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A selective and sensitive kinetic method for the determination of procaine and benzocaine in pharmaceuticals

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Abstract: A simple stopped-flow method for the determination of procaine and benzocaine which is suitable for their routine analysis in pharmaceutical samples is reported. The method is based on a condensation reaction between each compound and 4-dimethylaminocinnamaldehyde, which yields coloured compounds and which are monitored spectrophotometrically. The calibration graph generated is linear over the range 0.5–20 μg ml^{-1} (RSD 0.73%) for procaine and 0.5–15 μg ml^{-1} (RSD 0.40%) for benzocaine, and the selectivity is very high in both cases. The proposed methods were satisfactorily applied to the determination of the two drugs in various pharmaceutical samples. A procedure for resolution of procaine–benzocaine mixtures in mass ratios between 15:1 and 1:7 with a precision of *ca* 1% was developed in order to determine one compound in the presence of the other with no interference.

Keywords: *Procaine; benzocaine; pharmaceuticals; stopped-flow; spectrophotometry.*

Introduction

Local anaesthetics are special compounds which cause a reversible loss of sensation by preventing transmission of sensory nerve impulses near the site of application or injection. The chemical structure of these compounds is relatively similar. Procaine and benzocaine are two esters of *p*-aminobenzoic acid and are widely used as local anaesthetics. These compounds are available as injectables, ophthalmic and otic solutions, creams, ointments and topical solutions. These pharmaceutical preparations are analysed, according to the U.S. Pharmacopeia [1], by methods including the potentiometric titration of benzocaine with sodium nitrite using a calomel-platinum electrode and an ultraviolet spectrophotometric assay which requires several liquid–liquid extraction steps to remove the sample matrix for procaine. The former method is tedious, while the latter is scarcely selective and both are time-consuming.

Increasing effort has recently been devoted to the development of reliable analytical methods for the determination of these drugs and their dosage forms. In the last few years, polarographic [2], gas chromatographic [3], HPLC [4] and, particularly spectrophotometric methods, have been reported. Spectrophotometric determinations of these drugs are based on the ability of their primary amine group to yield chromogens on condensation [5, 6], diazo coupling [7, 8], oxidative coupling [9], etc. However, the colour development generally takes quite a long time (about 10–60 min), so these methods are unsuitable for routine analysis.

This work was aimed at developing a simple, rapid kinetic-spectrophotometric method for the routine determination of these local anaesthetics by using the stopped-flow technique for the mixing of sample and reagents and applying it to drug substances and some pharmaceutical preparations. The method is based on a condensation reaction of the drug with 4-dimethylaminocinnamaldehyde (4-DACA) in a methanol medium; the concentration of the drugs in the sample is directly proportional to the rate of appearance of the condensation product, which is monitored spectrophotometrically at 547.5 nm. Although the method is very tolerant to other related drugs, the only severe interference arises from the presence of the other compound, i.e. either procaine or benzocaine. For this reason, mixtures of these local anaesthetics were assayed using a differential reaction-rate method.

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Experimental

Reagents

All chemicals used were of analytical reagent grade. All diluted solutions were prepared immediately prior to use. Solutions of procaine hydrochloride and benzocaine containing 1000 μg ml^{-1} of either drug were prepared by dissolving 100.0 mg of each (Sigma) in 100 ml of methanol. A 1.425×10^{-2} M solution of 4-dimethylaminocinnamaldehyde (4-DACA) (Merck) solution was prepared by dissolving 249.7 mg of the reagent in methanol and diluting to 100 ml. A solution of 40% (w/v) trichloroacetic acid (Merck) in methanol was also prepared.

Apparatus

Spectrophotometric measurements were made on a Hewlett–Packard spectrophotometer (Model 8452A) fitted with a stopped-flow module [10]. The temperature of the spectrophotometric cell compartment was kept at 40°C by circulating water from a thermostatted water bath. Computational aspects, the hardware and software used for implementation of kinetic measurements are described elsewhere [11, 12]. A Radiometer PHM62 pH meter, equipped with a combined glass-calomel electrode, was used for pH measurements.

Procedures

Stopped-flow determination of the local anaesthetics. The two drive syringes of the stopped-flow module were filled with the reagent and sample solution and were prepared as follows: the reagent solution contained 5.0 ml of 1.42×10^{-2} M 4-DACA and 5.0 ml of 40% (w/v) trichloroacetic acid in 10 ml volumetric flask; the sample solution contained between 5 and 200 μg of procaine hydrochloride or between 5 and 150 μg of benzocaine and was diluted to volume with methanol in 10 ml volumetric flask. The two solutions were mixed in the mixing/observation cell and the reaction was monitored by following the increase in absorbance at 547.5 nm. The temperature was kept constant at 40 ± 0.1°C throughout the analyses. The computer system recorded the full signal vs time curve and calculated the initial rate (over a period of *ca* 3 s) and the concentration of each compound.

