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Kinetic fluorimetric determination of promethazine by a stopped-flow mixing technique

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Abstract: The oxidation of promethazine to its corresponding fluorescent sulphoxide was used to develop a novel kinetic fluorimetric method for the determination of this drug. The use of a stopped-flow mixing technique makes use of an oxidizing reagent unnecessary because the oxidation is rapidly carried out by dissolved oxygen. The method is simple and fast as it only requires a few seconds to obtain kinetic data which allows ready application to routine analyses. The calibration graph is linear over the range 0.5–80 μg ml^{-1} and the precision (%RSD) is close to 2%. The method was applied to the determination of promethazine hydrochloride in two pharmaceutical preparations.

Keywords: *Promethazine; stopped-flow mixing technique; fluorimetry.*

Introduction

Although phenothiazine is too toxic for use in human medicine, many phenothiazine derivatives are employed as antihistamine drugs and tranquillizers. All of them can be readily oxidized to sulphoxide derivatives which possess appropriate absorptiometric or fluorimetric properties, and many methods for their determination rely on such oxidation. Promethazine is the phenothiazine derivative most frequently used as an antihistamine drug, though even it also has a marked sedative effect and local anaesthetic properties. Available methods for its determination include titrimetric [1], chromatographic [2, 3] and photometric [4–6] ones. Some derivative [7] and synchronous [8] spectrofluorimetric methods have also been used. Most of these methods rely on the oxidation of promethazine to its sulphoxide derivative by using potassium ferricyanide [1], tungstophosphoric acid [4] or chloramine-T [5]. Also, a flow injection analysis (FIA) procedure was recently developed for the photometric determination of promethazine with cerium(IV) as oxidant [9].

No analytical methods for promethazine based on reaction rate measurements have yet been reported. Kinetic methodology, particularly in association with the stopped-flow mixing technique, currently offers major advantages over equilibrium methodology as it provides a means of accomplishing automation and rapid handling of reagents in routine analyses. The usefulness of this technique in analytical chemistry was recently reviewed [10].

In this work the stopped-flow mixing technique was used to develop a simple, fast kinetic fluorimetric method for the determination of promethazine based on the formation of its sulphoxide derivative and measurement of the reaction rate. Unlike other methods for promethazine, no oxidant need be added to the reaction medium. Due to some intrinsic features of the stopped-flow mixing technique, which allows rapid, thorough mixing of the streams from the drive syringes in the flow-cell, collisions between promethazine and dissolved oxygen molecules are promoted, so the oxidation is rapidly completed.

Experimental

Instrumentation

A Perkin–Elmer Model LS-50 luminescence spectrometer fitted with a stopped-flow module [11] supplied by Quimi-Sur Instrumentation was used for kinetic measurements. The spectrofluorimeter was controlled via a Hewlett–Packard Model Vectra computer. Reaction rate data were obtained by applying the 'Kinetic Obey' application program. The excitation and emission slits were adjusted to provide a 15-nm band-pass and the observation cell of the stopped-flow module had a path

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length of 1.0 cm. The spectrofluorimeter was adjusted daily by using a series of fluorescent polymer samples (Perkin–Elmer) to compensate for changes in the source intensity. The solutions in the stopped-flow module and the cell compartment were kept at a constant temperature (20°C) by circulating water from a thermostated tank.

Reagents

A stock solution of promethazine hydrochloride (Sigma) was prepared at a concentration of 1 mg ml^{-1} in 4×10^{-2} M hydrochloric acid. This solution was stored in amber glassware and kept refrigerated at 0–4°C. Working solutions were prepared daily by appropriate dilution. A 0.1 M phthalate buffer was prepared from potassium acid phthalate and adjusted to pH 2.9 with 0.1 M hydrochloric acid. All chemicals used were of analytical-reagent grade.

Procedure

The two 10 ml reservoir syringes of the stopped-flow module were filled with the same solution, prepared previously and contained 10 ml of 0.1 M phthalate buffer (pH 2.8) and a standard or sample solution of promethazine hydrochloride in a final concentration range 0.5–80 μg ml^{-1}, and a final volume of 25 ml. After the two 2 ml drive syringes were filled, equal volumes (0.15 ml) of the solution were mixed in the mixing chamber in each run. The variation of the fluorescence intensity throughout the reaction was monitored at λ_{ex} 330 nm and λ_{em} 370 nm. Fluorescence values were obtained over 15–20 s intervals and processed by linear regression using the microcomputer and software for application of the initial-rate method ('Kinetic Obey'). The reaction rate was determined in about 10 s and each sample was assayed in triplicate. The blank signal was found to be negligible. All measurements were carried out at 20°C. The concentration of promethazine in the samples was determined by interpolation from the working curves prepared from standard solutions of promethazine hydrochloride (final concentration 0.5–80 μg ml^{-1}) in water.

