J. Pharm. Pharmacol. 1994, 46: 951–955
Received July 15, 1993
Accepted February 1, 1994

Chemical Incompatibility Between Procainamide Hydrochloride and Glucose Following Intravenous Admixture

A. SIANIPAR, J. E. PARKIN AND V. B. SUNDERLAND

School of Pharmacy, Curtin University of Technology, PO Box U1987, Perth, WA 6001, Australia

Abstract—The chemical reaction between procainamide hydrochloride and glucose following admixture to glucose infusion has been investigated. Substantial amounts (10–15% after 10 h at room temperature) of the procainamide is lost with the formation of a mixture of the corresponding α- and β-glucosylamines. The chemical identity of the latter compounds was confirmed by ^{13}C and ^{1}H nuclear magnetic resonance spectroscopy.

Procainamide hydrochloride is frequently administered as an intravenous admixture in glucose intravenous infusion. This is despite studies which have shown that there is a rapid loss of procainamide following its addition to glucose solutions (Das Gupta 1982, 1983; Raymond et al 1988; Riley 1988; Riley & Junkin 1991; Henry et al 1991). The authors of these studies have suggested that the loss is ascribable to the formation of a reversible complex between the sugar and procainamide. Das Gupta (1982, 1983) demonstrated that the formation of this derivative was reversible in the presence of hydrochloric acid, was dependent on the concentration of glucose, appeared to have an optimum pH for formation in the region 4–5·2, and was also formed between procainamide and lactose and maltose, which are reducing sugars, but not with fructose and sucrose which are non-reducing, or with other polyols such as propylene glycol. Raymond et al (1988) showed that this derivatization was greatly reduced in neutralized 5% glucose injection and recently Henry et al (1991) proposed that the complex was irreversible in plasma, the latter authors concluding that it behaved like a new compound rather than as a reversible complex. However, they did not draw conclusions as to the nature of this compound.

This paper presents data which demonstrates that the compound formed is a mixture of the α- and β-glucosylamines (Fig. 1) and studies the rate of formation of these compounds following admixture of a commercial injection of procainamide hydrochloride to glucose infusion at 25 and 30°C.

Materials and Methods

Materials

Procainamide hydrochloride (Sigma, USA) and D-glucose-1-hydrate (Ferak, Germany) were used as supplied. Procainamide Hydrochloride Injection 100 mg mL^{-1} (Pronestyl, Squibb, Australia) and Glucose 5%, 500 mL bags (Abbott, Australia) were purchased commercially. All other reagents were either analytical grade or high-performance liquid chromatography (HPLC) quality.

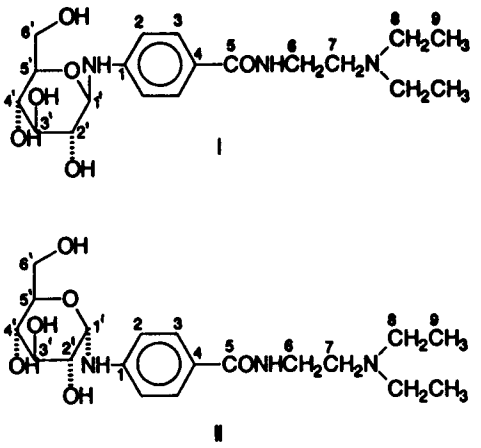

FIG. 1. I: β-glucosylamine and II: α-glucosylamine.

Nuclear magnetic resonance (NMR) study

Glucose 99·1 mg (5×10^{-4} mol) and procainamide hydrochloride 135·9 mg (5×10^{-4} mol) were dissolved in deuterium oxide (2 mL) and the solution was distributed equally into five 2-mL ampoules. The solutions were evaporated to dryness in a stream of dry nitrogen at 37°C. The ampoules were purged of traces of exchangeable protons by redissolving the contents three times in deuterium oxide (0·2 mL) and evaporating the contents. The ampoules were then dried by storage in a vacuum desiccator at 37°C for two days and sealed. An ampoule was submitted to analysis by HPLC.

The deuterated buffer for reconstitution was prepared by evaporating 0·5 mL 1 M phosphate buffer pH 7·0 to dryness at 90°C and purging the system of protons by addition of 3 vol 0·1 mL deuterium oxide followed by evaporation after each addition. The sample was prepared for NMR by dissolving the contents of one ampoule in deuterium oxide (1 mL) previously used to dissolve the contents of a buffer

Correspondence: J. E. Parkin, School of Pharmacy, Curtin University of Technology, PO Box U1987, Perth, WA 6001, Australia.

ampoule. The ^1H and ^{13}C NMR spectra were recorded at 500 MHz using 2,2-dimethyl-2-silapentane-5-sulphonate as the chemical shift standard (Brukker ARX-500, Germany).

