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The reaction of procainamide with glucose following admixture to glucose infusion

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Abstract

The kinetics of the reaction of procainamide with glucose to form glucosylamines was investigated over the pH range 1.73–6.00 at 30°C and 5% w/v (0.278 M) glucose concentration. The reaction showed reversible pseudo first-order kinetics with the equilibrium position being controlled by glucose concentration, temperature and pH. Activation parameters for the forward (k_f) and reverse (k_r) reactions were 50.1 and 72.2 kJ mol^{-1} respectively. The data indicate the rates of both the forward and reverse reactions are markedly influenced by pH giving maximum rates at approximately pH 3.0 and 1.5 respectively. These data confirm that the loss of procainamide would be substantial if admixed with glucose infusion.

Keywords: Procainamide; Dextrose; Glucose; Glucosylamines kinetics

1. Introduction

Procainamide hydrochloride may be administered as an intravenous admixture to glucose infusion. Studies have shown that there is a rapid loss of procainamide with time following admixture such that losses approach 20–30% over 24 h at room temperature (Sianipar et al., 1994). This loss was initially ascribed to the formation of a complex between the drug and glucose (Das Gupta, 1982, 1983; Raymond et al., 1988; Riley, 1988; Riley and Junkin, 1991; Henry et al., 1991) but studies utilising nuclear magnetic resonance spectroscopy and high-performance liquid chromatography (HPLC) have shown that the loss of procainamide is associated with the formation of a mixture of the corresponding α- and β-*N*-glucosylamines (*N*-glucosides) (Sianipar et al., 1994).

N-glucosylamines have undergone considerable study and it has been demonstrated that they are formed and are hydrolysed readily under acidic conditions being formed via the intermediate imine (Ellis and Honeyman, 1955; Ledl and Schleicher, 1990). Simple para-substituted aromatic

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glucosylamines exist as an anomeric mixture the ratio of which at equilibrium is 9:1 with the more stable β-anomer predominating (Capon and Connett, 1966). This equilibrium ratio was identical to that found for the glucosylamines of procainamide, which is a para-substituted primary aromatic amine, by integration of the nuclear magnetic resonance spectrum of the equilibrated mixture (Sianipar et al., 1994).

This paper presents detailed information concerning the kinetics of formation of these compounds under varying conditions of pH, temperature and carbohydrate concentration. These data provide further evidence of the instability of procainamide hydrochloride injection with glucose in solution.

2. Materials and methods

2.1. Materials

Procainamide hydrochloride (Sigma, St Louis, MO) and glucose-1-hydrate (Ferak, Germany) were used as supplied. All other chemicals were either analytical reagent grade or HPLC grade.

2.2. Instrumentation

Chromatographic conditions and instrumentation were identical to those employed for the analysis of procainamide in the stability assessment following intravenous admixture as previously reported by Sianipar et al. (1994). Spectrophotometric measurements were made using a photodiode-array spectrophotometer (Model 8452A, Hewlett Packard, USA).

2.3. Kinetic experiments

All kinetic experiments were performed by addition of 10^{-3} M procainamide hydrochloride solution (10 ml) to a solution of double strength buffer (50 ml) to which was added an appropriate amount of glucose followed by dilution to volume in a 100-ml volumetric flask. All solutions were pre-equilibrated to temperature and the procainamide solution added at zero time and the mixture made to volume with water pre-equilibrated to temperature. This provided a 10^{-4} M solution of the drug in buffer. Buffers used were pH 1.73 hydrochloric acid and pH 2.18, 2.50, 3.00 and 6.00 phosphate and pH 3.50, 4.00 and 4.50 acetate all of 0.05 M and adjusted to a total ionic strength of 0.5 with sodium chloride. The solutions were assayed for procainamide content by HPLC at regular time intervals.