Sample treatment. The pharmaceutical samples assayed were treated as follows: a given number of tablets were ground to a fine powder (drop solutions and aerosols were shaken). Accurately weighed samples were then stirred with methanol until complete dissolution and the solution was diluted with methanol in a 100 ml volumetric flask. If a residue was formed, it was filtered off and washed with portions of methanol, and the filtrate and washings were diluted to volume with this solvent in a 100 ml volumetric flask. In all cases, aliquots of these solutions were treated as described previously.

Simultaneous analysis for procaine and benzocaine. Two solutions were prepared in order to fill the drive syringes of the stopped-flow module. One was the reagent solution and the other was prepared by mixing local anaesthetic solutions containing 1–15 μg ml^{-1} procaine hydrochloride and 1–7 μg ml^{-1} benzocaine. The procedure was then continued as described above. From the kinetic graph recorded at a data collection rate of 150 ms per point, the absorbance data measured over the first 3 s of the reaction (initial rate) and again when colour development was complete, were treated by the proportional-equation method [13].

Results and Discussion

Amino-compounds can be determined kinetically in a number of ways, both individually [14–22] and in mixtures by using differential methods [23–30]. These determinations are based on a large variety of reactions; however, the ability of the primary amine group to form condensation products with aldehydes and ketones has scarcely been used to develop kinetic determinations in contrast with equilibrium methods. In this work, the condensation reactions between two local anaesthetics, procaine and benzocaine, and 4-dimethylaminocinnamaldehyde in an acid medium were exploited to develop a reliable kinetic analytical method for the determination of these compounds. These fast reactions required application of the stopped-flow technique in order to monitor the reaction rate spectrophotometrically. Figure 1 shows the absorbance vs time curves obtained for the two local anaesthetics with the aid of the data acquisition system used. As can be seen, benzocaine reacts much faster than procaine

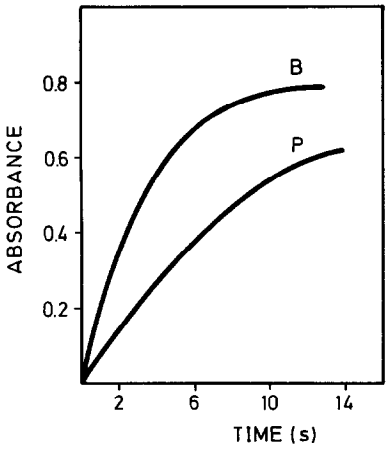

Figure 1
Absorbance vs time graphs run at 547.5 nm. B, benzocaine and P, procaine hydrochloride. Local anaesthetic concentration, 7.5 μg ml^{-1} in both cases. Other experimental conditions as described in Procedures.

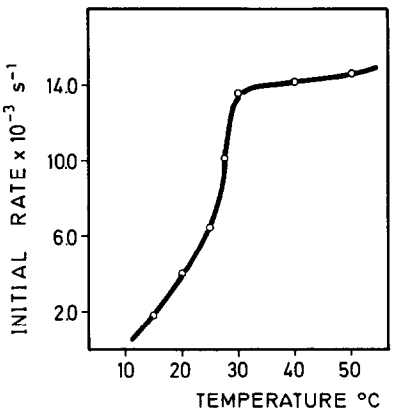

Figure 2
Influence of temperature on the initial rate. Experimental conditions: $C_{4\text{-DACA}}$, 9.5 × 10^{-4} M; $C_{\text{trichloroaceticacid}}$, 8.3% (w/v); C_{procaine}, 2.5 μg ml^{-1}.

hydrochloride. This differential behaviour was exploited for the kinetic determination of both compounds individually and in mixtures.

Effect of variables on the analytical procedure

In order to achieve the maximum possible sensitivity with the proposed kinetic methods, a number of factors such as temperature, acidity, concentration of the condensation reagent and methanol percentage were optimized. Due to the similar structure of both compounds the reaction variables were only optimized for procaine hydrochloride and all concentrations given below are initial concentrations in the drive syringes of the stopped-flow module.

The effect of temperature on the reaction rate was examined between 15 and 50°C (Fig. 2). The reaction rate increased exponentially with increasing temperature over the range 15–30°C, the relative change being smaller above 30°C; a temperature of 40°C was thus chosen for subsequent experiments. An activation energy of 94.8 kJ mol^{-1} was obtained by plotting the logarithm of the initial rate against the reciprocal of the absolute temperature.