Chromatographic equipment and conditions

The HPLC system consisted of a pump (501, Waters Associates, USA), 20-μL loop injector (7125, Rheodyne, USA), variable wavelength absorbance detector (486, Waters Associates) and integrating recorder (3396 A, Hewlett-Packard, USA) with a column of cyanopropyl-bonded silica, 10 μm particle size, 8 mm i.d. × 10 cm (Waters RCM 8 × 10). The mobile phase consisted of acetonitrile-water (12 : 88) containing 0·6% glacial acetic acid with the pH adjusted to 5·2 with sodium hydroxide. The flow rate was 1·5 mL min^{-1} and the monitoring wavelength was 279 nm. Spectra of eluted peaks were obtained using a photodiode-array absorbance detector (991, Waters Associates).

Rate of reaction of procainamide with glucose at 30°C in pH 4 buffer

A solution of procainamide hydrochloride (10^{-4} M) in 0·05 M acetate buffer pH 4·0 containing glucose (5% w/v) was equilibrated to 30°C. The concentration of procainamide and glucosylamines were determined at 15-min intervals for 8 h and then irregularly to 18 h by HPLC analysis.

Rate of hydrolysis of glucosylamines at 30°C in pH 4 buffer

A concentrated sample of glucosylamines was prepared by dissolving glucose (0·991 g) (5×10^{-3} mol) and procainamide hydrochloride 1·359 g (5×10^{-3} mol) in water (4 mL) and the resulting solution was divided equally among 10 ampoules. The solutions were evaporated to dryness under a stream of nitrogen and dried in a vacuum desiccator at 37°C for 24 h. The contents of one ampoule were dissolved in 25 mL 0·5 M phosphate buffer pH 8·0. Preparative HPLC was performed on the sample using a 200-μL loop injector and chromatographic conditions as described previously. The eluate fraction containing the glucosylamines arising from three injections was collected into 8 mL 0·5 M phosphate buffer pH 8·0 and the sample made to 10 mL with buffer.

Five millilitres of this solution of glucosylamines was transferred to 40 mL 0·05 M acetate buffer (pH 4·0) previously equilibrated to 30°C and the volume was adjusted to 50 mL with pH 4·0 buffer. After mixing, a sample was assayed immediately for procainamide and glucosylamines and at 15 min intervals to 10 h and at 24 h.

Reaction of procainamide hydrochloride in glucose infusion bags

To a 500-mL infusion bag equilibrated to temperature Pronestyl injection (10 mL) was added by syringe to give a nominal concentration of 0·2% procainamide hydrochloride. The contents of the bag were thoroughly mixed and 10-mL samples were withdrawn for analysis at regular intervals to 48 h. The pH was recorded and a sample diluted 1 mL to 100 mL with 0·5 M phosphate buffer (pH 8·0) and procainamide and glucosylamines determined by HPLC. The experiment was performed in triplicate at 25°C and at 30°C.

Results and Discussion

Reducing sugars are known to react readily under mild conditions with compounds containing primary amino-groups to form glucosylamines (Ellis & Honeyman 1955; Ledl & Schleicher 1990). This is a reversible reaction; however, such compounds may react further undergoing the irreversible Amadori rearrangement to form the corresponding 1-amino-1-deoxyketose sugars (Hodge 1955). We suggest that the observations made by previous workers concerning the stability of procainamide in dextrose infusion may be a reaction of this type rather than formation of a complex.

Attempts to synthesize a pure sample of the possible glucosylamines failed to achieve a crystalline product. Studies using NMR were then undertaken of the reaction product mixture formed when a 1 : 1 mol ratio of glucose and procainamide hydrochloride was allowed to react in deuterium oxide with removal of the solvent. The product formed was non-crystalline.

Assay by HPLC of the mixture demonstrated that 65·7%

Table 1. Chemical shifts and coupling constants of ^1H NMR signals†.