The rate constants for the forward and reverse reactions (k_f and k_r) were determined from linear plots of $\ln(C_t - C_\infty)$ versus time by the least squares method (Eq. (1)) where C_t, C_∞ and C_o were the concentrations of procainamide at time t, infinity and time zero respectively and k_f and k_r the rate constants for the forward and reverse reactions. The value of C_∞ was determined using the re-iterative program of Irwin (1990):

$$\ln(C_t - C_\infty) = \ln(C_o - C_\infty) - (k_f + k_r)t \quad (1)$$

Since

$$K = \frac{k_f}{k_r} = \frac{C_o - C_\infty}{C_\infty} \quad (2)$$

then

$$C_\infty = \frac{k_r C_o}{(k_f + k_r)} \quad (3)$$

hence

$$k_r = \frac{C_\infty (k_f + k_r)}{C_o} \quad (4)$$

and

$$k_f = \frac{C_\infty (C_o - C_\infty)(k_f + k_r)}{C_o} \quad (5)$$

Values of k_f and k_r were obtained by substitution into Eqs. (4) and (5) respectively.

2.4. Determination of the apparent dissociation constant

The apparent dissociation constant for the primary aromatic amino-group of procainamide was determined by the spectrophotometric method of Albert and Serjeant (1971) using hydrochloric acid (pH 1.50 and 1.75) and phosphate buffers (pH 2.00, 2.25 and 2.50) all at an ionic strength of

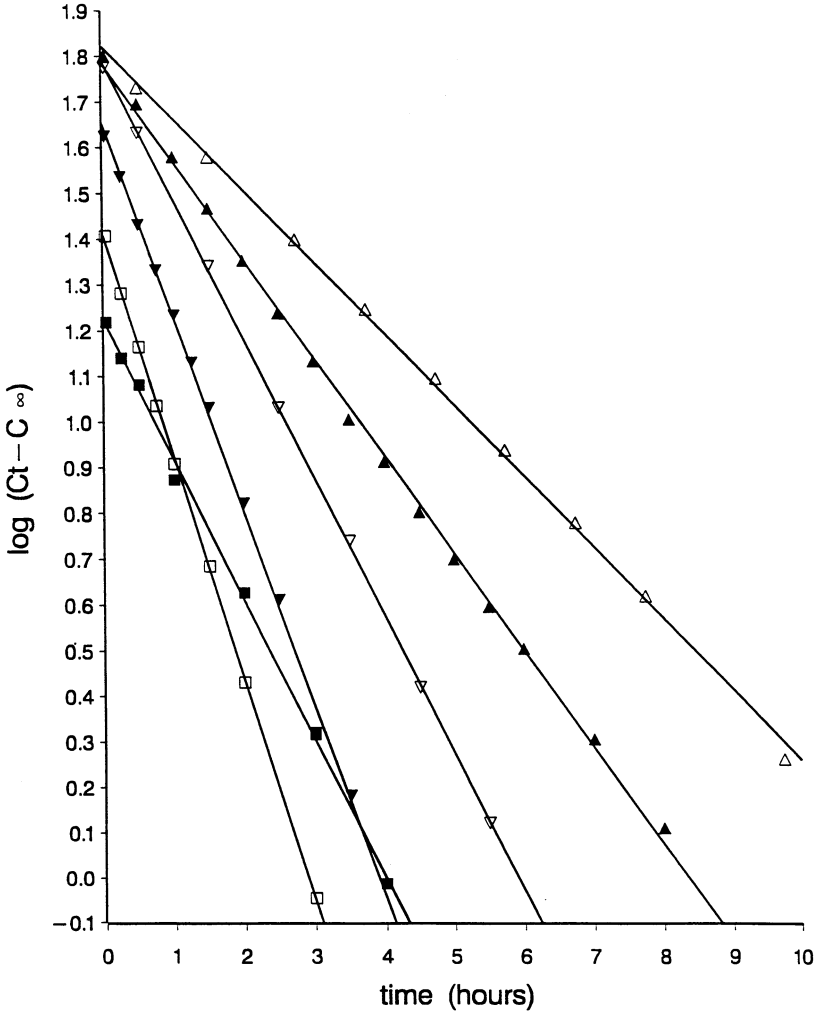

Fig. 1. Representative pseudo first-order plots of the data derived from the reaction procainamide hydrochloride (10^{-4} M) in glucose (5% w/v) at 30°C. pH: ■, 1.73; □, 2.18; ▼, 2.50; ▽, 4.00; ▲, 4.50 and △, 5.00.

