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Stopped-flow determination of procaine hydrochloride in pharmaceutical preparations

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Abstract: A stopped-flow photometric method for the quantitative determination of procaine hydrochloride is proposed. It is based on the measurement of the rate of formation of a coloured product between procaine and sodium 1,2-naphthoquinone-4-sulphonate. The conversion of an equilibrium method to a kinetic method by the stopped-flow technique results in an improved detection limit and linear range, and in considerably increased sample throughput. A simple modular stopped-flow system has been used for this purpose. The results obtained on application of this method to the determination of procaine in various pharmaceutical preparations, show the suitability of the stopped-flow technique for routine pharmaceutical analyses.

Keywords: *Procaine hydrochloride; stopped-flow technique; pharmaceutical preparations; colorimetry.*

Introduction

Despite its outstanding virtues (high sampling rate and precision and low sample consumption), application of the stopped-flow technique has been limited by the high cost of commercial stopped-flow instruments. To overcome this disadvantage, a simple, inexpensive modular stopped-flow system [1] has been developed which can be coupled to any spectrophotometer or spectrofluorimeter. The use of this module makes the stopped-flow technique affordable by any laboratory. The usefulness of this module for routine determinations has been demonstrated by application to the analysis of copper in serum [2] and iron in wine [1].

In the present paper this stopped-flow module has been applied to pharmaceutical analyses, namely, the determination of procaine hydrochloride in various pharmaceutical preparations. Procaine is a local anaesthetic that is widely used in a variety of pharmaceutical preparations. Although several electrochemical methods [3–5] have been reported for the determination of procaine, this drug is usually determined spectrophotometrically [6–8] by equilibrium methods. No kinetic methods have been described for such a determination.

The colour-formation reaction between procaine hydrochloride and sodium 1,2-naphthoquinone-4-sulphonate has been chosen; this reaction was previously used to

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develop an equilibrium method for the determination of procaine hydrochloride [8]. The conversion of this method to a kinetic method by the stopped-flow technique substantially improves the features of the determination; for example, the kinetic method has an increased sampling rate and a wider linear range of the calibration graph; in addition, there is no need for a blank solution. The present study demonstrates the suitability of the stopped-flow technique for routine pharmaceutical analysis.

Experimental

Apparatus

A Perkin–Elmer spectrophotometer (Model Lambda 5) equipped with the stopped-flow module, was used for reaction rate measurements. The module, depicted in Fig. 1, was designed in the author's laboratory [1] and is marketed by Quimi-Sur Instrumentation. It includes two three-way valves which allow fractions of the prepared samples to be taken automatically and mixed with the reagent in the flow-cell (mixing chamber), which is introduced into the spectrophotometer cell compartment. A checking valve placed after the flow-cell exit provides a back pressure during the delivery operation and avoids the generation of fine bubbles. Each syringe delivers 0.25 ml of prediluted sample and reagent, and three flushes are used to prevent carryover. The spectrophotometer cell compartment is thermostatted by an electronic Peltier system and the solutions in the stopped-flow module are thermostatted using a circulating water-bath. The module is controlled by the associated electronics and a Hewlett–Packard 98640 A computer.

Reagents

A stock solution (1 g l^{-1}) of procaine hydrochloride (Sigma) was prepared in distilled water and stored at 0–4°C. Standard solutions of lower concentrations were prepared daily by diluting to the appropriate volume with distilled water.

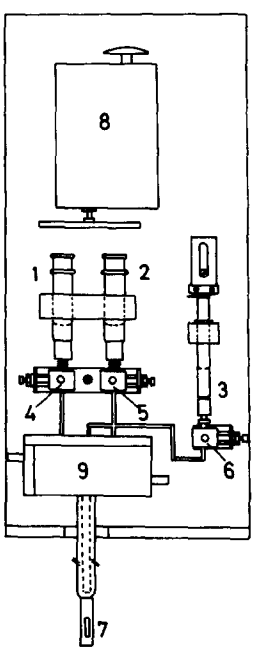

Figure 1
Scheme of the stopped-flow module: 1 and 2, drive syringes; 3, stop-syringe; 4, 5 and 6, three-way stopcock delivery valves; 7, flow-cell; 8, propulsion system; 9, thermostatic system.