Chemical shift (ppm)	Signal with relevant coupling constant	Proton assignment	Integration relative to C_9 methyl group (= 6H)	Mol fraction of components
1·29	Overlapping triplets	C_9 PA and α- and β-GA	6·000	
3–4	Overlapping multiplets	$C_{2'}$-$C_{6'}$; C_6-C_8	14·100	
4·62	Doublet, J = 8·0	$C_{1'}$ β-glucose	0·193	
4·79	Doublet, J = 8·9	$C_{1'}$ β-GA	0·613*	0.685 as α- and β-GA—0.613 as β-GA and 0.072 as α-GA; 0.315 as α- and β-glucose—0.193 as β-glucose and 0.122 as α-glucose
5·21	Doublet, J = 3·9	$C_{1'}$ α-glucose	0·122	
5·32	Doublet, J = 5·0	$C_{1'}$ α-GA	0·072	
6·82		C_2 PA	0·670	0·338 as free PA, 0·577 as β-GA and 0·085 as α-GA
6·89		C_2 β-GA	1·142	
6·98		C_2 α-GA	0·168	
7·62		C_3 PA	0·677	0·335 as free PA, 0·665 as α- and β-GA
7·68		C_3 α- and β-GA	1·323	

†PA = procainamide, GA = glucosylamine.
*Overlapped by water peak, integration calculated by difference assuming there is one anomeric $C_{1'}$ proton as α- and β-glucose and α- and β-GA.

Table 2. Chemical shifts of ^{13}C NMR signals†.

Chemical shift (ppm)		Assignment	Comments
10·88	C_9	PA; α- and β-GA	Signals overlapping
37·63	C_8	PA; α- and β-GA	Signals overlapping
50·64	C_7	PA; α- and β-GA	Signals overlapping
53·79, 53·76	C_6	PA; α- and β-GA	Signals overlapping
63·39, 63·25, 63·19	$C_{6'}$	α- and β-Glucose; α- and β-GA	Two signals overlapping
72·07–79·46	$C_{2'}$–$C_{5'}$	α- and β-Glucose; α- and β-GA	16 signals no specific assignment made
83·61	$C_{1'}$	α-GA	Lit. value of α-*N*-phenylglucosylamine: 82·84[a]
86·59	$C_{1'}$	β-GA	Lit. value of β-*N*-phenylglucosylamine: 85·29[a]
94·75	$C_{1'}$	α-Glucose	Lit. value: 94·86[b]
98·57	$C_{1'}$	β-Glucose	Lit. value: 98·75[b]
116·08, 116·13, 117·74	C_2	PA, α- and β-GA	114·1 using additivity rules[c]
124·54, 124·80, 125·09	C_4	PA, α- and β-GA	124·7 using additivity rules[c]
131·69, 131·79, 131·82	C_3	PA, α- and β-GA	128·0 using additivity rules[c]
152·41, 152·57, 153·91	C_1	PA, α- and β-GA	151·4 using additivity rules[c]
173·56, 173·66	C_5	PA, α- and β-GA	Two signals overlapping

†PA = procainamide; GA = glucosylamine.
[a] Chavis et al (1983), [b] Bailey & Butterfield (1981), Breitmaier & Voelter (1990).

of the procainamide had reacted under these conditions and that, therefore, the mixture would consist at equilibrium of the α- and β-anomers of glucose, a mole equivalent amount of procainamide and 65·7% of the procainamide as a mixture of the α- and β-anomers of the glucosylamines (Fig. 1). A detailed study of 500 MHz ^1H and ^{13}C spectra confirmed this hypothesis.

All protons in the ^1H spectrum fall between 3 and 4 ppm except for the terminal methyl group of procainamide and the α- and β-glucosylamines, the aromatic protons of these compounds and the anomeric 1′-proton of α- and β-glucose and the α- and β-glucosylamine (Table 1). The identity of the glucose 1′-anomeric protons was confirmed by adding an authentic sample of glucose to the sample. The magnitude of the coupling constants of the 1′-proton of the glucosylamine anomers confirms the assignment, the β-anomer showing diaxial coupling and the α-anomer axial-equatorial coupling in keeping with the Karplus equation (Silverstein et al 1991) (α-anomer-dihedral angle 60°:5·0 Hz; β-anomer-dihedral angle 180°:8·9 Hz). These values are similar to those of the α- and β-anomers of glucose (Table 1) and those reported for the corresponding phenylglucosylamines (α-anomer: 4 Hz; β-anomer: 8 Hz) by Chavis et al (1983).