The effect of varying the 4-DACA concentration over the range 4.75 × 10^{-4}–2.85 × 10^{-3} M was also studied. An increase in the reagent concentration resulted in a linear increase in the initial rate over the range 1.42 × 10^{-3}–2.85 × 10^{-2} M as shown in Fig. 3(a). Higher concentrations of 4-DACA could not be used because they required using oversaturated stock solutions of this compound

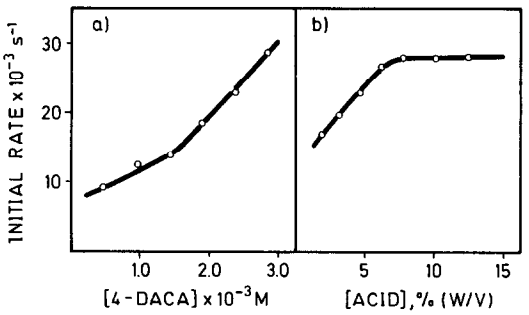

Figure 3
Effect of (a) condensing agent and (b) medium acidity on the reaction rate. Experimental conditions: temperature 40°C; $C_{\text{trichloroaceticacid}}$, 8.3% (w/v); C_{procaine}, 2.5 μg ml^{-1}.

in preparing the reagent solution for the drive syringe. However, under these conditions, the mole ratio of 4-DACA to procaine was about 300, which assured first-order conditions for the determination of this local anaesthetic. A 4-DACA concentration of 2.85 × 10^{-3} M was selected accordingly which was obtained by adding 5.0 ml of a 1.42 × 10^{-2} M solution of this compound to prepare 10 ml of reagent solution.

The effect of the medium acidity on the condensation reaction was evaluated by using trichloroacetic acid solutions at concentrations over the range 1.56–12.5% (w/v). An exponential dependence was found up to a concentration of about 7.8%, above which the initial rate remained constant (Fig. 3b). A concentration of 8% was chosen as optimal which was obtained by adding 5.0 ml of 40% (w/v) methanolic trichloroacetic acid to prepare the reagent solution.

The amount of methanol used was crucial to the development of the condensation reaction. Solvents, other than methanol, were not tested since it is generally used in this reaction. The effect of this solvent was tested in the two solutions used to fill the drive syringes of the stopped-flow module (reagent and sample solution). Reagent solution containing smaller amounts of this solvent yielded irreproducible results or even caused precipitation to occur, on the other hand, a methanol content of 50% (v/v) in the sample solution resulted in a decrease of the initial rate by about 50%. Lower methanol contents gave irreproducible results and precipitation occurred on mixing of the sample and reagent solutions in the mixing-observation cell of the stopped-flow module. For these reasons, the sample and reagent solutions were prepared in methanol.

Absorbance–time curves were recorded for solutions containing different amounts of procaine or benzocaine under the above-described experimental conditions. The reaction was found to be first-order with respect to both local anaesthetics. The following kinetic equation was used for the determination of the analytes:

$$\text{d[Condensation product]}/dt = k\text{[4-DACA][Local anaesthetic]},$$

where k is the rate constant.

Stopped-flow determination of the local anaesthetics

The absorbance vs time curves obtained at different concentrations of procaine and benzocaine under the optimal working conditions were analysed by two methods: initial-rate (kinetic mode) and absorbance (equilibrium mode). In the latter, the analytical signal corresponded to the difference between the initial absorbance ($t = 0$) and that obtained on reaction completion ($t = \infty$).

The key analytical features of the two determinations are summarized in Table 1. The analytical sensitivity was taken as the slope of each calibration plot and the detection limit was calculated by the IUPAC procedure [31]. The precision (expressed as the RSD) was determined by analysing 11 samples containing 2.5 μg ml^{-1} of procaine or benzocaine each, and the sample throughput was calculated for the time required for three replicate analyses including changeover of the sample solution in the drive syringe of the stopped-flow unit.

The initial-rate and absorbance method provided similar results; however, the former is more recommendable for the routine determination of these local anaesthetics on account of its higher sample throughput. Also, the determination of benzocaine is more sensitive than that of procaine, although its dynamic linear range is narrower, particularly when determined by the absorbance method.

Simultaneous determination of procaine and benzocaine

According to the results obtained so far, the proposed methods are useful for the determination of these local anaesthetics; however, procaine interferes severely with the determination of benzocaine and vice versa. This shortcoming could be overcome by using a separation technique which, however, would make the method too complicated for routine analytical use. Alternatively, a differential kinetic method can be used to determine both compounds simultaneously in a given sample using a single aliquot.