A sodium 1,2-naphthoquinone-4-sulphonate (Aldrich) solution (3.84×10^{-2}M) was prepared by dissolving 1.0 g of the reagent in 100 ml of distilled water.

A K_2HPO_4–KH_2PO_4 buffer solution (total concentation 0.1 M, pH 7.0) was also prepared.

All chemicals used were analytical reagent grade.

Procedure

One of the two 10-ml reservoir syringes was filled with a previously prepared aqueous solution containing 2 ml of phosphate buffer (pH = 7.0) and 4 ml of sodium 1,2-naphthoquinone-4-sulphonate reagent and diluted with distilled water to a final volume of 10 ml; the other syringe was filled with a standard or sample solution of procaine hydrochloride at a concentration of 1–200 μg ml^{-1}. Once the 2-ml drive syringes had been filled, 0.25 ml of both solutions from these syringes were mixed in the mixing chamber in each run. The variation of the absorbance was monitored at 486 nm and followed through a chart recorder operating at 20 mm min^{-1}. All measurements were carried out at 40°C. The absorbance values were collected at each 0.6 s and processed by the microcomputer, provided with a program for application of the initial-rate method. The reaction rate was determined in about 6 s, and each sample was assayed in triplicate. The blank signal was found to be negligible.

Determination of procaine hydrochloride in pharmaceutical preparations

This determination required only appropriate dilution of the sample to obtain a concentration within the range of concentrations in the calibration graph. No sample pretreatment was needed and the diluted samples were treated as described under Procedure.

Results and Discussion

Procaine hydrochloride forms an orange-red product with sodium 1,2-naphthoquinone-4-sulphonate [8] by a condensation reaction. Although this method has been proposed for routine analysis of official preparations of procaine hydrochloride, it requires waiting for 60 min before absorbance measurements are made. To avoid this dead-time, the method has been modified by measuring the rate of formation of the coloured product instead of the final absorbance. The rapid and thorough mixing of sample and reagent in the flow-cell of the stopped-flow module used for this purpose, allows the immediate development of the coloured product, which can be monitored photometrically by kinetic measurements.

Optimization of variables

In order to optimize the variables potentially affecting the reaction rate, each was changed in turn while the others were kept constant. The effect of pH, buffer solution, temperature, reagent concentration, dielectric constant and ionic strength was evaluated. The optimum values chosen were those yielding the minimum possible relative standard deviation for the initial-rate measurements, under conditions where the reaction order with respect to the variable concerned was zero or close to zero. All concentrations indicated are the initial concentrations in the syringes (two-fold the actual concentrations in the reaction mixture at time zero after mixing). The kinetic results given are the mean of three measurements.

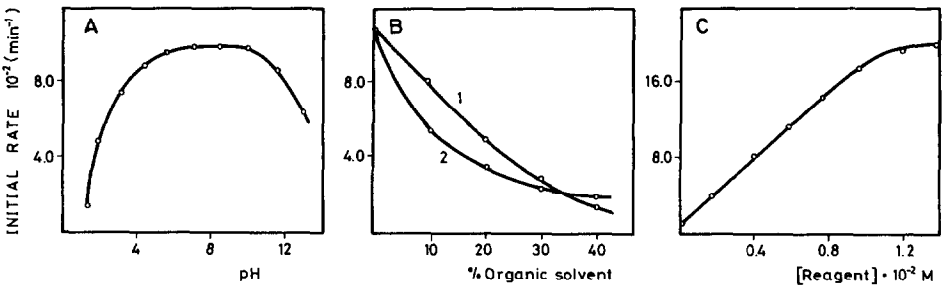

Figure 2
Effect of variables on the reaction rate: (A) pH. (B) % Organic solvent, 1, ethanol; 2, dimethylformamide. (C) Concentration of sodium 1,2-naphthoquinone-4-sulphonate.

