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A Kinetic Study on the Loss of Sulfamethoxazole in Honey Solutions

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Abstract

A study on the kinetic of sulfamethoxazole in acetate buffer pH 3.6 containing 50% honey at various temperatures has been done. Samples were taken at time intervals until the concentration of sulfamethoxazole remaining was constant. As control, a solution of sulfamethoxazole in buffer solution without honey was treated at similar conditions. The concentration of sulfamethoxazole remaining was determined spectrophotometrically by using Bratton – Marshall method.
Results showed that the decrease in the concentration of sulfamethoxazole in buffer solution containing 50% honey followed pseudo first-order reversible kinetics with the forward rate constant of 0.0803; 0.1932; 0.2937/hr, the reverse rate constants of 0.0595; 0.1423; 0.2084/hr respectively at 60°, 70° and 80° C. Reaction followed the Arrhenius equation with the Ea value of 20.5459 kcal/mole and 21.1272 kcal/mole for the forward and reverse reaction respectively. The shelf life (t_{90}) of sulfamethoxazole in honey solution pH 3.6 at room temperature was 56.6769 hours.

Keywords : kinetic study, sulfamethoxazole, honey solution, glycosylamines

Introduction

Compounds with an amino group were known to readily react with reducing sugars to form glycosylamines. The reaction was the early step of a series of reactions known as Maillard reactions which was associated with the formation of characteristic color and odor in baking foods (Ledl and Schleicher, 1990).

Sulfamethoxazole was found to react with glucose and other reducing sugars such as lactose, maltose and galactose. It also reacts with reducing sugars found in milk, orange juice, apple juice and a carbohydrate containing drink. Lucida (1998) reported that there was a decrease in sulfamethoxazole concentration in those drinks which was associated with the formation of glycosilamines.

Honey is a source of carbohydrate which is consumed daily or taking as and with drug. Honey contains ± 82.2% of total carbohydrate ie. 38.2% fructose, 31.3% dextrose, 1.3% sucrose, 7.3% maltose, 1.5% oligosacharide etc (Crane, 1986).

Low et al. reported the loss of sulfathiazole in concentrated carbohydrate solutions which composition was similar to honey. The absorbance of sulfathiazole decreased 57–83% over 65 hour at 80°C. The authors described the loss of sulfathiazole was due to a complex formation in carbohydrate containing solution.

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There was a body of literatures that sulfamethoxazole could react with carbohydrate in honey and this reaction might have an effect on the drug concentration if taken with honey. This paper reports the kinetic of the loss of sulfamethoxazole in honey solution at various temperatures which allowed prediction of the shelf life of the drug in honey solution.

Materials and Methods

Reagents

All chemicals were reagent grade or better: sulfamethoxazole (Indofarma), naturally occurred honey (collected from Jambi area), sodium nitrit (Merck), ammonium sulfamate, N-(1-naphtyl)ethylenediamine dihydrochloride, sodium hydroxide, hydrochloric acid, methanol, acetic acid glacial acetic acid, sodium acetate, aethanol, hexane, chloroform and aquadest.

Apparatus

Spectrophotometer UV-visible (Shimadzu UV-160), Thin layer chromatography plates (silica gel 60 F254, Merck), pH meter, water bath.

Qualitative and quantitative analysis of sulfamethoxazole in honey solution

In a 100 mL volumetric flask, 50 mg of sulfamethoxazole was dissolved in 5 mL of methanol, 50 mL naturally

occurred honey was added and the solution was diluted with 0.2 M acetate buffer pH 3.6 to volume. As standard, 50 mg of sulfamethoxazole was dissolved in 5 mL methanol and diluted to 100 ml with 0.1 M acetate buffer pH 3.6. These solutions were stored for 24 hours at room temperature. Fifty µL of each solution was pippetted and then was spotted on a thin layer chromatoghraphy plate. The plate was placed in a solvent saturated chromatographic chamber with the mobile phase consisted of aethanol–methanol (95:5); hexane–chloroform–glacial acetic acid (2.5:2.3:2.5:0.7). The sample and the standard were eluted, the spots formed were then observed under the 254 nm UV lamp. The new spot with different retention time than that of standard sulfamethoxazole solution was identified as the reaction products of sulfamethoxazole with reducing carbohydrates in honey.

