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Kinetic study of the reaction of sulfamethoxazole and glucose under acidic conditions I. Effect of pH and temperature

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Abstract

The kinetics of the reaction of sulfamethoxazole (SMX) in 5% w/v glucose to form the corresponding α- and β-glucosylamines over the pH range of 0.80–6.88 at 37°C has been investigated. The identity of the glucosylamines was determined by ^{1}H-nuclear magnetic resonance spectroscopy of an authentic sample of the α-glucosylamine (USP) and the reaction products, and by interconversion of this compound to the corresponding β-anomer. The reaction followed pseudo first-order reversible kinetics and involved specific acid and general acid–base catalysis. The pH-rate profile demonstrated that over the pH range of 0.80–2.90 and 5.50–6.88 the reactions were dependent on H^+ concentration but pH independent between pH 3.00–5.45, which reflects the influence of ionization of SMX and the glucosylamines on the reversible reaction. Interpretation of the data with respect to kinetic models and rate equations for the formation and hydrolysis of the glucosylamines was investigated. Temperature dependence studies followed the Arrhenius equation with an Ea of 49.28 kJ mol^{-1} for the forward and 63.46 kJ mol^{-1} for the reverse reaction at pH 2.89 respectively.

Keywords: Dextrose; Glucose; Glucosylamines; Kinetics; Reversible reactions; Sulfamethoxazole

1. Introduction

Primary aromatic amines have been known to readily react with reducing sugars such as glucose to form the corresponding glucosylamines (Ellis and Honeyman, 1955; Capon and Connett, 1965a). Such reactions have been reported for procainamide following admixture to glucose infusion solution causing a significant loss of the drug (Sianipar et al., 1994). It was also found to occur in lactating cows dosed with sulfonamides, the reaction with lactose in the milk resulting in the formation of lactose conjugates of the sulfonamides (lactosylamines) (Paulson et al., 1992). Heinze et al. (1991) demonstrated a considerable retardation in the absorption of sulfonamides in calves when the drugs were administered orally concurrently with milk resulting in decreased blood levels and bioavailability. Glucosylamines have been identified as major components of sul-

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fonamide residues found in swine tissues following administration of sulfamerazine (Giera et al., 1982a,b; Fisher et al., 1992).

A limit test has been applied for sulfamethoxazole N_4-glycoside (glucosylamine) for the oral suspension of the combination of sulfamethoxazole (SMX) and trimethoprim by the US Pharmacopeial Convention (1994). This product arises from the reaction of sulfamethoxazole with glucose when carbohydrates are used as flavouring agents in this formulation. The reaction between SMX and reducing sugars such as lactose and maltose in solid physical mixtures has also been reported, but the nature of the reaction products has never been investigated (Tarjanyi et al., 1971; Ghanem et al., 1980; Meshali, 1984).

A recent study on the influence of reducing sugars in beverages on SMX bioavailability in humans showed that despite a delay in drug absorption there was no significant change in the overall bioavailability of SMX when administered with a 10% glucose solution (Parkin et al., 1997). However, dissolution data demonstrated that SMX reacted with reducing sugars in beverages under acidic conditions at a significant rate in dissolution media containing carbohydrate rich drinks.

It is considered important to understand the nature of the reaction of SMX with glucose over the pH range encountered physiologically. This study reports the kinetics of the reaction of SMX and glucose in the formation of glucosylamines under acidic conditions at 37°C.

2. Materials and methods

2.1. Materials

D-glucose, AR anhydrous (Chem-Supply, Australia), D-glucose monohydrate (BDH, UK), SMX (Sigma Chemical Company, USA) and SMX N_4-glucoside, USP Reference standard (US Pharmacopeial Convention, 1994) were used. All materials and reagents were of analytical reagent or HPLC grade and used as purchased. All solutions were prepared using Milli-Q water (Millipore, Australia).

2.2. HPLC analysis

The chromatographic system consisted of a Model 501 HPLC pump (Waters Assoc. USA), a Model 7152, loop injector (Rheodyne, USA), a Model 484 tunable absorbance detector (Waters Assoc.) and an HP 3396A integrator (Hewlett Packard, USA). Photodiode array ultraviolet spectrophotometry was also used to monitor the eluate, which consisted of a Model 991 photodiode array detector (Waters Assoc.), Profound 386SX monitor and a Model 5200 printer plotter (Waters Assoc.).

The analysis was made using a 300×3.9 mm I.D. Bondclone 10 C18 column (Phenomenex, USA) for SMX and glucosylamines.

The mobile phase consisted of methanol-0.1 M phosphoric acid (20:80 v/v) containing 0.1% v/v triethylamine and adjusted to pH 6.0 with sodium hydroxide. The flow rate was 1.5 ml min^{-1}, the monitoring wavelength 263 nm and the injection volume 20 μl.

The stability indicating nature of the assay was investigated by reacting glucose and SMX and also SMX alone in acid (0.16 M HCl) and alkali (0.16 M NaOH) at 70°C for 8 and 19 h respectively. No degradation was detectable in the absence of glucose. Considerable loss of SMX was evident in the presence of glucose and the HPLC traces indicated three new peaks from acid and four from alkaline degradation. There was evidence of glucose degradation. None of these peaks interfered with the SMX peak which, in both cases, was quantitatively restored on the addition of the calculated concentrations of SMX.

2.3. Nuclear magnetic resonance (NMR) study

Solution A (reactant mixture): This was prepared by dissolving SMX (0.250 g, 10^{-3} mol) and anhydrous glucose (0.178 g, 10^{-3} mol) in methanol (10 ml) and transferring 1 ml of this solution to each of ten 5 ml ampoules.

Solution B (catalyst): This was prepared by dissolving oxalic acid (0.100 g, 1.1×10^{-3} mol) in deuterium oxide (1 ml) and transferring 50 μl (equivalent to 5 mg of oxalic acid) to each of ten 5 ml ampoules.

These solutions were evaporated to dryness under a stream of nitrogen and redissolved three times in deuterium oxide (0.2 ml) followed by evaporation under a stream of nitrogen to dryness. The ampoules were then stored in a desiccator and sealed.

Solution C (deuterated buffer): This was prepared by evaporating to dryness at 90°C 1 M phosphate buffer pH 8.0 (0.5 ml in 5 ml ampoules). These were redissolved three times in deuterium oxide (0.1 ml) followed by evaporation at 90°C in an oven. The ampoules were then sealed.

Solutions of glucosylamine were prepared by admixture of solutions A and B. Ampoules of A were dissolved in deuterated methanol (1 ml), and the solution transferred to ampoules B and incubated at 37°C. The reaction was monitored by dilution of a sample with methanol and analysis by HPLC at regular intervals for 2 days. After 2 days the solution was evaporated to dryness and stored in a desiccator at 4°C until NMR analysis. For the NMR study, these ampoules were dissolved in deuterium oxide (1 ml) containing the deuterated buffer. The ^{1}H spectra were recorded at 500 MHz (a model ARX-500 NMR spectrometer, Brukker, Germany) using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the chemical shift standard.