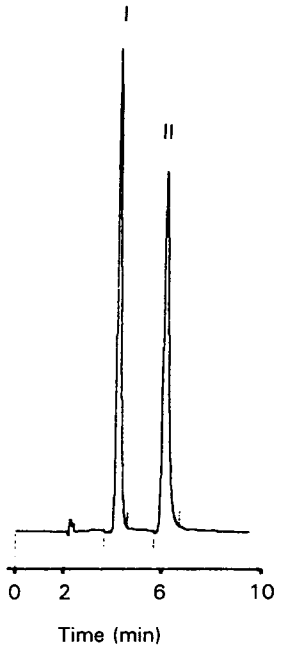

FIG. 2. Representative chromatogram derived from the assay. I: glucosylamines; II: procainamide.

The compositions calculated from the data in the aromatic region and the data arising from the 1′-anomeric proton were consistent (Table 1). The proportion of procainamide reacted to form the glucosylamines was identical to that found by HPLC, the unreacted glucose forming a mixture of anomers (61·2% β, 38·8% α) in the proportions expected (Breitmaier & Voelter 1990) and the glucosylamines forming a mixture of anomers (88·0% β, 12·0% α). As expected the β-anomer is more favoured as the substituent exists in an equatorial conformation (Chavis et al 1983). The proportion of anomers of the glucosylamines is similar to that found by Capon & Connett (1966) when they followed the anomerization and hydrolysis of a range of aryl glucosylamines by polarimetry and found that the equilibrium mixture consisted of about 10% of the α- and 90% of the β-anomer.

The ^{13}C NMR spectrum confirmed this interpretation. Carbons near the procainamide-glucose bond (C_1–C_5, $C_{6'}$ and $C_{1'}$) all showed the appearance of multiple signals in keeping with their being procainamide and the α- and β-anomers of glucose and glucosylamine present (Table 2). The $C_{1'}$ anomeric carbon displayed four distinct signals in keeping with the presence of α- and β-glucose and α- and β-glucosylamines, the chemical shifts being confirmed by their relationship to authentic values for α- and β-glucose and to the analogous phenylglucosylamines (Bailey & Butterfield 1981; Chavis et al 1983).

The evidence strongly supports the conclusion that procainamide reacts with glucose to form a mixture of the α- and β-glucosylamines with the β-anomer predominating. Chromatagraphic studies were then undertaken to study the kinetics of formation of these products.

The existing method of Das Gupta (1983) was modified and afforded peaks for both the glucosylamines and pro-

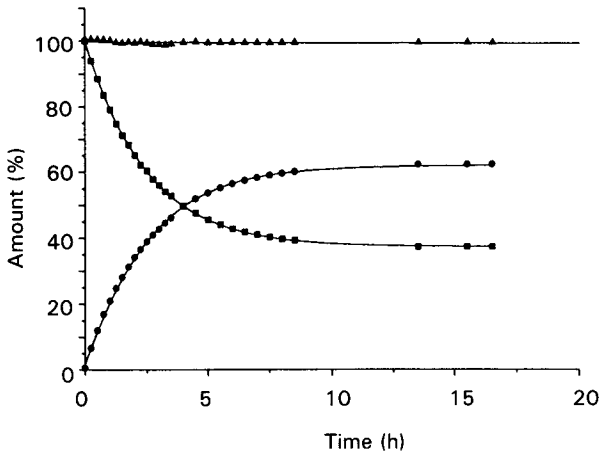

FIG. 3. Reactions of procainamide (10^{-4} M) with glucose 5% w/v in 0·05 M acetate buffer pH 4·0 at 30°C. ■ Procainamide, ● glucosylamines, ▲ total of the two compounds.

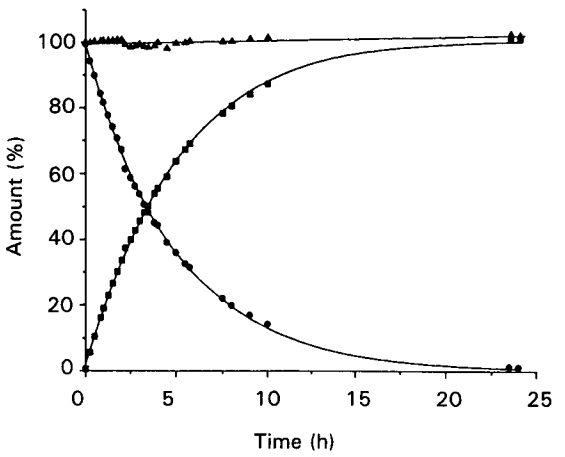

FIG. 4. Hydrolysis of glucosylamines ($0·7 \times 10^{-4}$ M) in 0·05 M acetate buffer pH 4·2 at 30°C. ■ Procainamide, ● glucosylamines, ▲ total of the two compounds.

cainamide (Fig. 2). It should be noted that the glucosylamines form a single peak and there is no evidence of resolution of the anomers under the chromatographic conditions employed in this study. This is not unexpected as α- and β-glucosylamines would be expected to have very similar polarities.