Taking into account the kinetic behaviour observed in the two local anaesthetics and described in the previous section, we chose the differential kinetic proportional-equations method, a widely used mathematical approach for the resolution of closely related species [32] in order to resolve the drug mixture. Practical application of this method entails measuring the analytical signal at two reaction times in order to establish two equations. Not only time but also other parameters can be used to establish the simultaneous equations from which the required concentrations are to be calculated. Thus, we used the initial rate (IR) and the absorbance measured after the reaction has developed (A_∞) for the individual calibration plots of the two local anaesthetics. Thus, two linear calibration graphs (IR vs [local anaesthetic] and A_∞ vs [local anaesthetic]) were run for each compound separately. Table 2 lists the principal data derived from these plots, from which the following simultaneous equations were established for the resolution of procaine–benzocaine mixtures:

$$\text{IR} = -4.70 \times 10^{-4} + 8.41 \times 10^{-3}\,\text{[Procaine]} - 6.67 \times 10^{-3} + 2.33 \times 10^{-2}\,\text{[Benzocaine]},$$

$$A_\infty = -1.89 \times 10^{-2} + 8.67 \times 10^{-2}\,\text{[Procaine]} - 5.32 \times 10^{-2} + 1.23 \times 10^{-1}\,\text{[Benzocaine]},$$

Table 1
Analytical features of the methods

	Procaine		Benzocaine	
Feature	Initial rate	Absorbance	Initial rate	Absorbance
Linear dynamic range	0.5–20 $\mu g\ ml^{-1}$	1.0–15 $\mu g\ ml^{-1}$	0.5–15 $\mu g\ ml^{-1}$	1.0–7.0 $\mu g\ ml^{-1}$
Analytical sensitivity	8.41×10^{-3} ml $\mu g^{-1}\ s^{-1}$	8.67×10^{-2} ml μg^{-1}	2.33×10^{-2} ml $\mu g^{-1}\ s^{-1}$	1.23×10^{-1} ml μg^{-1}
Detection limit	100 ng ml^{-1}	300 ng ml^{-1}	30 ng ml^{-1}	200 ng ml^{-1}
Precision (RSD)	0.73%	0.56%	0.40%	0.72%
Sample throughput	85 h^{-1}	30 h^{-1}	100 h^{-1}	35 h^{-1}

Table 2
Principal data derived from the calibration graphs generated for implementation of the proportional-equation method

Local anaesthetic	Measured parameter	Slope	Intercept	Corr. coeff. (n = 8)
Procaine	Initial rate	8.41×10^{-3} ml μg^{-1} s^{-1}	4.70×10^{-4} s^{-1}	0.998
	A_∞	8.67×10^{-2} ml μg^{-1}	-1.89×10^{-2}	0.999
Benzocaine	Initial rate	2.33×10^{-2} ml μg^{-1} s^{-1}	-6.67×10^{-3} s^{-1}	0.999
	A_∞	1.23×10^{-1} ml μg^{-1}	-5.32×10^{-2}	0.998

Table 3
Analysis of synthetic mixtures of procaine and benzocaine

Local anaesthetic (μg ml^{-1})					
Procaine	Benzocaine	Procaine found (μg ml^{-1})	Error (%)	Benzocaine found (μg ml^{-1})	Error (%)
5.00	5.00	4.92	−1.60	4.89	−2.20
2.50	1.00	2.45	−2.00	1.01	1.00
5.00	1.00	4.94	−1.20	1.01	1.00
7.50	1.00	7.68	2.40	0.97	−3.00
10.00	1.00	9.97	−0.30	1.02	2.00
12.50	1.00	12.59	0.72	0.98	−2.00
15.00	1.00	15.06	0.40	0.98	−2.00
1.00	3.00	1.01	1.00	3.16	5.33
1.00	5.00	1.01	1.00	5.09	1.80
1.00	7.00	0.99	−1.00	6.67	−4.71

where the concentration of each local anaesthetic is given in μg ml^{-1}. Hence, only one kinetic curve need be acquired by the computer system under the optimal experimental conditions for the resolution of procaine–benzocaine mixtures. From this, IR and A_∞ were calculated and the concentrations of the two local anaesthetics determined from the above equations.