The reaction rate is not affected by variations in the pH in the range 6.0–10.2, but is decreased outside this range (Fig. 2A). The pH of the samples was adjusted with K_2HPO_4–KH_2PO_4 buffer solution (pH = 7.0), the concentration of which has no appreciable effect on the reaction rate, at least in the range 5×10^{-3}–6×10^{-2}M. There was a linear increase in the initial rate with increasing temperature in the range 15–30°C; such a rate was a maximum and independent of temperature in the range 30–60°C. A temperature of 40 ± 0.1°C was selected.

The reaction rate is markedly affected by the dielectric constant of the solution when this is decreased by the use of organic solvents such as ethanol or dimethyl formamide (Fig. 2B). Various ionic strengths up to 0.4 (adjusted with KCl, $NaClO_4$ or KNO_3) had no effect on the reaction rate. Figure 2C shows the variation of the reaction rate with the concentration of sodium 1,2-naphthoquinone-4-sulphonate. The reaction rate increases with concentration up to 1.2×10^{-2}M and remains constant above this concentration of reagent.

The initial slopes indicate a first-order reaction with respect to procaine. In the working conditions, chosen from the optimization study, all reagents showed a pseudo-zero kinetic dependence; thus, the following kinetic equation is suggested:

$$v = k\,(\text{procaine}),$$

where v is the rate of formation of the coloured reaction product and k is the conditional rate constant.

Features of the analytical method

Under the optimum conditions, absorbance–time curves for different amounts of procaine hydrochloride were processed by the initial-rate method. The calibration graph was linear over the concentration range 1–200 μg ml^{-1} of procaine hydrochloride, with a Pearson's coefficient (r^2) of 0.998. The detection limit, as defined by IUPAC [9], was 0.7 μg ml^{-1} of procaine hydrochloride. These results show that the use of the stopped-flow technique results in a considerably broadened linear range in comparison to that of the equilibrium method [8] (6–20 μg ml^{-1}). Although no detection limit was calculated for the equilibrium method [8], the lower limit of the calibration graph suggests that the kinetic method was better than the equilibrium method in this respect. The relative standard deviation ($P = 0.05$, $n = 10$) for 10 μg ml^{-1} of procaine hydrochloride was

±1.23% in the kinetic method. The total time for development of the reaction using the stopped-flow technique is 25 s. Although in the equilibrium method [8] this time is stated to be 60 min; the authors of the present paper have verified that by using the optimum reagent concentration for the kinetic method but carrying out the measurement by the conventional mode, the reaction attains equilibrium in only 3 min. This short reaction time can be further decreased by using the stopped-flow technique because of the rapid mixing of procaine and the reagent in the flow-cell. With this method, the time elapsed in three replicate determinations performed on the same sample was about 25 s since each kinetic curve was monitored for only 6 s.

To determine the selectivity of the stopped-flow method, several species usually present with the analyte in commercial pharmaceutical preparations were tested at higher concentrations than those usually present in such preparations. The results obtained (Table 1) showed that none of the species assayed except epinephrine (adrenaline) interfered with the determination of procaine, at least at a concentration 100 times higher than that of the analyte. The epinephrine/procaine hydrochloride

Table 1
Tolerated concentrations of various species in the determination of 10 μg ml^{-1} of procaine hydrochloride

Tolerated concentration (μg ml^{-1})	Species added
1000	Glycerol, lidocaine (lignocaine), tetracaine (amethocaine), ascorbic acid, cocarboxylase, urea, thiamine, methionine, dimethylsulphoxide, boric acid, Mg^{2+}, Zn^{2+}, Co^{2+}, SO_4^{2-}, Cl^-
5	Epinephrine (adrenaline)