As a pure sample of glucosylamines was unavailable, it was not possible to prepare a direct calibration curve for these compounds. There was no chromatographic or NMR evidence that any other products were formed during the reaction and, therefore, the peak area response of the glucosylamines peak was related to the concentration of procainamide by relating loss of the area of the procainamide peak to the area of formation of the glucosylamine peak during a kinetic study of the reaction in pH 4 acetate buffer. Over the time course of the reaction it was found that, at the monitoring wavelength, the glucosylamines display a greater absorbance than procainamide ($1·344 \pm 0·017$ (n = 26) of the peak area of the procainamide lost) and, therefore, the concentration of the glucosylamines was determined relative to the procainamide standard by applying a factor of 0·744. Later studies on the hydrolysis of the glucosylamines and their formation in the i.v. infusion bags afforded results in which the total concentration of glucosylamines and procainamide remains constant, demonstrating the validity of this approach (Figs 3, 4, Table 3). A linear relationship exists for the mixed glucosylamines and procainamide over the concentration range to 1×10^{-4} M and the assays were fully validated for both compounds.

Using a photodiode-array spectrophotometric detector it was possible to show the glucosylamines had similar spectroscopic characteristics to the procainamide having a λ_{max} of 286 nm. The retention time of the glucosylamines and the spectrum was identical to that of the diluted sample used in the NMR studies.

An investigation was also undertaken to assure that the reaction was adequately quenched by dilution of a sample with pH 8 buffer. Under room-temperature conditions (21°C) the hydrolysis of the glucosylamines and formation of procainamide proceeded slowly (0·19% h^{-1} for formation when procainamide was in the presence of 5% w/v glucose in the buffer and 0·12% h^{-1} for hydrolysis when an equilibrated solution of procainamide in glucose was diluted with the buffer). This assures that samples derived from the later studies using i.v. infusion bags could be diluted without substantial errors provided the sample is submitted to analysis within a few hours.

Table 3. Reaction of procainamide hydrochloride with glucose following admixture of a commercial injection to glucose infusion.

Time (h)	Procainamide remaining (%)		Glucosylamine formed (%)		Total	
	25°C	30°C	25°C	30°C	25°C	30°C
0	100·0	100·0	–	–	100·0	100·0
1	99·6 ± 1·5	95·8 ± 0·8	1·6 ± 0·04	2·3 ± 0.07	101·3	98·0
2	95·5 ± 2·4	94·6 ± 1·2	3·0 ± 0·04	4·3 ± 0·09	98·6	98·9
3	94·3 ± 2·4	92·8 ± 1·4	4.4 ± 0·12	6·2 ± 0·15	98·7	99·0
4	93.1 ± 1·6	91·1 ± 1·7	5·8 ± 0·15	8·2 ± 0·18	98·9	99·2
5	91·7 ± 1·9	88·5 ± 1·0	7.1 ± 0·16	9·9 ± 0·07	98·9	98·4
7	88·9 ± 3·9	85·5 ± 1·0	9·7 ± 0·40	13·4 ± 0.22	98·6	98·9
9	86·2 ± 2·0	82·2 ± 1·9	12·2 ± 0·30	16·6 ± 0·54	98·4	98·8
12	83·0 ± 1·4	77·7 ± 1·4	15·8 ± 0·33	21·0 ± 0·46	98·8	98·7
24	71·1 ± 1·7	63·2 ± 1·4	27·9 ± 0·70	34·0 ± 0·91	99·0	97·7
36	61·8 ± 2·2	53·9 ± 1·8	36·6 ± 1·2	43·2 ± 1·6	98·2	97·0
48	55·1 ± 1·7	–	46·5 ± 1·4	–	98·6	–