The results obtained for several synthetic mixtures of procaine and benzocaine are summarized in Table 3. The determination of procaine–benzocaine mixtures is feasible over the weight ratio range 15:1 to 1:7 with an accuracy better than ±5.0%. Application of the proposed procedure to 11 samples containing 5.0 μg ml^{-1} procaine and 1.0 μg ml^{-1} benzocaine yielded a RSD of 0.83% for the former and 1.36% for the latter. We can thus conclude that the determination of benzocaine tolerates procaine at 15-fold concentrations, while the latter tolerates benzocaine in sevenfold amounts.

In order to evaluate the influence of other compounds on the determination of these local anaesthetics we carried out a study of potential interferences from related substances such as local and general anaesthetics, sympathomimetics, antidepressants, opioid analgesics, anxiolytic sedatives, hypnotics, etc. Thus, solutions of these local anaesthetics and each tested compound were mixed to obtain samples containing 2.5 μg ml^{-1} procaine and benzocaine and up to 250 μg ml^{-1} of interferent. The apparent procaine and benzocaine concentrations in these samples were measured and the tolerated limits were calculated, as the highest weight ratios yielding errors less than ±5% in the determination of the two local anaesthetics. The results found are given in Table 4. As can be seen, the proposed stopped-flow method for the determination of procaine and benzocaine is quite selective; therefore, it has a great potential for the analysis of these local anaesthetics in formulations. This is confirmed by the results given in the next

Table 4
Tolerated ratios of related compounds in the stopped-flow determination of 2.5 μg ml^{-1} procaine and benzocaine

Compound	Tolerated mass ratio of compound to local anaesthetic
Thiopental, epinephrine, norepinephrine, amileine ketamine, cocaine, morphine, etomidate, droperidol	100
Tetracaine	90
Mepivacaine, lidocaine	80

Table 5
Determinations of procaine and benzocaine in pharmaceutical preparations by the stopped-flow technique

Sample	Local anaesthetic	Amount		Mean recovery (%)
		Certified by the manufacturer	Found[a]	
Eupnol Desinfectante[b]	Procaine	0.52 mg tablet^{-1}	0.52 (0.19)	99.5
Dentol Tópico[c]	Procaine	10 mg ml^{-1}	9.37 (2.92)	98.6
Otosedol[d]	Procaine	12.60 mg ml^{-1}	12.12 (1.27)	99.5
Dynamin[e]	Procaine	5.00 mg tablet^{-1}	5.05 (0.30)	99.8
Sanaden Reforzado[f]	Benzocaine	25 mg ml^{-1}	25.40 (0.76)	98.9
Otocerum[g]	Benzocaine	30 mg ml^{-1}	30.48 (0.62)	99.3
Hibitane Oral[h]	Benzocaine	2.0 mg tablet^{-1}	1.99 (0.10)	99.2
Topicaina Miró[i]	Benzocaine	140 mg ml^{-1}	141.12 (0.42)	99.7

[a] Average of four determinations with RSD in brackets. [b–i] Coexisting substances (in mg) include: [b] benzethonium chloride (0.32), cineole (3.90), cherry laurel water (4.16), terpineol (2.60), menthol (1.95); [c] alcanfor (250), saffron (50), clove oil (1.0), chloral carnitine (50), menthol (20); [d] phenazone (63); [e] cyanocobalamin (0.002), cocarboxylase (10), folic acid (5), pyridoxine (5), haemotoprophyrin (0.20), serine phosphate (15), gluthatione (0.50), inosine (25), adenosine triphosphate (5), uridine-diphosphate-glucose (15), ribonucleic acid (25), orotic acid (2.5), magnesium molybdate (10.10), magnesium pemoline (10); [f] creosote (150), eugenol (50), gommier oil (25), lidocaine (10); [g] benzilo-*p*-phenol (10), chlorobutanol (0.05), turpentine oil (0.15); [h] chlorhexidine hydrochloride (5); [i] benzalkonium chloride (5), butacaine sulphate (10), butoforme (60), cetrimonium bromide (0.05), tetracaine hydrochloride (20).

section, which reports on the analyses of these compounds in pharmaceutical preparations.

Analysis of pharmaceutical samples

The proposed procedure for determination of procaine and benzocaine described above was applied to various pharmaceutical formulations manufactured by Spanish laboratories. All pharamceuticals contained only one of the analytes as no Spanish laboratory appears to manufacture any preparations containing both. In order to evaluate the potential influence of other ingredients, the recoveries of these local anaesthetics in various samples were determined. The results found are summarized in Table 5, which lists the nominal local anaesthetic contents quoted by the manufacturers, those obtained by the stopped-flow method and the mean recovery, for each analysed sample. As can be seen, the satisfactory recovery values obtained, between 98.6 and 99.8% confirm the good performance of the proposed kinetic method for the determination of the two local anaesthetics in these types of sample.

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