Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 12, pp. 1435–1440, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

A modular stopped-flow system for use in routine pharmaceutical analysis*

D. PÉREZ-BENDITO,† A. GÓMEZ-HENS, M. SILVA, M. C. GUTIÉRREZ and M. CARMONA

Department of Analytical Chemistry, University of Córdoba, 14071 Córdoba, Spain

Abstract: A modular stopped-flow system for routine pharmaceutical analysis is presented. It consists of an inexpensive stopped-flow module which is fitted to a spectrophotometer or spectrofluorimeter and controlled by a simple computer. The automatic technique developed with this system is suitable for the individual and simultaneous determination of various pharmaceuticals (anti-asthmatics, psychotropics, hormones, analgesics, anaesthetics and antiseptics) with satisfactory results.

Keywords: *Kinetics; stopped-flow; photometry; fluorimetry; automatic analysis; drugs.*

Introduction

The use of reaction-rate methods in chemical analysis has gained popularity in recent years, especially with the laboratory where speed and selectivity are mandatory. The stopped-flow technique is the automatic approach most commonly used when fast reactions are involved. However, the high cost of the associated instrumentation hindered its use in routine analyses — so far this technique has chiefly been applied to physicochemical studies. To overcome this limitation we designed an inexpensive stopped-flow mixing module that can be readily fitted to any available spectrophotometer or spectrofluorimeter and controlled on-line by means of a simple computer [1]. Valuable features of the stopped-flow technique include the ability to mix sample and reagent solutions automatically, the acquisition of kinetic data from the mixed solution and their processing to obtain the desired information, which can be delivered as required. It can also be applied to kinetic measurements on slower reactions with the substantially increased sample throughput, reproducibility and accuracy required by modern control laboratories.

This paper shows the suitability of this modular stopped-flow system for routine pharmaceutical analyses. Thus, various pharmaceuticals such as anti-asthmatics, psychotropics, hormones, analgesics, anaesthetics and antiseptics were analysed both individually and in mixtures with satisfactory results. For these purposes, different redox (catalysed and uncatalysed) and condensation reactions were used with photometric or fluorimetric detection.

*Presented at the "Third International Symposium on Drug Analysis", May 1989, Antwerp, Belgium.
†To whom correspondence should be addressed.

Experimental

Instrumentation

The instrumental set-up consisted of a stopped-flow module, a detector and a data acquisition system. The stopped-flow module, designed by the authors [1] and marketed by "Quimi-Sur Instrumentation", was fitted to a spectrophotometer (Perkin–Elmer, model Lambda 5) or a spectrofluorimeter (Perkin–Elmer, model 650–10S), depending on the optical signal required to monitor the reaction under study. Kinetic data (absorbance or fluorescence versus time plots) were collected and treated by a 64-K Commodore computer furnished with an IEEE-488 interface (Binary System Precision) and an 8-bit Fluke 8840A analog-to-digital converter or by a Hewlett–Packard 98561A computer provided with a 16-bit Hewlett–Packard analog-to-digital converter depending on the half-life of the reaction. The software used was written by the authors for application of kinetic determinative methods and differential reaction-rate methods used for the resolution of mixtures of these drugs. The solutions in the stopped-flow module and in the detector cell compartment were kept at a constant temperature by circulating water from a thermostatted tank.

General procedure

One motor-driven syringe of the stopped-flow module was generally filled with the sample solution and the other with the reagent, generally at the working pH. Occasionally, the reagent solutions had to be distributed in the two syringes owing to stability problems, appearance of blank signals, etc. After the 2 ml drive syringes had been filled, equal volumes (0.2 ml) of the solutions were mixed in the flow-cell in each run. The absorbance or fluorescence was monitored at the appropriate wavelengths and the data collection rate was selected according to the half-life of the reaction under study. All measurements were carried out at a controlled temperature. The initial rate, the measured parameters needed for the application of the differential reaction-rate methods and the analyte concentration(s) were automatically acquired by the computer system.