Quantitative analysis for the concentration of sulfamethoxazole remaining was done by taking the spot of sulfamethoxazole from the plate, and addition of the Bratton-Marshall reagent. The absorbance of the complex was measured by using spectrophotometric method at the range of 400 – 800 nm. The spot was taken, diluted in 5 mL of 0.1 N NaOH, and centrifused at 3000 rpm for 3 minutes. One mL of the supernatant was transferred quantitatively into a 25 mL volumetric flask, then 0.5 mL of 4N hydrochloric acid and 1mL 0.1% sodium nitrite solution were added. The solution was shaken and leaved 2 minutes for standing. One mL of 0.5% ammonium sulfamate solution was added and leaved 3 minutes for standing. Then 1 mL of 0.1% N-(1-naphtyl) ethylenediamine was added and leaved 10 minutes for standing. The solution was diluted to 25 mL by addition of aquadest. The absorbances of the coloured solution was measured at the wavelength between 400 and 800 nm to get the maximum wavelength of the complex.

Determination of the glucose contents of naturally occurred honey

The concentration of glucose in honey was determined by using acidic phenol colorimetric method at 489 nm. Twentyfive mL of honey was diluted with aquadest in a 100 mL volumetric flask. Five µL of the solution were pippeted, 1 mL of 5% phenol and 5 mL H_2SO_4 glacial were added, and shaken with vortex. This solution was leaved 20 minutes for standing until the solution turned orange. The absorbance of this solution was measured at 489 nm. The concentration of glucose was calculated based on calibration curve of pure glucose solutions determined by the same method.

The kinetic studies of sulfamethoxazole in honey solution

In a 1L volumetric flask, 400 mg sulfamethoxazole was dissolved in 50 mL methanol, diluted to volume with 0.2 M acetate buffer pH 3.6 to obtain a concentration of sulfamethoxazole of 400 µg/mL. For sample solution, 50 mL of the solution was transferred into a 100 mL volumetric flask, added to volume with naturally occurred honey and placed in a waterbath at 60°C. As standard, 50 mL of sulfamethoxazole solution was transferred into a 100 mL volumetric flask and made to volume with aquadest, placed in the same conditions as sample. At zero time, 0.5 mL of each of the sample and the standard solution was taken, the Bratton-Marshall reagent was added and make to 25 mL with aquadest. The absorbances of these solutions were measured. Samples were also taken at time intervals (at 15 min, 30 min, 1 , 2, 3, 4, and 5 hour) and analysed quantitavely for the remaining sulfamethoxazole. The same procedure was also done at temperatures 70 and 80 °C respectively.

Data analysis

Kinetic data were analyzed using the equation for first-order reversible kinetics reaction to determine the k_f and k_r values :

$$\log(A_t - A_{eq}) = \log(A_o - A_{eq}) - \frac{(k_f + k_r)t}{2.303} \qquad (1)$$

Data at various temperatures were then plotted according to Arrhenius equation to obtain the rate constants at room temperature :

$$\log k = \log A - \frac{Ea}{2.303RT} \qquad (2)$$

The shelf life of sulfamethoxazole in solution containing 50% honey was calculated using equation:

$$t_{90} = \frac{2.303 \log \frac{(A_o - A_{eq})}{(0.9A_o - A_{eq})}}{(k_f + k_r)} \qquad (3)$$

Results and Discussion

A modified Bratton-Marshall colorimetric method was used in this study to determine the stability of sulfamethoxazole in the presence of reducing sugars. The relationships between absorbance and sulfamethoxazole concentration was linear over the concentration range 1.0 – 3.0 µg/mL ($r = 0.9998$).

Qualitative analysis of sulfamethoxazole in solution containing 50% honey showed two spots with the retention times of 0.45 and 0.83 respectively. The TLC analysis of sulfamethoxazole standard solution at pH 3.6 showed one

spot with the retention time of 0.83. Therefore the broaden spot at 0.45 was predicted as that of the reaction product of sulfamethoxazole with carbohydrates in honey (glycosylamines). The glycosylamines standard was not available commercially. The spot with the retention time of 0.83 was taken, eluted and reacted with Bratton-Marshall reagent for the analysis of sulfamethoxazole remaining at time intervals for the kinetic study. The maximum wavelength of the sulfamethoxazole complex with the reagent was 541.5 nm.