2.4. pH measurements

The pH was measured with a Model 8417 Hanna Instruments (Hanna, Singapore) calibrated with primary buffer solutions of pH 4.00 and 7.00 or a Metrom 632 pH-meter (Selby Scientific) calibrated with 0.05 M potassium tetraoxalate and temperature compensated for measurement at 37°C (Perrin and Dempsey, 1974).

The pH of the reaction mixtures were measured at the beginning, in the middle and at the end of each experiment. The pH values reported in the results were obtained at the end of the reaction, which was always less than 0.02 pH units from the initial pH. The measured pH corresponds to the hydrogen ion activity (a_{H^+}) rather than the concentration (Perrin and Dempsey, 1974), thus in the pKa determination and all kinetic equations [H^+] will refer to the activity of H^+.

2.5. pK_a determination

The pK_a values were determined using the ultraviolet-visible (UV) spectrophotometric method of Albert and Serjeant (1984) under the conditions of the kinetic study. All measurements were made at 37 ± 1°C using a 1 cm cell (A model 8452 Hewlett Packard (HP) diode array spectrophotometer). The absorbance of the molecular (A_{SMXH}), protonated (A_{SMXH^+}) and deprotonated (A_{SMX^-}) forms of SMX were determined in solutions of pH 3.82 (0.1 M acetate buffer, $\mu = 0.50$), 2 M hydrochloric acid and pH 7.85 (0.067 M phosphate buffer, $\mu = 0.50$) (37°C), respectively where the pure species are present (μ = ionic strength).

2.6. Kinetic studies

2.6.1. Buffer systems

The buffer systems used in the kinetic studies were phosphate (pH 2.00–3.00 and 5.50–6.80), pyridine (pH 4.00) and acetate (pH 4.00 and 5.00) adjusted with sodium chloride to the ionic strength (μ) of 0.50. At low pH values (0.80–1.55) a mixture of hydrochloric acid and sodium chloride ($\mu = 0.50$) were used. The pH of this solution was determined using the equation (Perrin and Dempsey, 1974):

$$\mathrm{pH} = -\log[\mathrm{HCl}] - 0.16 \quad (1)$$

For the interconversion of the glucosylamines, the buffer systems used were pH 5.80, 6.00, 6.80 and 7.20 phosphate buffer ($\mu = 0.50$) adjusted with sodium chloride.

2.6.2. Analysis of kinetic data

The kinetic study was performed under pseudo first-order conditions by maintaining the glucose in high molar excess over SMX. The forward reaction is the formation of the glucosylamines and their subsequent hydrolysis is the reverse reaction (Eq. (2)).

$$\mathrm{SMX} \underset{k_r}{\overset{k_f}{\Leftrightarrow}} \mathrm{GA} \quad (2)$$

where SMX is sulphamethoxazole, GA the glucosylamines and k_f and k_r are the forward and reverse rate constants, respectively. This equation is a simplification of the total reaction since the

interconversion of α- and β-glucose and that of the α- and β-glucosylamines is much faster than the formation and hydrolysis of the glucosylamines and the equilibrium ratios of the two anomers of glucose and glucosylamines are kept constant during the reaction (Kharmats and Afanas'ev, 1968).

A solution of the α-glucosylamine (USP) was unstable, hence this could not be used as a standard. Therefore the concentration of the glucosylamines was calculated from a comparison to the concentration of SMX standard solution by application of a correction factor. This is possible because the photodiode-array data demonstrate that the two anomers of the glucosylamine have similar spectroscopic characteristics to that of SMX with higher molar absorptivities at the monitoring wavelength (λ 263 nm). A correction factor was derived using the method Sianipar et al., (1994) which gave a value of (0.7345 ± 0.0137). By introducing this value to all the kinetic data at various pH values the total concentration of reactants and products corresponded within (100 ± 5)% of the initial SMX concentration.

The concentration data were analyzed using a direct least squares fit of Eq. (3):

$$\ln \{[A_t] - [A_{eq}]\} = \ln \{[A_0] - [A_{eq}]\} - (k_f + k_r)t \quad (3)$$

where A_0, A_t and A_{eq} are the SMX concentration at zero, time (t) and at equilibrium, respectively and k_f and k_r are the forward and the reverse rate constants. A_{eq} is determined from the computer program developed by Irwin (1990). The observed pseudo-first order rate constants ($k_f + k_r$) for the reversible reaction were obtained from the slope of the linear relationship derived from Eq. (3). The individual rate constants were calculated from the equation for the equlibrium constant (Eq. (4):

$$K = \frac{[GA_{eq}]}{[SMX_{eq}]} = \frac{[A_t] - [A_{eq}]}{[A_{eq}]} = \frac{k_f}{k_r} \quad (4)$$

Experiments involving interconversion of the α-glucosylamine were performed in the presence of 5% w/v glucose to suppress hydrolysis of the glucosylamine. The concentration data were treated similarly as for those of the formation of the glucosylamines.

2.6.3. Formation of the glucosylamines

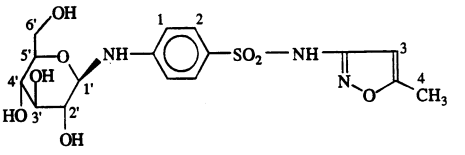
Glucose-1-hydrate (5.5 g, equivalent to 5 g of anhydrous glucose) was dissolved in 50 ml of the appropriate double strength buffer solution ($\mu = 1.00$) in a 100 ml volumetric flask and equilibrated to 37°C. A freshly prepared SMX stock solution (2.5×10^{-3} M) in water containing 4% v/v methanol was also pre-equilibrated to temperature. At zero time, 20 ml of this stock solution was added to the glucose and buffer solution and the flask made to volume with water pre-equilibrated to temperature to obtain a solution of 5×10^{-4} M SMX and 5% w/v glucose in the buffer with the ionic strength (μ) of 0.50 M. The solution was thoroughly mixed and at regular time intervals samples from the solution were withdrawn and submitted to HPLC analysis. The pH of the reaction mixtures was recorded.

2.6.4. Interconversion of the glucosylamines

Glucose-1-hydrate (2.75 g, equivalent to 2.50 g of anhydrous glucose) was dissolved in 25 ml of the appropriate double strength buffer solution ($\mu = 1.00$) in a 50 ml volumetric flask and equilibrated to 37°C. A freshly prepared stock solution of α-glucosylamine (1.25×10^{-3} M) in 0.1 M Na_2HPO_4 solution was also pre-equilibrated to temperature. At zero time, 10 ml of this stock solution was added to the buffer solutions and the flasks made to volume with water pre-equilibrated to temperature to afford a solution of 2.5×10^{-4} M glucosylamine and 5% w/v glucose in the buffer with the ionic strength (μ) of 0.50 M. The solutions were thoroughly mixed and at regular time intervals samples of the solution were withdrawn and submitted to HPLC analysis. The pH of the reaction mixtures was recorded.

2.6.5. Activation parameters

The kinetic experiments of solutions in pH 2.89 (0.1 M phosphate buffer) were performed at 27, 32, 37 and 42°C. Data were treated according to the Arrhenius equation and the van't Hoff equation.