Results and Discussion

The proposed stopped-flow modular system is a useful means of accomplishing the automation and rapid handling of reagents for the immediate acquisition of analytical results in routine drug analyses. It consists of a stopped-flow module which is placed upright on the spectrophometer or spectrofluorimeter and a microcomputer (Fig. 1). The stopped-flow module in turn has three components: the mixing system, the propelling syringe system and the thermostatted mixing chamber. The mixing system is composed of two drive syringes which deliver sample and reagents at a high speed through the mixing chamber (a commercially available flow-cell), the reactant flow being abruptly stopped by a third syringe (stop syringe). The extent of reaction is monitored by measuring the analytical signal over time with the aid of the computer, which processes and displays acquired data.

Photometric and fluorimetric methods were developed for the analysis of various drugs in different types of pharmaceutical formulations using this modular stopped-flow system. Some of these methods are based on the advantageous transformation of the conventional kinetic or equilibrium methods into stopped-flow methods. Differential

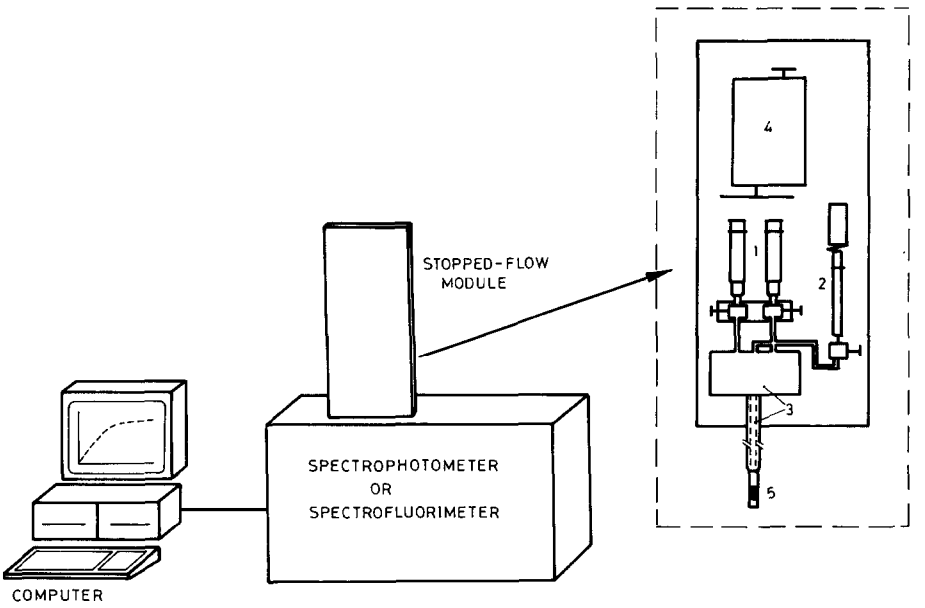


Figure 1
Scheme of the modular stopped-flow system and detail on the stopped-flow module. (1) Driving syringes; (2) stop syringe; (3) thermostatting system; (4) pneumatic propelling system; (5) mixing and observation chamber.

kinetic methods were also developed for the simultaneous determination of mixtures of some of these compounds with good results as described below.

Determination of a single species

A number of drugs in different pharmaceutical preparations were analysed by the stopped-flow technique. Table 1 summarizes the results obtained and some analytical features of the corresponding methods. The overall results obtained show how suitable this simple, inexpensive and rapid approach is for routine pharmaceutical analyses.

Most of these determinations were based on redox reactions. Thus, by using the classical Sandell–Kolthoff reaction between Ce(IV) and As(III), which had not been previously studied by the stopped-flow technique, several methods for the determination of iodide [2, 3], thyroxine (T_4) and tri-iodothyronine (T_3) [4], which catalyse this