The kinetic study showed that there was a decrease in the remaining sulfamethoxazole concentration with an increase in temperature (Figure 1). The reaction followed a pseudo first-order reversible kinetics which was agree with the reaction of sulfamethoxazole with glucose and other reducing sugars. Data analysis using equation (1) showed linear plots with the correlation coefficient at 60°, 70°, and 80° C of -0.9886, -0.9882 and -0.9961 respectively (Figure 2). These indicated that the reaction was pseudo first-order reversible.

Analysis of data using equation (2) showed that the reaction followed the Arrhenius equation with the correlation coefficient of -0.9992 and -0.9981 for the forward and the reverse reactions respectively (Figure 3). The energy of activation for the forward (Ea_f) and the reverse (Ea_r) reactions were 20.5459 kcal/mole and 21.1272 kcal/mole respectively. These results implied that the reverse reaction was more temperature dependent. Extrapolation of the Arrhenius plot at room temperature obtained the forward and reverse reaction constants (kf_{25} and kr_{25}) of 0.0015 and 0.0018/hr respectively. The shelf-life of sulfamethoxazole in 50% honey solution obtained by equation (3) was 56.6797 hours. This was much longer than the shelf-life of sulfamethoxazole in a 5% glucose solution at the same pH value and room temperature ie. 1.05 hour (Lucida, et al.). This result agreed with the fact that the reaction of sulfamethoxazole with glucose was much more rapid than with other mono and disaccharides (Lucida, 1998).

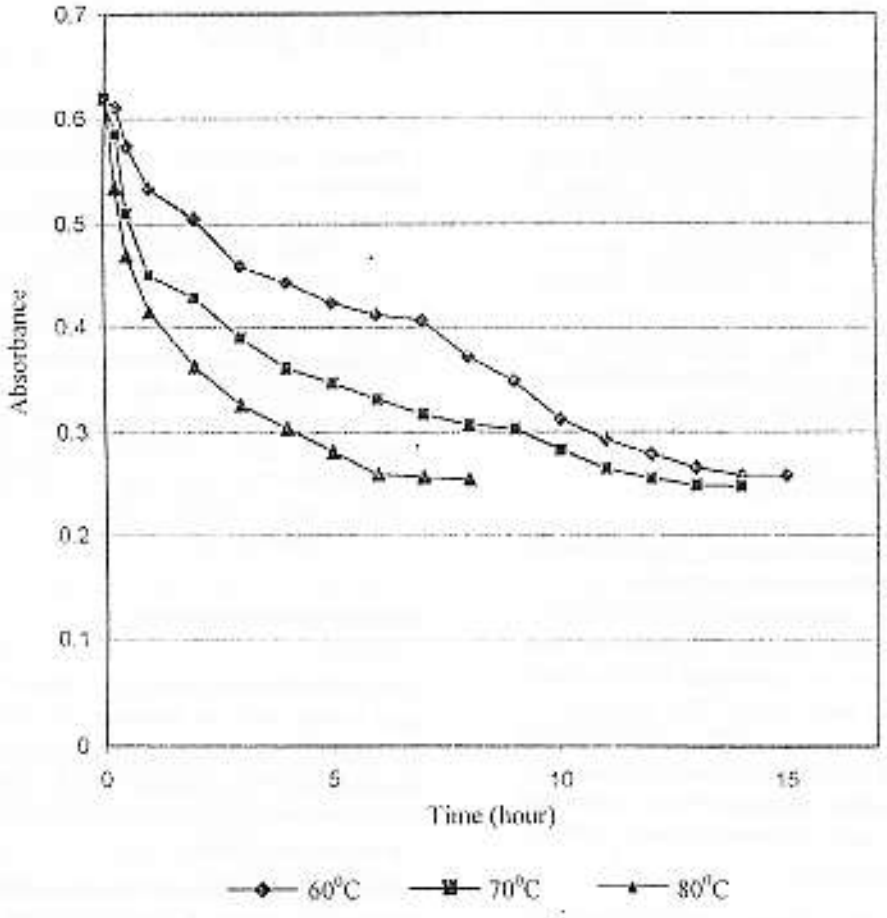

Figure 1. The decrease in sulfamethoxazole concentration in buffer solution pH 3.6 containing 50% honey at various temperatures