Fig. 3 shows the rate of formation of glucosylamines in glucose at pH 4·0 and 30°C. The reaction follows pseudo first-order reversible kinetics (forward rate constant = $0{\cdot}259\,h^{-1}$; reverse reaction rate constant = $0{\cdot}156\,h^{-1}$; r = 0·999) which rapidly achieve an equilibrium containing 62·3% of procainamide as the glucosylamines. Using preparative HPLC a concentrated sample of glucosylamines in pH 8 buffer was prepared (HPLC analysis demonstrated only 0·54% of free procainamide). When this was diluted with pH 4·0 buffer it produced a buffered solution with a pH of 4·2 and the kinetics of the reverse reaction could be separately assessed (Fig. 4). This follows pseudo first-order kinetics (rate constant = $0{\cdot}199\,h^{-1}$; r = 0·999), in acceptable agreement with the rate constant found under reversible conditions and further confirms the reaction is reversible and indicates that the compound formed is glucosylamine and not an Amadori rearrangement product.

These studies were extended to admixture studies of a commercial injection of procainamide (Pronestyl) to commercial glucose 5% i.v. infusion bags at 25 and 30°C, which are temperatures likely to be encountered under clinical conditions (Table 3). The reaction proceeds rapidly and these data demonstrate that a substantial proportion of the procainamide is lost with the formation of glucosylamines in a period of time under which these admixture products would be prepared and administered.

These studies were not analysed kinetically since, at both temperatures, there was a fall in pH of the admixed product of approximately 0·2 pH units. This may be accounted for by the presence of sodium metabisulphite in the Pronestyl injection which would lower the pH of the unbuffered admixture upon contact with oxygen by oxidation to sulphuric acid. In these studies, there was a small loss of total procainamide which may be accounted for by sorption to the plastic bags over the time-course of the study. However, accepting a shelf-life where $\geq 90\%$ of the procainamide concentration remained then the admixture would retain its potency for approximately 5 h at 25–30°C.

These investigations demonstrated that there is a potentially clinically significant fall in concentration of procainamide when a procainamide hydrochloride injection is added to glucose infusion and that glucose infusion is not a suitable diluent for procainamide hydrochloride injection. The fate of the α- and β-glucosylamines in the body are unknown and warrants further investigation.

Acknowledgements

We wish to thank Dr L. Byrne of the University of Western Australia for the NMR spectra.

References

Bailey, K., Butterfield, A. G. (1981) Structure of condensation products between isonicotinylhydrazine and reducing sugars by ^{13}C NMR spectroscopy. Can. J. Chem. 59: 641–646

Breitmaier, E., Voelter, W. (1990) Carbon–13 NMR Spectroscopy. 3rd edn, VCH, Cambridge, pp 380–381

Capon, B., Connett, B. E. (1966) The mechanism of the hydrolysis of *N*-aryl-*D*-glucosylamines. J. Chem. Soc. 4497–4502

Chavis, C., de Gourcy, C., Dumont, F., Imbach, J.-L. (1983) NMR studies of D-ribosylamines in solution: derivatives of primary amines. Carbohydr. Res. 113: 1–20

Das Gupta, V. (1982) Complexation of procainamide with dextrose. J. Pharm. Sci. 71: 994–996

Das Gupta, V. (1983) Complexation of procainamide with hydroxide-containing compounds. J. Pharm. Sci. 72: 205–207

Ellis, G. P., Honeyman, J. (1955) Glucosylamines. Adv. Carbohydr. Chem. 10: 95–167

Henry, D. W., Lacerte, J. A., Klutman, N. E., Riley, C. M. (1991) Irreversibility of procainamide-dextrose complex in plasma in vitro. Am. J. Hosp. Pharm. 48: 2426–2429

Hodge, J. E. (1955) The Amadori rearrangement. Adv. Carbohydr. Chem. 10: 169–205

Ledl, F., Schleicher, E. (1990) New aspects of the Maillard reaction in foods and in the human body. Angew. Chem. (Int. Ed.) 29: 565–594

Raymond, G. G., Reed, M. T., Teagarden, J. R., Story, K., Geberbauer, C. W. (1988) Stability of procainamide hydrochloride in neutralized 5% dextrose injection. Am. J. Hosp. Pharm. 45: 2513–2517

Riley, C. M. (1988) Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection. Am. J. Hosp. Pharm. 45: 2079–2091

Riley, C. M., Junkin, P. (1991) Stability of amrinone and digoxin, procainamide hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures. Am. J. Hosp. Pharm. 48: 1245–1252

Silverstein, R. M., Bassler, G. C., Morrill, T. C. (1991) Spectrometric identification of organic compounds. 5th edn, Wiley, New York, pp 196–197