×10^{-3}	150.91×10^{-3}

decreased (Table-2). It verifies that at high hydrogen ion concentrations hydrolysis take place which results increase in rate constant.

On comparing the sulfamethoxazole and co-trimoxazole it was found that sulfamethoxazole degrade through solvolytic process which means reaction in solution having heat of activation of sulfamethoxazole at pH 3-10 at 25, 37, 50 and 60 ºC were 4.39, 4.31, 3.88, 3.56, 4.41, 2.98, 2.96 and 2.84, respectively. While in co-trimoxazole the heat of activation under the same conditions at pH 3-10 at 25, 37, 50 and 60 ºC were 4.12, 3.49, 1.98, 0.81, 2.40, 2.79, 2.60 and 3.27, respectively (Figs. 3 and 4).

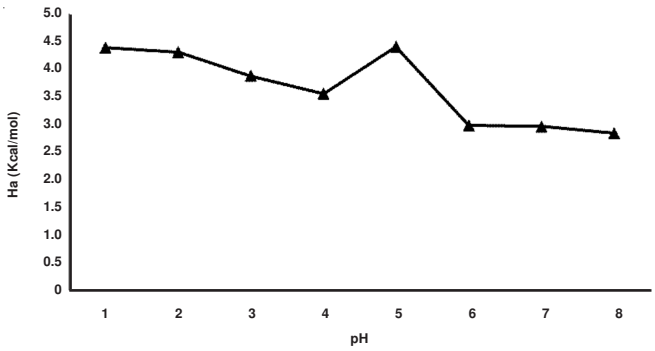

Fig. 3. Heat of activation (Kcal/mol) of sulfamethoxazole degradation at different pH values (3-10)

When heat of activation was calculated it was observed that heat of activation decreased from pH 3-pH 6 and increased at pH 7 and then decreased up to pH 10 in sulfamethoxazole. While in co-trimoxazole heat of activation decreased from pH 3-pH 6 and increased from pH 7-pH 10. It shows that trimethoprim has changed the heat of activation pattern. Due

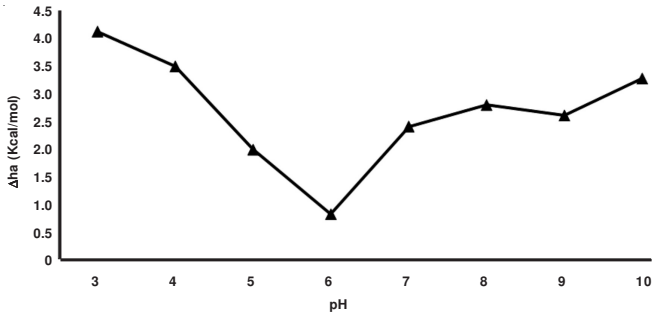

Fig. 4. Heat of activation (Kcal/mol) of sulfamethoxazole degradation in Co-trimoxazole at different pH values (3-10)

to low heat of activation of co-trimoxazole at pH 6-pH 7, photolysis can be rate-determining step of reaction[8].

In pharmaceutical field, the time required for 10 % of the drug to degrade is an important value to know, it represents a reasonable limit of degradation of active ingredients. When $t_{10\,\%}$ was calculated at pH 3-pH 10 at 25-60 ºC it was observed that $t_{10\,\%}$ decreased from pH 3-pH 6, maximum at pH 7 and then decreased gradually at pH 8-pH 10 in sulfamethoxazole (Fig. 5).

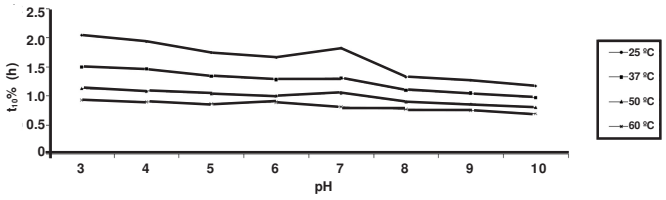

Fig. 5. $t_{10\,\%}$ (h) of sulfamethoxazole *versus* different pH at different temperatures

In co-trimoxazole it was found that $t_{10\,\%}$ decreased from pH 3-10 from 25-60 ºC. These results signify that trimethoprim

TABLE-2
LOG OF OBSERVED PSEUDO-FIRST ORDER RATE CONSTANT (K h^{-1}) OF SULFAMETHOXAZOLE DEGRADATION IN CO-TRIMOXAZOLE (Septran Susp.) AT DIFFERENT TEMPERATURES AT DIFFERENT pH

pH	Temperature (ºC)			
	25	37	50	60
3	60.45×10^{-3}	80.57×10^{-3}	103.63×10^{-3}	231.75×10^{-3}
4	62.32×10^{-3}	70.52×10^{-3}	90.47×10^{-3}	115.37×10^{-3}
5	61.32×10^{-3}	65.32×10^{-3}	75.61×10^{-3}	85.66×10^{-3}
6	70.52×10^{-3}	72.23×10^{-3}	85.33×10^{-3}	80.57×10^{-3}
7	75.61×10^{-3}	93.73×10^{-3}	85.32×10^{-3}	119.46×10^{-3}
8	80.44×10^{-3}	96.56×10^{-3}	105.33×10^{-3}	136.57×10^{-3}
9	82.44×10^{-3}	95.62×10^{-3}	121.32×10^{-3}	130.91×10^{-3}
10	90.47×10^{-3}	92.32×10^{-3}	108.49×10^{-3}	142.62×10^{-3}

has modified the degradation pattern and has increased that $t_{10\,\%}$ at all pH and temperatures values (Fig. 6). Our $t_{10\,\%}$ results are in agreement with Deans *et al.*[9].

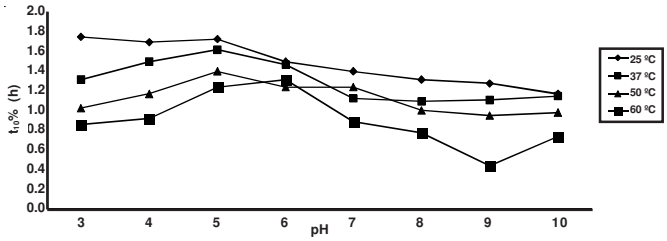

Fig. 6. $t_{10\,\%}$ (h) of sulfamethoxazole degradation *versus* different pH at different temperatures in co-trimoxazole

Conclusion

When percentage residual drug of sulfamethoxazole and co-trimoxazole was studied, they constituted different pattern. Rate of degradation with time in co-trimoxazole was more than sulfamethoxazole. It was concluded from study that stability of sulfamethoxazole in co-trimoxazole is better than sulfamethoxazole alone at different accelerated temperature conditions. Sulfamethoxazole in combination with trimethoprim is more stable at lower pH *e.g.*, pH 5.5 as compared to sulfamethoxazole at pH 7. Its half life can be prolonged by appropriate choice of vehicles besides control of other parameters such as pH, stabilizers and temperature.

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