Results and Discussion

The oxidation of promethazine to its corresponding sulphoxide derivative is very fast in slightly acid medium and in the presence of an oxidant such as hydrogen peroxide. The oxidation can also be effected by dissolved oxygen, but the reaction is much slower. In the presence of hydrogen peroxide the reaction rate is roughly twice as fast as in its absence if the batch technique is used. However, in the stopped-flow mixing technique, the presence of hydrogen peroxide does not modify the reaction rate obtained in its absence which is roughly four times higher than that obtained by using the batch technique in the presence of hydrogen peroxide. Figures 1 and 2 show the fluorescence spectra and kinetic curves obtained in the presence and absence of hydrogen peroxide, respectively. The profiles

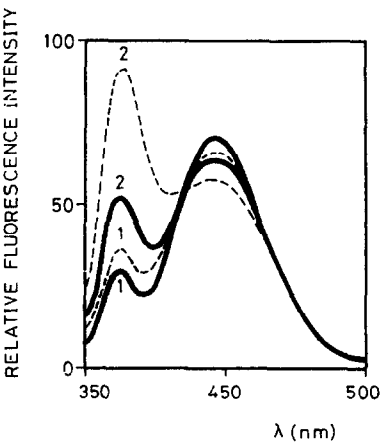

Figure 1
Emission spectra (λ_{ex} 330 nm) of promethazine in the absence (---) and presence (———) of hydrogen peroxide (1.6×10^{-2} M). Time: curves 1 = 1 min, curves 2 = 5 min. [Promethazine·HCl] = 20 μg ml^{-1}; temperature = 20°C. (All spectra were obtained by the batch technique.)

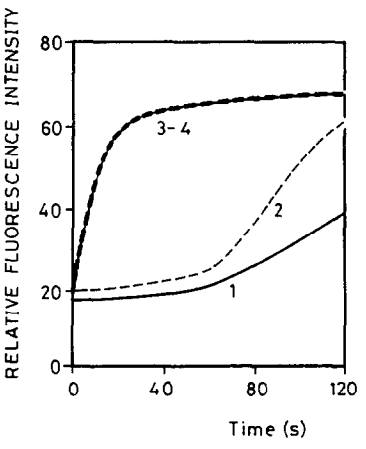

Figure 2
Kinetic curves (λ_{ex} 330, λ_{em} 370 nm) obtained for promethazine in the absence (---) and presence (———) of hydrogen peroxide (1.6×10^{-2} M) by the batch (curves 1, 2) and stopped-flow (curves 3, 4) techniques. [Promethazine·HCl] = 20 μg ml^{-1}; temperature = 20°C.

of the kinetic curves are very different; those provided by the batch technique are subject to an induction period, so analytical data can only be obtained after *ca* 70 s.

The higher reaction rate obtained by using the stopped-flow technique is a result of the rapid, thorough mixing of the streams from the drive syringes in the flow-cell, which promotes collisions between the promethazine and dissolved oxygen molecules so that oxidation is rapidly completed and addition of an oxidizing reagent is unnecessary. This simplifies the method because only a pH adjustment of the sample is required. Also, both drive syringes of the stopped-flow module can be filled with the same sample solution to avoid dilution in the mixing chamber.

Effect of variables

The system was optimized by altering each variable in turn while keeping all others constant. All concentrations given are final concentrations in the flow-cell, i.e. the same as in the drive syringes because, as both syringes were filled with the same solution, there was no sample dilution (as is usually the case with the stopped-flow technique). Each kinetic result was the average of three measurements.

The most important variable of this system is the sample pH. The analytical signal was maximal and independent of pH over the range 1.8–3.2, outside which it was somewhat smaller [Fig. 3(A)]. A phthalate buffer at pH 2.9 was chosen to adjust the pH of the samples. The concentration of this buffer did not affect the reaction rate at least up to a 1.5×10^{-2} M concentration level [Fig. 3(B)]. A concentration of 10^{-2} M was thus chosen.

The temperature did not affect the reaction rate over the range 20–30°C [Fig. 3(C)], but higher temperatures resulted in decreased rates. The decrease in the dielectric constant of the solutions resulting from addition of ethanol caused a slight decrease in the reaction rate up to a 20% ethanol content and a more considerable effect at higher contents [Fig. 3(D)]. Variations in the ionic strength over the range 0.1–0.4 (adjusted with sodium perchlorate) had no effect on the system.