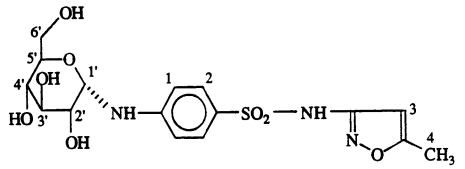


Fig. 1. Chemical structure of the glucosylamines of SMX.

3. Results and discussion

3.1. Identification of the products

For the NMR study, the glucosylamines (Fig. 1) were prepared by reacting equimolar (10^{-4} mol) amounts of SMX and glucose under acidic conditions (5.5×10^{-5} mol oxalic acid) in methanol at 37°C followed by removal of the solvent when equilibrium had been achieved. Oxalic acid was used as the catalyst as it has no non-exchangeable protons and so would not appear in the ^{1}H NMR spectrum (Kemp, 1991). For NMR measurements, this mixture was redissolved in pH 8.0 deuterated phosphate buffer to avoid hydrolysis of the glucosylamines during data acquisition. Signal assignments are listed in Table 1.

HPLC analysis of the equilibrated mixture demonstrated that under these conditions, 88.8% of SMX had reacted and thus existed as the α- and β-anomers of the glucosylamine, leaving 11.2% of free SMX and a mole equivalent of glucose as a mixture of the α- and β-anomers.

Signals at 4.66 (doublet, $J = 8.83$ Hz) and 5.19 ppm (doublet, $J = 4.79$ Hz) were assigned as those of the anomeric proton at $C_{1'}$ of the β- and α-glucosylamine, respectively. On the basis of coupling constants and chemical shift values previously reported for analogous compounds and

Table 1
^{1}H-NMR signal assignments for the reaction mixture[a]

Chemical shift (ppm)	Signal with relevant coupling constant	Proton assignment	Integration relative to C_4 protons (H = 3)	Mol. fraction of components
2.01		C_4(SMX)	0.41	
2.04		C_4(α-+β-GAs)	2.59	
3.15−3.80	Multiplets	C'_2–C'_6(α-+β-Glu and α-+β-GAs)	6.10	
4.56	Doublet ($J = 7.98$)	C'_1(β-Glu)	0.11	0.813 as α- and β-GA (0.678 as β-GA and
4.66	Doublet ($J = 8.83$)	C'_1(β-GA)	0.65	0.135 as α-GA); 0.187 as α- and β-glucose
5.14	Doublet ($J = 3.76$)	C'_1(α-Glu)	0.07	(0.117 as β-glucose and 0.070 as α-glucose)
5.19	Doublet ($J = 4.79$)	C'_1(α-GA)	0.13	
5.58		C_3(SMX)	0.14	
5.60		C_3(α-+β-GAs)	0.85	
6.69		C_1(SMX)	0.43	0.198 as free SMX, 0.664 as β-GA and
6.76		C_1(β-GA)	1.33	0.138 as α-GA
6.84		C_1(α-GA)	0.28	
7.50		C_2(SMX)	0.44	0.217 as free SMX, 0.783 as α- and β-GAs
7.56		C_2(α-+β-GAs)	1.59	

[a] SMX, sulfamethoxazole; GA, glucosylamine; Glu, glucose.

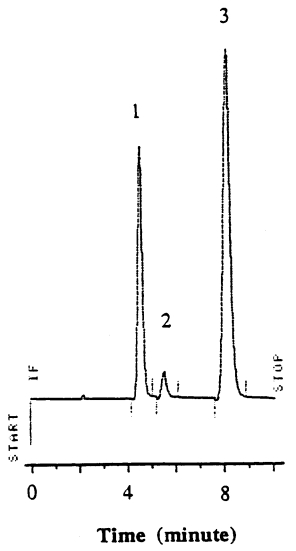


Fig. 2. A representative chromatogram for the β-and α-glucosylamines (1 and 2) and SMX (3).

from these signals the equilibrium ratio was derived (Chavis et al., 1983; Silverstein et al. 1991).

The ratio of the α- and β-glucosylamines found by NMR (16.6% α-:83.4% β-) is consistent with that found by HPLC (15.5% α-:84.5% β-). This ratio is different to that determined from the kinetic data (10.0% α-:90.0% β-) as the equilibrium was reached in a different solvent (methanol). The data showed that the β-anomer was favoured. The results reported by Sianipar et al. (1994) were 12.9% α-:87.1% β- for the glucosylamines of procainamide in aqueous solution, and by Capon and Connett (1965a,b), who found 10% α-:90% β- for the analogous para-substituted *N*-aryl-glucosylamines. The anomers of glucose were found in the proportions expected (37.5% α-:62.5% β-) (El Khadem, 1988).

The assignment of ^{1}H signals in the NMR spectrum of authentic USP SMX N_4-glycoside under similar conditions demonstrated that it consists of the less stable α-anomer of the glucosylamine and confirms the identity of the signal assignments in the reaction mixture.

3.2. The experimental pK_a values

The pK_a values for SMX were calculated from the UV absorption data at the analytical wavelengths of 267 and 244 nm for protonation of the amino-group (pK_{a1}) and deprotonation of the sulphonamido group (pK_{a2}), respectively. The average values of $1.69 + 0.005$ (pK_{a1}) and $5.57 + 0.053$ (pK_{a2}) for SMX were obtained. These values are in good agreement with those reported by Koizumi et al. (1964), corrected for the ionic strength effect (p$K_{a1} = 1.60$ and p$K_{a2} = 5.64$; $\mu = 0.50$).

The dissociation constants of the glucosylamines were not determined due to the instability of these compounds under acidic conditions. Sianipar et al. (1994) proposed that the pK_a values for the protonation of the glucosylamines of procainamide were much lower than the corresponding pK_a values of the amine (p$K_a = 2.75$) and in solution of 0.5 M hydrochloric acid, the glucosylamines were substantially protonated. Considering this data and supported by the kinetic data, the pK_{a1} of SMX glucosylamines was proposed to be 0.50 or below and the pK_{a2} to be similar to that of SMX.

3.3. Kinetics of the formation of the glucosylamines

The analytical method used in the study afforded well resolved peaks for the β-, α-glucosylamines and SMX (Fig. 2). The two anomers of glucosylamine eluted earlier than SMX due to their more polar nature. There was no evidence of the presence of detectable levels of intermediate (the imine) under any conditions. The assay was stability indicating and fully validated for both SMX and the glucosylamines.

The HPLC data show a proportional increase of glucosylamines formed with the loss of SMX. In all cases the proportion of the two glucosylamine anomers was found to be 90% β- and 10% α- at equilibrium. For the kinetic data, the two anomers were calculated as total glucosylamine. The representative concentration-time profile for the reaction is shown in Fig. 3 and in all kinetic experiments, the observed rate of disappearance

of SMX strictly followed pseudo-first order reversible kinetics over at least three half-lives of the reaction (Fig. 4).

The reaction was buffer catalyzed under all conditions and a study of this buffer effect on the reaction rate will be reported in a later publication.