0.50. The spectra of the non-protonated species and the protonated species were obtained using phosphate buffer (pH 6.00) and hydrochloric acid (0.5 M).

3. Results and discussion

The procainamide concentrations were determined using the stability indicating HPLC method employed in the previous study (Sianipar et al., 1994). The method fully resolves the procainamide and the peak arising from the mixed glucosylamines which co-elute at a shorter retention time.

When primary amines react with glucose and other reducing sugars under mild conditions they react via the intermediate imine to form an anomeric mixture of the α- and β-glucosylamines according to Eq. (6):

$$G + PAH \leftrightarrows \text{imine} \rightleftarrows \text{mixed } PAH.G + H_2O \quad (6)$$

where G is glucose, PAH the monoprotonated form of procainamide and PAH.G is the corre-

sponding glucosylamine. The reaction from imine to glucosylamine is rapid and previous studies have shown no evidence of measurable levels of

Table 1
Kinetic parameters (forward and reverse first-order rate constants k_f and k_r) and equilibrium constant (K) for the reaction between procainamide and glucose under various conditions

Parameter	k_f (h^{-1})	k_r (h^{-1})	K
A. pH[a]			
1.73	0.1153 (± 0.0024)	0.5779 (± 0.0119)	0.1995 (± 0.0143)
2.18	0.3126 (± 0.0020)	0.9654 (± 0.0062)	0.3238 (± 0.0082)
2.50	0.3959 (± 0.0057)	0.5363 (± 0.0077)	0.7382 (± 0.0133)
3.00	0.4557 (± 0.0101)	0.3712 (± 0.0082)	1.2276 (± 0.0183)
3.50	0.4015 (± 0.0039)	0.2686 (± 0.0026)	1.4948 (± 0.0065)
4.00	0.3267 (± 0.0060)	0.1975 (± 0.0036)	1.6542 (± 0.0096)
4.50	0.2175 (± 0.0055)	0.1241 (± 0.0032)	1.7526 (± 0.0087)
5.00	0.0907 (± 0.0051)	0.0505 (± 0.0029)	1.7960 (± 0.0080)
6.00	0.0239 (± 0.0004)	0.0133 (± 0.0002)	1.7970 (± 0.0006)
B. Glucose concentration[b] (% w/v)			
1.0	0.0163 (± 0.0056)	0.0479 (± 0.0019)	0.3403 (± 0.0075)
3.0	0.0488 (± 0.0003)	0.0460 (± 0.0003)	1.0609 (± 0.0006)
5.0	0.0907 (± 0.0051)	0.0505 (± 0.0029)	1.7960 (± 0.0006)
7.0	0.1206 (± 0.0004)	0.0479 (± 0.0002)	2.6305 (± 0.0006)
9.0	0.1638 (± 0.0006)	0.0505 (± 0.0002)	3.2436 (± 0.0008)
C. Temperature[c] (°C)			
30.0	0.0907 (± 0.0051)	0.0505 (± 0.0029)	1.7960 (± 0.0080)
37.0	0.1264 (± 0.0028)	0.0848 (± 0.0042)	1.4906 (± 0.0070)
45.0	0.2252 (± 0.0028)	0.1901 (± 0.0023)	1.1846 (± 0.0051)
52.0	0.3379 (± 0.0115)	0.3395 (± 0.0115)	0.9953 (± 0.0230)

Standard errors in parentheses.
[a] 30°C, 5% w/v glucose and total $I = 0.5$.
[b] pH 5.0, 30°C and total $I = 0.5$.
[c] pH 5.0, 5% w/v glucose and total $I = 0.5$.