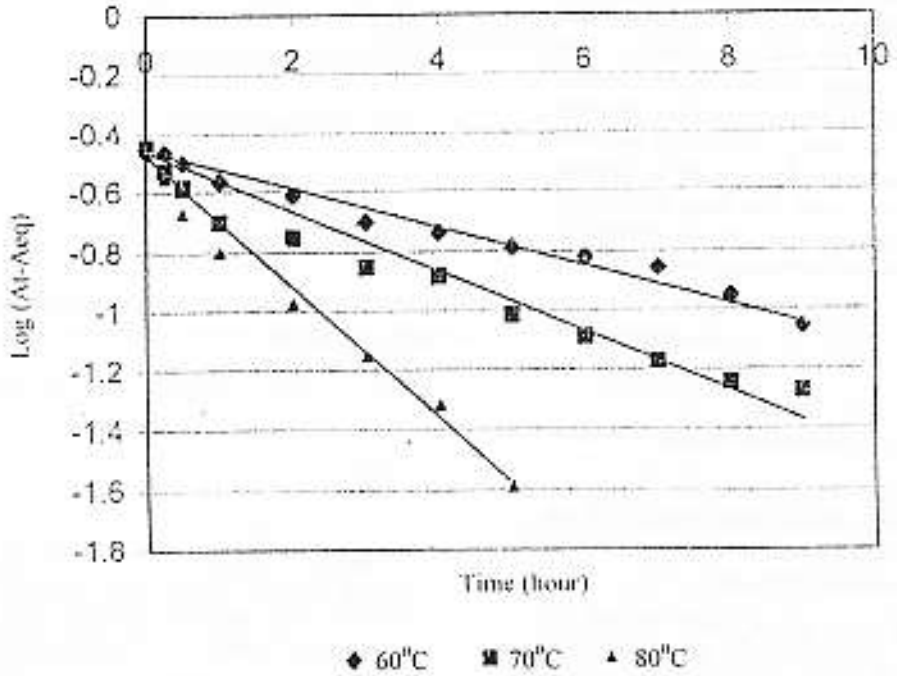

Figure 2. First order revesible kinetic plots of the stability of sulfamethoxazole in buffer solution pH 3.6 containing 50% honey at various temperatures

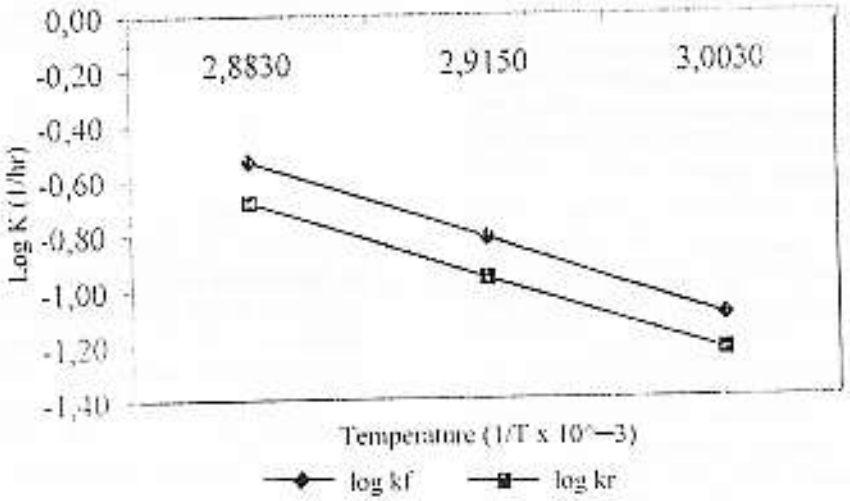

Figure 3. Arrhenius plots for the forward and the reverse reactions of sulfamethoxazole with carbohydrates in buffer solution pH 3.6 containing 50% honey

Determination of the glucose content of honey solution used in this study indicated that the glucose content was 24.4492% which was lower than that in literatures. This glucose content was the major reactant which reacted with sulfamethoxazole to form the reaction product. Other reducing sugars occurring in honey such as maltose and sucrose (which was hydrolyzed to glucose and fructose) may also react with the drug. This study implied that there was a possibility of an interaction between sulfamethoxazole with carbohydrates in honey.

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