Buffer independent rate constants (Table 2)

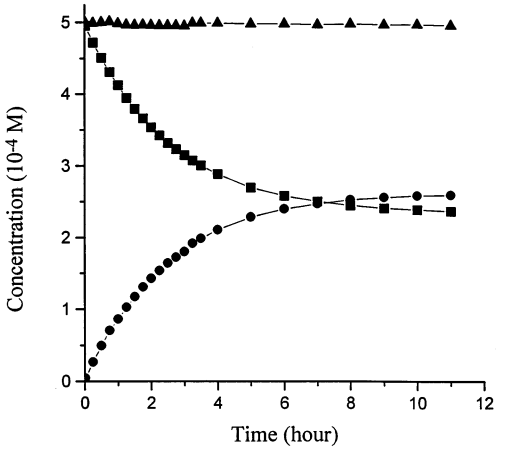


Fig. 3. Representative plot for concentration-time profile of the reaction of 5×10^{-4} M SMX with 5% w/v glucose in 0.1 M acetate buffer pH 3.82 ($\mu = 0.50$; 37°C). SMX: ■; the α-and β-glucosylamines: ●; total concentration: ▲.

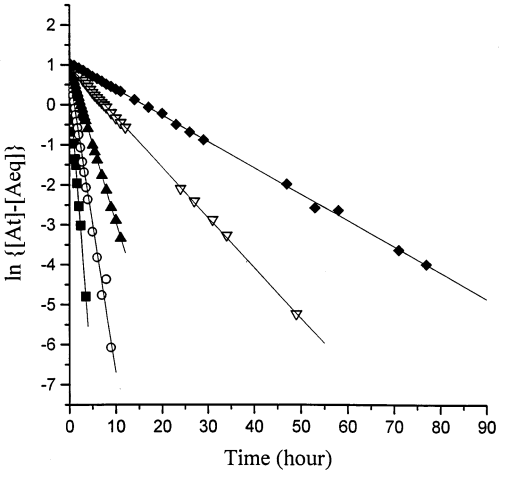


Fig. 4. Typical pseudo first-order plots for the reaction of 5×10^{-4} M SMX with 5% w/v glucose in buffer solution at various pH values ($\mu = 0.50$; 37°C). pH: ■: 1.55; ○:2.10; ▲:3.82; ▽: 5.05; ◆:6.09.

Table 2
Buffer independent rate constants for the reaction of 5×10^{-4} M SMX with 5% w/v glucose at various pH values ($\mu = 0.50$; 37°C)[a]

pH	k_i forward (h^{-1})	k_i reverse (h^{-1})	K
0.80	0.7487	4.0689	0.1840
1.09	0.7040	2.8195	0.2497
1.55	0.5373	0.9865	0.5446
2.10	0.1068	0.1088	0.9816
2.90	5.81×10^{-2}	5.43×10^{-2}	1.0707
3.93	6.58×10^{-2}	5.22×10^{-2}	1.2612
5.04	4.64×10^{-2}	4.01×10^{-2}	1.1589
5.50	4.25×10^{-2}	3.27×10^{-2}	1.2997
6.09	2.81×10^{-2}	2.13×10^{-2}	1.3218
6.68	7.70×10^{-3}	5.46×10^{-3}	1.4118
6.88	6.11×10^{-3}	4.66×10^{-3}	1.3116

[a] k_i, buffer independent rate constants; K, the equilibrium constant.

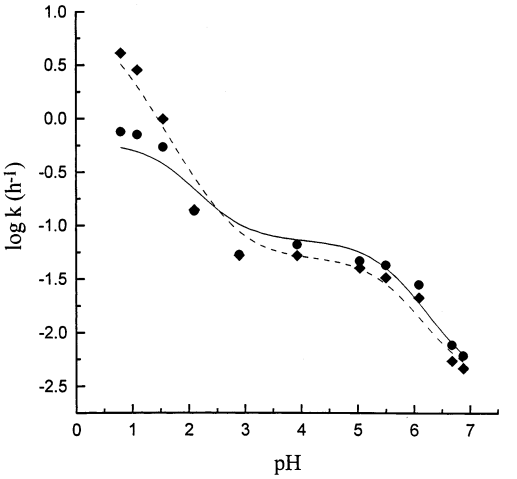


Fig. 5. Plot for pH-rate profile of the reaction of 5×10^{-4}M SMX with 5% w/v glucose in aqueous solution ($\mu = 0.50$; 37°C). k_f: —●—; k_r: —◆—.

were obtained from reaction mixtures which contained only hydrochloric acid or from the intercept of plots of the observed rate constants against the total buffer concentration for the forward and reverse reactions. The pH-rate profiles of the reactions (Fig. 5) show two inflections at pHs corresponding to the experimental pK_a of SMX. This reflects the different reactivities of the protonated ($SMXH^+$), molecular (SMXH) and deprotonated (SMX^-) species of SMX for the

forward and those of the glucosylamines (GAH^+, GAH and GA^-) over the pH range studied.

The pH-rate profile shows that at a pH around pK_{a1}, k_f decreased but k_r increased. This may be interpreted as the result of the protonation of the amino-group, which perturbed the equilibrium position of the reaction (K). It is a mechanistic requirement for the reaction that the amine must have available lone pair electrons to attack the carbonyl group (Jencks, 1964). Therefore, $SMXH^+$ may be assumed to be unreactive. As the proportion of this species increases with the decrease in pH, the equilibrium position of the reaction shifts to the reactants which leads to reduced formation of glucosylamines at lower pH. That is, the formation of the glucosylamines decreases but their hydrolysis would increase and may then decrease at pH values around the pK_a of the glucosylamines.

This effect is demonstrated by a non linear best-fit of the equilibrium constant (K) against pH. K can be described as the sum of terms in Eq. (5) for the equilibrium position at pH below and above 3.5.

$$K = \frac{[GA]_{eq}}{[SMX]_{eq}} = \frac{[GA]_{eq}}{([SMXH]_{eq} + [SMXH^+]_{eq})} + \frac{[GA]_{eq}}{([SMXH]_{eq} + [SMX^-]_{eq})} \quad (5)$$

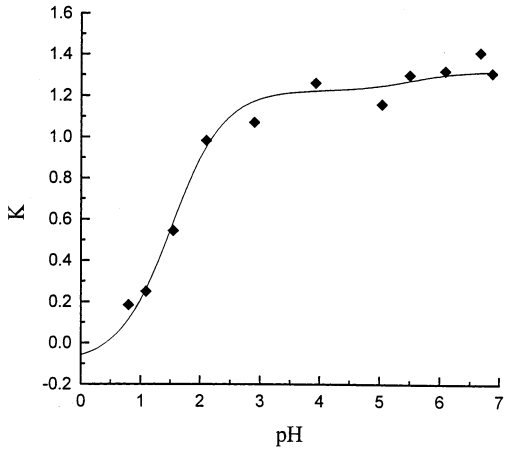


Fig. 6. Plot of the equilibrium constant (K) against pH.

where $[GA]_{eq}$, $[SMXH]_{eq}$, $[SMXH^+]_{eq}$ and $[SMX^-]_{eq}$ are the concentrations of the glucosylamines, the molecular, the protonated and the deprotonated forms of SMX at equilibrium, respectively. $[SMX]_{eq}$ is the concentration of total SMX at equilibrium.