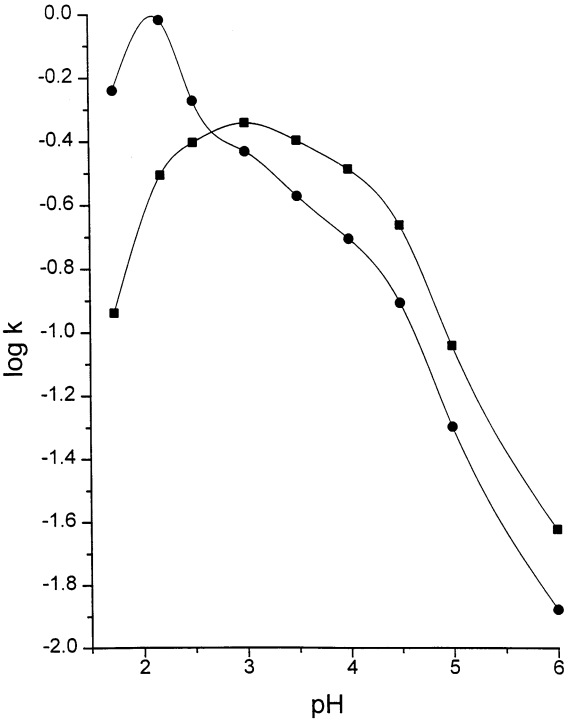

Fig. 2. Plot of the forward (k_f) and reverse (k_r) rate constants for the reaction of procainamide hydrochloride (10^{-4} M) with glucose (5% w/v) at 30°C in buffers of $I = 0.50$; k_f, ■; k_r, ●.

imine at equilibrium (Capon and Connett, 1966; Sianipar et al., 1994). Since glucose and water are in large excess with respect to procainamide and the pH conditions are maintained during the experiment the equation may be simplified to

$$\mathrm{PAH} \underset{k_r}{\overset{k_f}{\leftrightarrows}} \mathrm{PAH.G} \qquad (7)$$

and which is described by reversible pseudo first-order kinetics.

For all of the experimental conditions the data clearly show the reversible loss of procainamide. Previous studies have shown that this loss is associated with the concomitant formation of a mole equivalent of the α- and β-glucosylamines (Sianipar et al., 1994).

The kinetics of the reaction of procainamide with glucose (5% w/v) over the pH range 1.73–6.00 was investigated and it was found that the data demonstrated reversible first-order kinetics for up to three half-lives of the reaction (Fig. 1). Both the rate and equilibrium position ($K = k_f/k_r$)