According to the slopes of the fluorescence–time curves for solutions containing different amounts of promethazine, the reaction is first order with respect to promethazine. Under the working conditions used here, the following kinetic equation is proposed: $v = k$ [promethazine], where v is the rate of formation of the sulphoxide and k is the conditional rate constant.

Features of the method

Under the optimum experimental conditions described above, the fluorescence–time curves

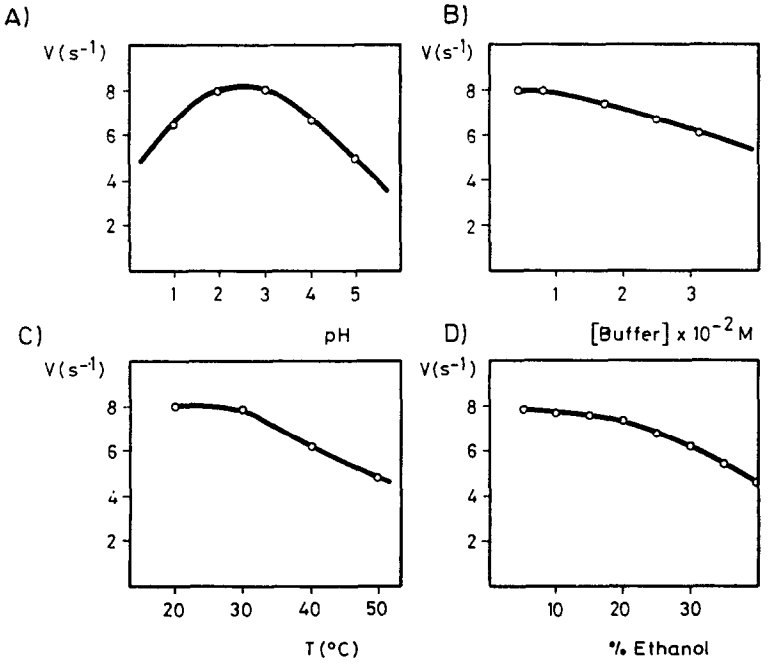

Figure 3
Effect of pH (A), buffer concentration (B), temperature (C) and ethanol content (D) on the initial rate.

Table 1
Determination of promethazine in pharmaceutical samples

Sample	Promethazine·HCl content		Promethazine·HCl		
	Stated	Found*	Added ($\mu g\ ml^{-1}$)	Found* ($\mu g\ ml^{-1}$)	Recovery (%)
Frinova† (ampoules)	25	25.4	5	5.2	104.0
			10	10.2	102.0
			20	20.9	104.5
Frinova‡ (tablets)	25	24.3	5	4.9	98.0
			10	9.7	97.0
			20	19.7	98.5

* Average of three determinations.
† mg ml^{-1}, ‡ mg $tablet^{-1}$.

obtained for different amounts of promethazine, with excitation and emission wavelengths of 330 and 370 nm, respectively, were processed by the initial-rate method. The calibration graph was linear over the concentration range 0.5–80 $\mu g\ ml^{-1}$, with a Pearson's correlation coefficient (r) of 0.999. The detection limit, as defined by IUPAC [12], was 0.3 $\mu g\ ml^{-1}$.

The relative standard deviation ($P = 0.05$, $n = 10$) obtained for 1 and 10 $\mu g\ ml^{-1}$ promethazine hydrochloride was 2.6 and 2.2%, respectively. A salient feature of this method is its speed (initial rate measurements are performed in about 10 s).

Applications

The proposed method was applied to the determination of promethazine hydrochloride in two commercial pharmaceutical preparations. Table 1 summarizes the results found and the recoveries achieved by adding different amounts of promethazine hydrochloride to each pharmaceutical and subtracting the results obtained for solutions prepared similarly but containing no promethazine. The mean recovery was 100.7%.

Conclusions

According to the results obtained, the joint use of kinetic methodology and the stopped-flow mixing technique provides a simple and fast fluorimetric method for the determination of promethazine requiring no reagent addition or sample dilution. The features of the stopped-flow technique ensure that the oxidation of promethazine by dissolved oxygen takes place in a few seconds, so kinetic data can be obtained quickly, a result which provides a useful means of accomplishing automation in routine analyses. On comparing the features of the proposed method with those of the FIA automatic method using cerium(IV) as oxidant [9], the following conclusions can be drawn: the stopped-flow method avoids the high cost of cerium(IV) salts and the environmental problems posed by sulphuric acid waste; also the quantification limit is lower and the linear range of the calibration graph is wider than that of the FIA method (10.3–51.3 $\mu g\ ml^{-1}$).

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