From the ionization of SMX, the following equations are obtained:

$$[SMXH^+] = [SMXH] \cdot 10^{(pK_{a1} - pH)} \quad (6)$$

$$[SMX^-] = [SMXH] \cdot 10^{(pH - pK_{a2})} \quad (7)$$

where the hydrogen ion activity $(a_{H^+}) = 10^{-pH}$ and $K_a = 10^{-pK_a}$. Analogously for the proportion of the species at the equilibrium, substitution of Eq. (6) and Eq. (7) to Eq. (5) gives the following equation for K over the pH range studied:

$$K = \frac{[GA]_{eq}}{\{[SMXH]_{eq}(1 + 10^{pK_{a1} - pH})\}} + \frac{[GA]_{eq}}{\{[SMXH]_{eq}(1 + 10^{pH - pK_{a2}})\}} \quad (8)$$

Applying Eq. (8) to the experimental data, a non-linear least squares best-fit of K as a function of pH was obtained (Fig. 6). A mathematical model for the best-fit is given in Eq. (9).

$$K = \frac{p_1}{1 + 10^{(p_2 - pH)}} + \frac{p_3}{1 + 10^{(pH - p_4)}} \quad (9)$$

where K is the equilibrium constant at zero buffer concentration and p the parameters ($p_1 = 1.32 \pm 0.05$; $p_2 = pK_{a1} = 1.52 \pm 0.10$; $p_3 = (-9.71 \times 10^{-2}) \pm 0.05$; and $p_4 = pK_{a2} = 5.53 \pm 1.38$). Statistical analysis showed that the p_3 value was not significantly different from zero.

The asymptotic value at low pH reaches zero (Fig. 6), indicating that the protonation of the amino-group (pK_{a1}) perturbs K. This supports the interpretation that $SMXH^+$ is unreactive and therefore makes no contribution to the overall reaction.

The fit for the data at high pH demonstrates that the ionization of the sulphonamido group (pK_{a2}) slightly affects k_f and k_r, and hence K. Data at this pH range were scattered, giving a high standard deviation for the calculated pK_{a2}. This may be due to the second ionisation of the glucosylamines, which was assumed to be similar

to that of SMX. The asymptotic value of K at high pH (Fig. 6) was 1.32, which corresponds to that of SMX–SMX$^-$ for the forward and GA–GA$^-$ for the reverse reactions. The calculated pK_a values from the fit (p$K_{a1} = 1.52$ and p$K_{a2} = 5.53$) were in acceptable agreement with the experimental values of 1.69 and 5.57, respectively (literature values: p$K_{a1} = 1.60$ and p$K_{a2} = 5.64$, $\mu = 0.50$) (Koizumi et al., 1964).

At intermediate pHs (3.5–5.5), the pH profile showed an independent region with respect to the H^+ concentration. In this region, SMXH and SMX$^-$ are present with SMXH predominating. At pH values above 5.5 where SMX$^-$ predominates the rate constants decrease markedly with increase in pH. This implies that the two species have different reactivities. SMX$^-$ seems to be much more reactive than SMXH, so that with a slight increase in the H^+ concentration at high pH the reaction rate increases significantly. At intermediate pH values where the H^+ concentration is much higher, the reactivity of SMX$^-$ is compensated by that of the less reactive SMXH, which results in a plateau on the pH-rate profile. At low pH values (below 3.5), the SMXH fraction decreases but with the higher H^+ concentration, the reaction rates increase markedly and slow down upon protonation of the amine.

The slope of the pH-rate profiles suggest that the overall velocity of the reaction is a summation of the H^+ and the water catalytic constants of SMXH and SMX$^-$ for the forward and correspondingly GAH and GA$^-$ for the reverse reaction. The following total mechanism was proposed for the forward (Eqs. (10)–(15)) and reverse (Eqs. (16)–(21)) reactions:

$$\mathrm{SMXH} + \mathrm{H}^+ \overset{K_{bS}}{\rightleftharpoons} \mathrm{SMXH}^+ \tag{10}$$

$$\mathrm{SMXH}^+ \overset{k_H}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{11}$$

$$\mathrm{SMXH} \overset{k_H}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{12}$$

$$\mathrm{SMX}^- + \mathrm{H}^+ \overset{K_{bS}^-}{\rightleftharpoons} \mathrm{SMXH} \tag{13}$$

$$\mathrm{SMXH} \overset{k_H^-}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{14}$$

$$\mathrm{SMX}^- \overset{k_0^-}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{15}$$

$$\mathrm{GAH} + \mathrm{H}^+ \overset{K_{bG}}{\rightleftharpoons} \mathrm{GAH}^+ \tag{16}$$

$$\mathrm{GAH}^+ \overset{k_{-H}}{\rightarrow} \text{Products} \tag{17}$$

$$\mathrm{GAH} \overset{k_{-0}^{H_2O}}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{18}$$

$$\mathrm{GA}^- + H^+ \overset{K_{bG}^-}{\rightleftharpoons} \mathrm{GAH} \tag{19}$$

$$\mathrm{GAH} \overset{k_{-H}^-}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{20}$$

$$\mathrm{GA}^- \overset{k_{-0}^-}{\underset{H_2O}{\rightarrow}} \text{Products} \tag{21}$$

where the superscript - on the rate constants (k) refer to the deprotonated species; no superscript refers to the molecular species. The subscripts 0 and H refer to the solvent and the H^+ catalytic constants for the forward reaction, the subscript -0 and -H refer to the solvent and H^+ catalytic constants for the reverse reaction. The model involves a pre-rate determining step involving protonation for each of the species followed by the addition of the amine. K_{bS}, K_{bS}^- and K_{bG}, K_{bG}^- refer to the equilibrium constants for the protonation of the respective SMX and glucosylamines species.

The overall rate of the reaction for the forward reaction over the pH range studied can be written as:

$$v = -\frac{\mathrm{d}[\mathrm{SMX}]_t}{\mathrm{d}t} = k_{obs} \cdot [\mathrm{SMX}]_t \tag{22}$$

where $[\mathrm{SMX}]_t = [\mathrm{SMXH}] + [\mathrm{SMX}^-]$. Introducing the terms in the kinetic model to Eq. (22), the following equation for the pH-rate profile is obtained for the forward reaction:

$$k_{f_{obs}} = \{(k_0 + k_H \cdot [\mathrm{H}^+])\, f_{\mathrm{SMXH}}\} + \{(k_0^- + k_H^- \cdot [\mathrm{H}^+])\, f_{\mathrm{SMX}^-}\} \tag{23}$$

Analogously, the following equation applies for the reverse reaction:

$$k_{r_{obs}} = \{(k_{-0} + k_{-H} \cdot [\mathrm{H}^+])\, f_{\mathrm{GA}}\} + \{(k_{-0}^- + k_{-H}^- \cdot [\mathrm{H}^+])\, f_{\mathrm{GA}^-}\} \tag{24}$$