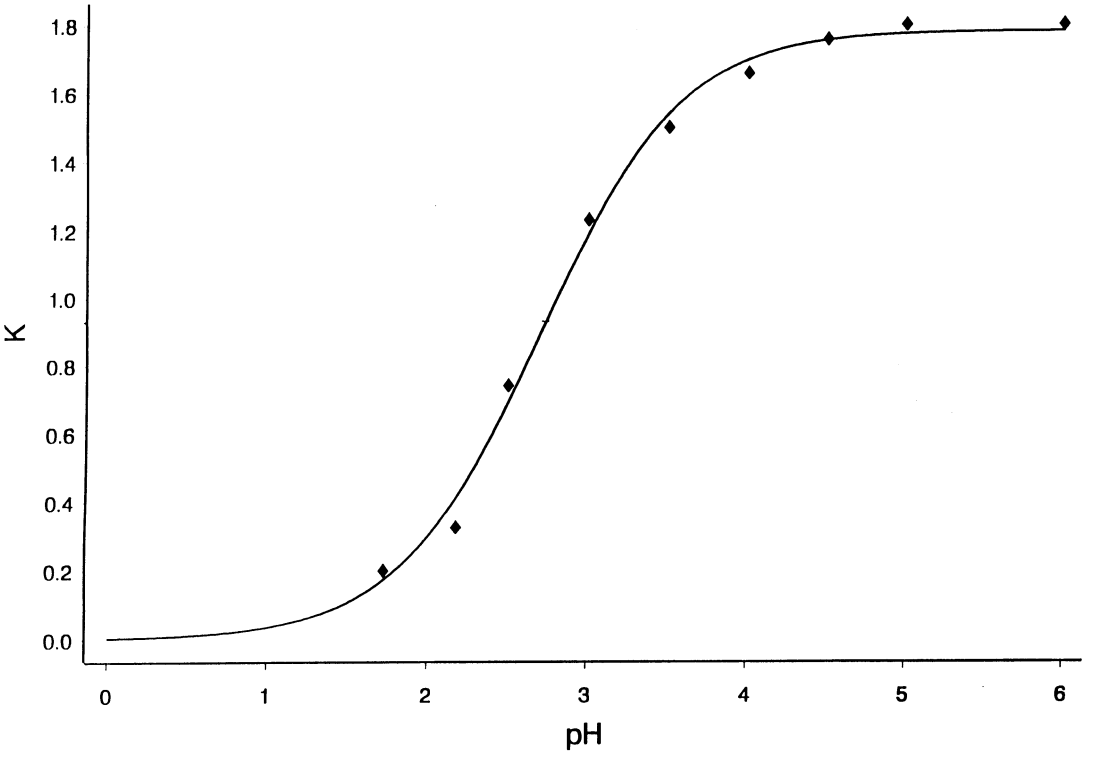

Fig. 3. Plot of the apparent equilibrium constant ($K_{(app)}$) as a function of pH derived from the data in Fig. 2 at $I = 0.50$.

was influenced by the pH and these data are reported in Table 1A. Fig. 2 shows the relationship of log k_f and log k_r versus pH, k_f showing a maximum at pH 3 and k_r a maximum at approximately 1.5. It should be noted that these data include any possible influence of the buffer species on k_f and k_r.

Possible effects of buffer catalysis on k_f and k_r precluded a quantitative analysis of these profiles. For example, the approximately linear region from pH 4.50 to 6.00 has a slope of 0.6. This could include mechanistic and catalytic influences of pH on the rate. As the pK_a for procainamide was found to be 2.75 ($I = 0.5$) the maximum in k_f is influenced by a much lower, or zero, reactivity of the diprotonated procainamide species, which dominates the reactant mixture below pH 2.75. The maximum value in the procainamide glucosylamine at much lower pH values indicates its weaker base characteristics.

Thus k_f at low pH values may be best interpreted as being influenced by intervention of protonation of the primary aromatic amino-group of the procainamide. It is recognised that a requirement for the reaction to proceed is that the amino group has a lone-pair of electrons to attack the carbonyl group (Jencks, 1964) and therefore the pH-dependent equilibrium may be described in terms of the equation.

$$PAH_2 \rightleftarrows PAH + G \leftrightarrows PAH.G \tag{8}$$

where PAH_2 is the diprotonated amine. Then the apparent equilibrium constant ($K_{(app)}$) for the species can be defined by the equation

$$K_{(app)} = \frac{k_f}{k_r} = \frac{[PAH.G]_{eq}}{([PAH]_{eq} + [PAH_2]_{eq})} \tag{9}$$

and therefore

$$K_{(app)} = \frac{[PAH.G]_{eq}}{[PAH]_{eq} + [PAH]_{eq}\ \text{antilog}(pK_a - pH)} \tag{10}$$

where $[PAH.G]_{eq}$ and $[PAH]_{eq}$ are the concentrations of the glucosylamine and unprotonated procainamide and $([PAH]_{eq} + [PAH_2]_{eq})$ the concentration of total procainamide at equilibrium. Applying Eq. (10) to the data a non-linear least squares best-fit could be derived for the equilibrium constants (K) versus pH (Fig. 3). The asymptotic value of $K_{(app)}$ at the high pH region was 1.84 which corresponds to that of uncharged amino-group. In the pH region where the amino-group was fully protonated the value approaches zero. The pK_a value derived from the pH of the mid-point value of $K_{(app)}$ (Eq. (6)) in Fig. 3 was 2.78. This shows acceptable agreement with the experimental value which was found to be 2.75 ± 0.06 ($n = 5$). It would be expected that any possible buffer effects on k_f and k_r would not influence the $K_{(app)}$ values owing to the principle of microscopic reversibility.