Table 2
Determination of procaine hydrochloride in pharmaceutical preparations

Sample*	Procaine hydrochloride content		
	Stated	Found†	Equilibrium method
1	12.6‡	12.8 ± 0.2	12.3
2	20.0‡	20.9 ± 0.2	20.0
3	20.0‡	21.0 ± 0.3	20.1
4	4.0‡	4.1 ± 0.2	4.1
5	15.0§	15.0 ± 0.2	15.5
6	10.0§	10.2 ± 0.2	10.6

*Name, laboratory and composition of samples:

1. Otosedol (Pensa): procaine hydrochloride (12.6 mg), phenazone (63.0 mg), excipient to 1.0 ml.
2. Anestesia local Miró (Andalucia Farma): procaine hydrochloride (20 mg), epinephrine (adrenaline) 0.1% solution (0.0125 ml), excipient to 1.0 ml.
3. Anestina Miró (Andalucia Farma): lidocaine hydrochloride (5 mg), procaine hydrochloride (20 mg), tetracaine (amethocaine) hydrochloride (1.25 mg), double-distilled water to 1.0 ml.
4. Sulmetin injectable intramuscular (Semar S.A.): magnesium sulphate hepta-hydrate (150 mg), procaine hydrochloride (4 mg), double-distilled water to 1.0 ml.
5. Otosmo (dr Aristegui): procaine hydrochloride (15.0 mg), analgesine (phenazone) (50 mg), glycerol to 1.0 g.
6. Otalgan (Instituto Berna de España S.A.): procaine hydrochloride (10.0 mg), phenyldimethylpirazolone (phenazone) (50.0 mg), glycerol to 1.0 g.

†Mean of three determinations ± standard deviation.

‡mg ml^{-1}.

§mg g^{-1}.

tolerated ratio was 1:2, but this catecholamine is generally present in pharmaceutical preparations at a very much lower concentration than that of procaine. Although the proponents of the equilibrium method [8] claim that epinephrine poses no interference, they do not give the tolerated concentration, and the samples analysed contain an epinephrine concentration one-thousandth that of procaine.

Applications

The stopped-flow method was satisfactorily applied to the determination of procaine hydrochloride in various commercial pharmaceutical preparations. The results obtained and the composition of the pharmaceutical preparations are summarized in Table 2. This table also shows the results obtained in the analysis of these samples by using the equilibrium method of Salama and Omer [8] as a reference method. The recovery study was carried out by adding different amounts of procaine hydrochloride to the

Table 3
Recovery of procaine hydrochloride from pharmaceutical preparations

Sample*	Procaine hydrochloride ($\mu g\ ml^{-1}$) Added	Found†	Recovery (%)
1	5.0	5.1	102.0
	10.0	10.1	101.0
	15.0	14.8	98.7
	30.0	29.8	99.3
	50.0	50.3	100.7
2	5.0	4.9	98.0
	10.0	10.4	104.0
	15.0	15.0	100.0
	30.0	28.7	95.6
	50.0	49.5	99.0
3	5.0	5.0	100.0
	10.0	10.4	104.0
	15.0	14.8	98.8
	30.0	29.6	98.6
	50.0	50.2	100.4
4	5.0	4.9	98.0
	10.0	10.3	103.0
	15.0	14.8	98.7
	30.0	30.2	100.6
	50.0	51.1	102.2
5	5.0	5.0	100.0
	10.0	10.6	106.0
	15.0	14.7	98.0
	30.0	29.5	98.3
	50.0	51.8	103.6
6	5.0	5.2	104.0
	10.0	10.1	101.0
	15.0	14.8	98.7
	30.0	30.8	102.7
	50.0	50.4	100.8

*See Table 2.
†Mean of three determinations.

pharmaceutical preparations and subtracting the results obtained for preparations made in a similar manner but to which no procaine was added. Table 3 lists the recoveries.

The sampling rate is about 140 samples h^{-1}. Each analysis was carried out by filling the drive syringes, recording the kinetic curve and then purging the stop syringe. These results show how readily the stopped-flow technique can be adapted to routine analyses for procaine hydrochloride in pharmaceutical preparations; such analyses are selective and fast.

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