The overall rate equation for the disappearance of sulphamethoxazole is given below:

$$k_{f_{obs}} = \frac{(k_0 K_{a1s}) + (k_0^- [\mathrm{H}^+]) + (k_H K_{a1s} [\mathrm{H}^+]) + (k_H^- [\mathrm{H}^+]^2)}{(K_{a1s} + [\mathrm{H}^+])} + \frac{(k_0^- K_{a2s}) + (k_0 [\mathrm{H}^+]) + (k_H^- K_{a2s} [\mathrm{H}^+]) + (k_H [\mathrm{H}^+]^2)}{(K_{a2s} + [\mathrm{H}^+])} \tag{25}$$

Table 3
Rate constants for the formation and hydrolysis of glucosylamines from the reaction of 5×10^{-4} M SMX and 5% w/v glucose ($\mu = 0.50$; 37°C) obtained from the $\log k_{obs}$ versus pH data

	k_0 (h^{-1})	k_h($dm^3\ mol^{-1}\ h^{-1}$)	k_h($dm^3\ mol^{-1}\ h^{-1}$)	k_h ($dm^3\ mol^{-1}\ h^{-1}$)
Forward reaction	3.46×10^{-3}	2.60×10^{1}	3.12×10^{-3}	3.53×10^{4}
Reverse reaction	1.74×10^{-2}	3.00×10^{1}	3.16×10^{-3}	1.20×10^{4}

Eq. (25) is also applicable for the reverse reaction, by substitution of the pK_a values of glucosylamines. The low pK_a value ($\ll 0.5$) for protonation to form GAH^+ would limit the influence of Eqs. (16) and (17) on the overall reaction rate.

Attempts to arrange the experimental data to a non-linear fit of Eq. (25) using a non-linear regression program gave poor results. The fit of the data at low pH values to the first term in Eq. (25), which is significant over this pH range, showed a better result giving an acceptable kinetic pK_{a1} for SMX, but not consistent with the model for the data at high pH (the second term in Eq. (25)). If the data at high pH were fitted separately, a good fit was obtained but resulted in the kinetic pK_{a2} being higher by a pH unit than the experimental value. The difference in the pK_a found might be interpreted by consideration of the presence of another reactive species over the pH range studied; the possibility of this occurring was excluded from the present study.

Indirect evidence from the literature indicates a possible change in the rate-determining step of the reaction from the addition of amine at low pH to dehydration of the intermediate (imine) at high pH (Cordes and Jencks, 1962; Jencks, 1964). The inconsistency of the fitted data at low and high pH in the present study could then be interpreted by consideration of this phenomenon. This means that the imine would be identifiable at high pH as it was dehydrated at a slower rate than its formation. There was, however, no direct evidence of the presence of an identifiable intermediate in the present study and therefore no experimental evidence could be found for a change in the rate-determining step over the pH range studied.

The rate constants were then derived by simplification of Eq. (25) and incorporation of the experimental pK_a values for SMX for the forward and the estimated pK_a values of the glucosylamines for the reverse reactions. Values for k_0 and k_H were obtained from the data at pHs less than 3.5 where the first term in Eq. (25) is significant. The contribution of these terms is not significant at pHs 6.68 and 6.88. At this pH range, terms: $\{(k_0^-.K_{2s}) + (k_H^-.K_{2s}[H^+])\}/(K_{2s} + [H^+])$ are significant because the SMX^- fraction is predominant, therefore k_0^- and k_H^- can be obtained. The data point at pH 3.93 is important because $\pm$ 98% of the species presents is SMXH. At pH 5.50, the two fractions have the same contribution as $f_{SMXH} \approx f_{SMX^-}$. The same method was applied to derive the rate constants for the reverse reaction.

The derived rate constants (Table 3) were substituted in Eq. (25) to obtain the calculated rate constants for the reaction over the pH range of 0.80–6.88. The fitting of the $\log k_{obs}$ against pH data for the forward and reverse reactions in Fig. 5 shows a good fit for the data at high pH, however, the trend of the data from the model at all pH values are consistent with the experimental data.

The kinetic data suggest that the addition of SMX to the aldehydic form of glucose is rate determining over the pH range studied, due to the fact that SMX is a weak base. This is consistent with the A_{dN} mechanism proposed for the reaction (Capon and Connett, 1965b; Kharmats and Afanas'ev, 1968). Thus the intermediate (imine) dehydration to form the products was faster than its formation and therefore it remained undetectable during the course of reaction. This is despite reports alluded to earlier that dehydration of the imine which is also acid catalyzed becomes rate determining at high pH. The evidence that supports this change in rate determining step was found for reactions with stronger amines and was shown to satisfactorily fit the bell-shaped curve of the pH-rate profile for the reaction of aniline and isoniazid

with glucose (Cordes and Jencks, 1962; Devani et al., 1985). There was no experimental evidence in this study that supports the change in rate determining step over the pH range investigated.

In a study of the reaction of a series of substituted primary aromatic amines such as 4-aminobenzoic acid ($pK_a = 2.41$), 3-bromoaniline ($pK_a = 3.51$) and aniline ($pK_a = 4.58$) with glucose, Kharmats and Afanas'ev (1968) interpreted that the addition of the amine was rate determining but employed the steady-state approximation to solve the rate equation without taking into account the reverse reaction. The bell-shaped curve of the pH-rate profile with the maxima close to the pK_a of the amines was satisfactorily fitted with this data treatment. Sianipar et al. (1994) reported a bell-shaped curve for the pH-rate profile for the reaction of procainamide ($pK_a = 2.75$) and glucose under acidic conditions. The maxima were found to correspond with the pK_a of the amine and the glucosylamines for the forward and reverse reaction respectively. No data can be found for the reactions of weaker bases in the literature.

The pH-rate profile for the reaction of SMX would show a bell-shaped curve with the maximum around the pK_{a1} of the aromatic amine (1.69) or that of the glucosylamines (0.50 or below). The trend in the experimental data supports this profile. Data at very low pH values could not be obtained as the reaction was too fast to measure.

The pH-rate profile for the forward and reverse reactions were consistent with the kinetic model proposed by incorporation of the experimentally determined pK_as of sulphamethoxazole and the estimated pK_as of glucosylamines. The fit for the experimental data showed reasonable results which confirmed that these pK_a values were reasonable and that the inflection in the pH-rate profiles (pH 3–4) was due to the ionization of the SMX for the forward and that of the glucosylamines for the reverse reaction.

A kinetic study at various SMX concentrations showed that the reaction followed pseudo first-order kinetics and was independent of the initial SMX concentration. The reaction in various glucose concentrations demonstrated that the forward rate constant was proportional to the glucose concentration and the reaction was first-order with respect to glucose. These give an overall second-order reaction with respect to both reactants, consistent with the A_{dN} mechanism.