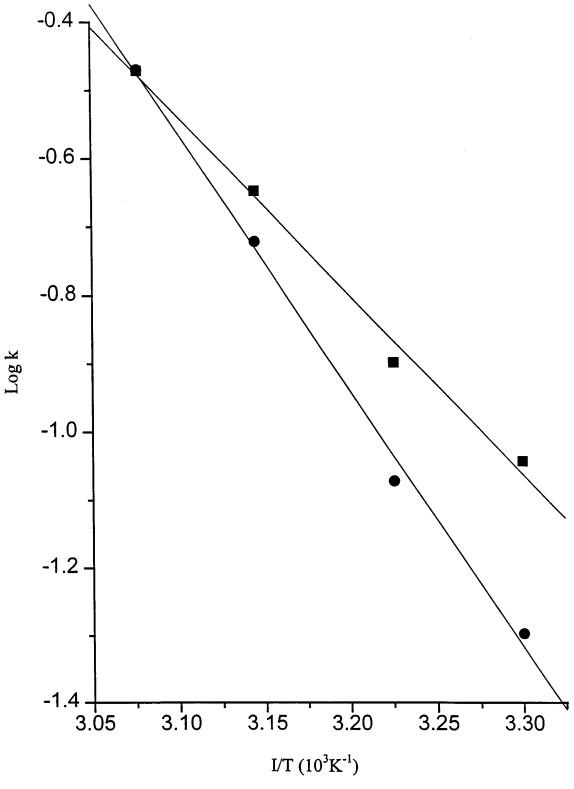

Fig. 4. Arrhenius plot of the forward (■) and reverse (●) rate constants for the reaction of procainamide (10^{-4} M) with glucose (5% w/v) at pH 5.0.

The reaction of procainamide and glucose in various concentrations in acetate buffer pH 5.00 at 30°C was also investigated (Table 1B). A plot of glucose concentration against the forward rate constant afforded a linear relationship passing through the origin demonstrating that the forward rate constant (k_f) is proportional to glucose concentration ($r = 0.998$; intercept $= -0.0036$). From the slope of the relationship a value of 0.33 l mol^{-1} h^{-1} for the second-order rate constant for the reaction was obtained.

The activation parameters for the forward and reverse reactions were also examined at pH 5.00 (Table 1C, Fig. 4), conditions under which the aromatic amino-group is unprotonated. The respective activation energy values (E_a) were determined from the Arrhenius relationship. It was found that E_a values for k_f and k_r were 50.1 and 72.2 kJ mol^{-1} respectively. These differences in activation energies indicate that the hydrolytic reverse reaction rate would be more temperature-dependent and that therefore the position of the equilibrium would be markedly influenced by temperature. A ΔH^o value of -22.2 kJ mol^{-1} is the heat of reaction which was obtained by application of the Van't Hoff equation to the temperature effect on the equilibrium constants. Activation energy data determined at lower pH values would be of a mixture of the charged and unprotonated species and therefore such studies were not pursued.

These data confirm the observations made previously that the mixed glucosylamines are formed rapidly when procainamide hydrochloride is admixed with commercial glucose infusion such that a 10% loss of procainamide ($t_{90\%}$) occurred in approximately 5 h, the rate of formation and equilibrium being influenced by temperature, carbohydrate concentration and pH of the solution. From data in the pH-rate profile the shelf-life ($t_{90\%}$) for procainamide in 5% glucose at pH 3.0 is 0.243 h and at pH 6.0, the value is 4.59 h.

As the pH of glucose infusion has an allowable pH range of 3.5–6.5 (US Pharmacopeia, 1990) and as the pH of the injection has been shown to fall following admixture of a commercial injection of procainamide hydrochloride (Sianipar et al., 1994) the rate of reaction and the extent of the

loss of procainamide with time is therefore dependent on these variables but will always be significant for admixtures stored and/or administered over a short time period.

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