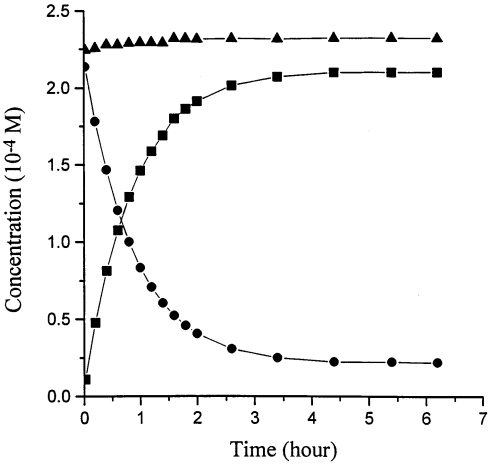


Fig. 7. Representative plot for concentration–time profile of the interconversion of 2.5×10^{-4} M SMX N_4-glycoside of the USP(α-glucosylamine) in the presence of 5% w/v glucose in 0.067 M phosphate buffer pH 5.86 ($\mu = 0.50$; 37°C). α-glucosylamine: ■; β-glucosylamines: ●; total concentration: ▲.

3.4. Kinetics of the interconversion of the glucosylamines

The NMR spectrum confirmed the identity of the USP reference standard SMX N^4-glycoside as being the α-anomer. This anomer was found to be less stable as it interconverted readily to the β-anomer to reach an equilibrium position which consisted of 10% α- and 90% β- (Section 3.3).

The interconversion of the two anomers was investigated by measuring the loss of the α-glucosylamine and the appearance of the products by HPLC analysis of the reaction mixtures. Solutions of the α-glucosylamines were prepared in an alkaline solution of Na_2HPO_4. Under these conditions solutions of the α-anomer were relatively stable.

Kinetic studies were performed over the pH range of 5.86–7.20 in phophate buffer ($\mu = 0.50$) at 37°C where the hydrolysis of the glucosylamines was still observed. Concentration data were corrected for the formation of the free SMX. The plot of the corrected α- and β-glucosylamine concentrations against time is shown in Fig. 7.

Table 4
Forward and reverse rate constants and the equilibrium constant for the interconversion of 2.5×10^{-4} M sulfamethoxazole N_4-glycoside of the USP α-glucosylamine) in the presence of 5% w/v glucose in buffer solution at various pH values ($\mu = 0.50$; 37°C).

No.	C buffer (M)	pH	k_f (h^{-1})	k_r (h^{-1})	K	%β-GA eq[a]	r[b]
1	0.067	5.86	1.0001	0.1040	9.6213	90.58	−0.9952
2	0.067	6.08	0.8609	8.94×10^{-2}	9.6304	90.59	−0.9979
	0.100	6.09	0.9252	9.33×10^{-2}	9.9108	90.83	−0.9979
	0.134	6.12	0.9917	0.01017	9.7547	90.70	−0.9992
	0.268	6.10	1.3587	0.1377	9.8686	90.80	−0.9999
3	0.067	6.89	0.1823	1.83×10^{-2}	9.9778	90.89	−0.9993
4	0.067	7.20	0.1065	1.07×10^{-2}	9.9411	90.86	−0.9999
	0.100	7.20	0.1281	1.29×10^{-2}	9.9727	90.89	−0.9999
	0.134	7.22	0.1737	1.68×10^{-2}	10.3620	91.20	−0.9999
	0.180	7.20	0.1907	1.91×10^{-2}	9.9491	90.87	−0.9994

[a] The proportion of β-glucosylamine at equilibrium.
[b] correlation coefficient of the first-order reversible plot of the data.

In all kinetic experiments the rate of interconversion of the α-glucosylamine followed first-order reversible kinetics ($r > 0.99$) over three to four half-lives of the reaction. The observed rate constants at various pH values and buffer concentrations are listed in Table 4.

The equilibrium constant (K) is consistent at various pH values which demonstrates that the same form of glucosylamines exist over the pH range studied. The percentage of the β-anomer at equilibrium (90.82 ± 0.18) is consistent with the proportion found for the formation of the glucosylamine (β-anomer = 90%) which confirms that the β-anomer of the glucosylamine is predominant.

The shape of the plot of the forward (k_f) and reverse (k_r) rate constants against pH (Fig. 8) indicates that the reaction is acid catalysed. In the presence of buffer, the rate constant is the sum of terms in Eq. (26).

$$k_{obs} = k_0 + k_{H^+}[H^+] + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^{2-}}[HPO_4^{2-}] \quad (26)$$

where k_{obs} is either k_f or k_r, k_0 the buffer independent rate constant, k_{H+} the acid catalytic rate constant, $k^-_{H_2PO_4}$ and $k^{2-}_{HPO_4}$ are the catalytic rate constants for the respective buffer species and $[H^+]$ refers to the hydrogen ion activity. The data were not all corrected to the buffer catalytic effect, hence were not interpreted further.

The effect of phosphate buffer on the observed rate constants was studied at pH 6.09 and 7.20. Over this pH range, the catalytic species are mainly $H_2PO_4^-$ and HPO_4^{2-} ions. Data in Table 4 demonstrate that the acidic component of the buffer has a more pronounced catalytic effect than its conjugate base as the slopes are larger at pH 6.09.

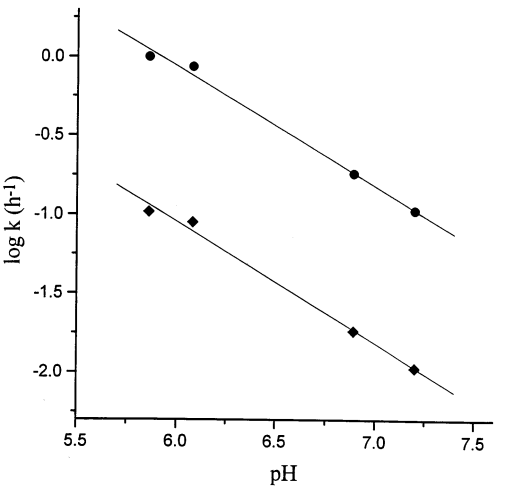


Fig. 8. Plot of the rate constants against pHs for the interconversion of 2.5×10^{-4} M SMX N_4-glycoside of the USP (α-glucosylamine in the presence of 5% w/v glucose in 0.067 M phosphate buffer ($\mu = 0.50$; 37°C). k_f: ●; k_r: ◆.

Table 5
Rate constants from the reaction of 5×10^{-4} M sulfamethoxazole with 5% w/v glucose in 0.067 M phosphate pH 2.89 at various temperatures ($\mu = 0.50$)

Temp. (°C)	Temperature ($1/T\ K \times 10^{-3}$)	k_f (h^{-1})	k_r (h^{-1})	K	r
27	3.3333	0.1263	9.80×10^{-2}	1.2895	−0.9998
32	3.2787	0.2011	0.1671	1.2035	−0.9996
37	3.2258	0.2476	0.2306	1.0737	−0.9996
42	3.1746	0.3346	0.3376	0.9910	−0.9999

Buffer catalytic rate constants were obtained from the slopes of the plot of the observed rate constants against buffer concentrations. The catalytic constant for $H_2PO_4^-$ ($k^-_{H_2PO_4}$) was found to be 3.14 M^{-1} h^{-1}, $k^{2-}_{HPO_4}$ was 6.18×10^{-2} M^{-1} h^{-1} for the forward reaction. Analogously k_{H2PO4}- and $k^{2-}_{HPO_4}$ were 3.10×10^{-1} and 5.41×10^{-3} M^{-1} h^{-1} for the reverse reaction respectively. These results imply a significant catalytic effect of the acidic species of phosphate buffer on the interconversion of the glucosylamines. This is consistent with the findings by Capon (1969) for the interconversion of L-arabinosylamine which was strongly catalyzed by acid. Smiataczowa et al. (1979) also found that the interconversion of a wide range of glycosylamines takes place according to the mechanism of general acid catalysis.

Data indicated that the interconversion of the glucosylamines proceeds more rapidly than their formation. For example, at pH 6.08 (0.067 M phophate buffer, $\mu = 0.50$ and 37°C) the ($k_f + k_r$) value was found to be 0.950 h^{-1} for the interconversion of the α-glucosylamine. The measured rate for the interconversion reaction from the β-glucosylamine was considered to be equal to this value (Capon and Connett, 1965b). Data indicate that, under similar conditions, ($k_f + k_r$) was 6.56×10^{-2} h^{-1} for the formation and hydrolysis of the glucosylamines, which was approximately 14 times slower.

The interconversion was too rapid to measure at pHs below 5.86. The half-life of the α-anomer at pH 5.86 (0.067 M phosphate buffer, $\mu = 0.50$ and 37°C) was found to be 0.73 h. The $10t_{50\%}$ value was 7.27 h when the interconversion reaction was considered essentially complete. This is much faster than the formation and hydrolysis of the glucosylamines. For instance, by the time the formation and hydrolysis reactions have taken place for 30.57, 21.50, 11.32 and 7.50% at pH 5.50, 6.09, 6.68 and 6.88 (0.067 M phosphate buffer, $\mu = 0.50$ and 37°C) respectively the interconversion is complete. First-order reversible plots of data of the kinetics of the formation and hydrolysis reactions at these pH values demonstrate that the slope (that is ($k_f + k_r$)) remains the same before or after the equilibrium between the two anomers has been achieved. These results demonstrate that the rate of the interconversion of the glucosylamines has no influence on the rate of their formation and hydrolysis, which may indicate that the two anomers have similar reactivities.

3.5. Effect of temperature

The rate constants at various temperatures in 0.067 M phosphate buffer pH 2.89 ($\mu = 0.50$) are shown in Table 5 and the Arrhenius plot in Fig. 9. Under these conditions the aromatic amino-group is substantially unprotonated. Ea_f was found to be 49.28 kJ mol^{-1} ($r = 0.9889$) and Ea_r 63.46 kJ mol^{-1} ($r = 0.9955$). Frequency factors of 5.06×10^7 h^{-1} and 1.14×10^{10} h^{-1} were calculated for the forward and reverse reactions respectively. The results indicate the more temperature dependent nature of the reverse reaction.

The change in the concentration of SMX at equilibrium and hence the equilibrium position (K) with temperature was observed. The plot of K against temperature according to the van't Hoff equation showed a linear relationship ($r = 0.9953$), the ΔH^0 value of -14.19 kJ mol^{-1} was obtained from the slope of the relationship.

These energies are similar to those observed for the reaction of procainamide with glucose ($Ea_f = 50.1$ kJ mol^{-1} and $Ea_r = 72.2$ kJ mol^{-1}) under similar conditions (Sianipar et al., 1994). These results suggest that both primary aromatic amines might undergo a similar pathway of reaction. The ΔH^0 value for the reaction of procainamide was -22.2 kJ mol^{-1} (Sianipar et al., 1994).

Extrapolation of the Arrhenius equation to 25°C results in k_f and k_r values of 0.1166 h^{-1} and 8.63×10^{-2} h^{-1} respectively in 0.067 M phosphate buffer pH 2.89 ($\mu = 0.50$). Under these conditions, the half-life of 15.42 h and the shelf life ($t_{90\%}$) of 1.05 h were obtained for the stability of sulphamethoxazole in 5% glucose solution.

The $t_{90\%}$ values were calculated from the data in Table 3 and gave the values of 0.23, 0.19, 0.22, 1.20, 1.89, 1.79, 2.34, 2.59, 3.96, 14.30 and 17.03 h for the reaction in buffer free solutions at pHs 0.80, 1.09, 1.55, 2.10, 2.90, 3.93, 5.04, 5.50, 6.09, 6.68 and 6.88 respectively at 37°C. These data demonstrate a very short shelf life for the drug when stored below pH 6.68 in a glucose infusion type solution. The low solubility of the drug at low pHs limits its use as a solution under these circumstances. The formulation of suspensions containing reducing sugars, however, should be avoided. It is possible that similar compounds may be formed with lactose when moist granulation procedures are employed in the preparation of solid dosage forms.

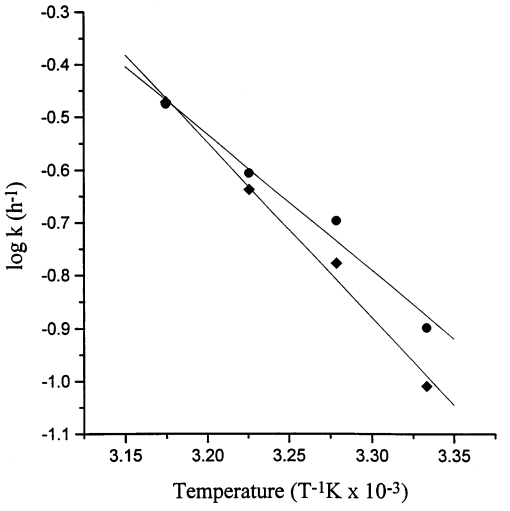


Fig. 9. Arrhenius plot for the reaction of 5×10^{-4} M SMX with 5% w/v glucose in 0.067 M phosphate buffer pH 2.89 ($\mu = 0.50$) and the reversible reaction under identical conditions. k_f: ●; k_r: ◆.

4. Conclusions

SMX reacts rapidly with glucose under acidic conditions with buffer species catalyzing the reaction at all pH values. The pH-rate profile shows the specific acid catalysis of the reaction (the slope ≈ -1) below pH 2.00 for the reverse reaction, and above pH 6.00 for both the forward and reverse reactions. These were due to the reaction of GAH below pH 2.00 and that of SMX^- for the forward and GA^- for the reverse reactions at high pH values. The reaction was pH independent at intermediate pHs. The correlation between the apparent equilibrium constants and pH indicates the effect of the ionization of SMX over the pH range studied. $SMXH^+$ is unreactive, SMX^- is more reactive than SMXH. The difference in reactivity of these species accounts for the inflection in the pH-rate profile. This was interpreted by consideration that for the weakly basic SMX, the addition step is rate determining over the pH range studied and the intermediate (imine) breaks down rapidly hence would be undetectable during the reaction. The reverse reaction is more temperature dependent than the forward reaction.

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