Table 1
Analytical features of drug determinations by the stopped-flow technique

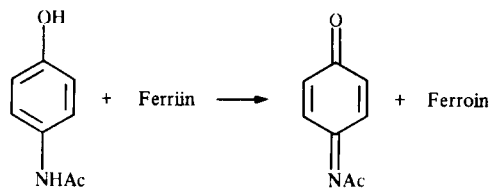
Analyte	Linear range	Precision (%)	Sampling rate (h^{-1})
Iodide	2–100 ng ml^{-1}*	2.6	60
	2– 80 ng ml^{-1}†	1.8	100
T_3, T_4*	2– 80 ng ml^{-1}	2.2	50
Theophylline†	1–250 μg ml^{-1}	1.5	100
Paracetamol*	0.25–25 μg ml^{-1}	2.6	60
Procaine*	1–200 μg ml^{-1}	1.2	100

*Photometry.
†Fluorimetry.

reaction, were developed. For the determination of iodide, which is commonly used as an antiseptic, two alternatives were assayed: the measurement of the absorbance decrease due to Ce(IV) [2] and that of the fluorescence increase due to the formation of Ce(III) [3]. The results found show that both methods are equally useful for the determination of iodide in pharmaceutical preparations. Regarding the two thyroid hormones, T_4 and T_3, only the photometric monitoring was possible by the stopped-flow technique owing to the effect of the acid used on the reaction rate [4].

Cerium(IV) has also been proposed as a reagent for the determination of theophylline, a bronchodilator commonly used in the treatment of asthma. The equilibrium method previously described [5] was satisfactorily transformed by us into a stopped-flow method for use in routine pharmaceutical analyses [6]. Although in the equilibrium method theophylline is reportedly oxidized by Ce(IV) and the fluorescence due to the resultant Ce(III) is measured, from the fluorescence spectra one can infer that, further in to the redox reaction, a complex is formed between the oxidation product of theophylline, probably alloxantin or purpuric acid, and Ce(III).

The reversible transformation of ferroin–ferriin complexes has been scarcely used for indication purposes in kinetic based analytical methods. This type of reaction has been used for the photometric stopped-flow determination of the analgesic acetaminophen (paracetamol) [7] in pharmaceutical formulations. A detailed study of the mechanism of this reaction was also carried out. Thus, according to the kinetic and equilibrium results, the oxidation of acetaminophen by ferriin gives rise to *N*-acetyl-*p*-quinoneimine:



Finally, procaine, which is a local anaesthetic widely used in a variety of pharmaceutical preparations, can also be determined by the stopped-flow technique. The proposed method [8] 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 the conventional equilibrium method [9] to a kinetic stopped-flow method results in improved detection limit and linear range, and in considerably increased sample throughput, which again shows the suitability of the stopped-flow technique for routine pharmaceutical analyses.

Resolution of mixtures

There are few literature references to the resolution of mixtures of drugs by kinetic methods. In this work, different mixtures of drugs were resolved by using various differential reaction-rate methodologies. Table 2 summarizes the features of the different methods. The determinations were accomplished at $\mu g\ ml^{-1}$ levels, with a great precision and over wide ratio ranges. The simultaneous determination of perphenazine and chlorpromazine [10], two psychotropic drugs, was based on the oxidation of these species to their corresponding fluorescent sulphoxides (λ_{ex} 340, λ_{em} 380 nm). Owing to the intrinsic characteristics of the stopped-flow technique, which allows the rapid and thorough mixing of reactants, the addition of any oxidizing reagent was unnecessary since the oxidation was effected by the dissolved oxygen itself. The proportional-

Table 2
Simultaneous determination of binary mixtures of drugs by the stopped-flow technique

Mixtures assayed	Dynamic range ($\mu g\ ml^{-1}$)	Ratio range	Precision (% RSD)
Perphenazine +	0.2–40	1:8–8.1	5.1
Chlorpromazine	0.2–40		1.9
Paracetamol +	5–40	6:1–1:2	4.2
Oxyphenbutazone	5–40		1.6
Epinephrine +	1–40	1:10–10:1	2.0
Norepinephrine	1–40		1.9

equation method was applied for the resolution of these mixtures, taking into account the additivity of the initial rate and maximum fluorescence intensity for both drugs.

The above-mentioned reversible transformation of ferroin–ferriin complexes was also exploited for the resolution of mixtures of drugs. Thus, paracetamol and oxyphenbutazone, two analgesic drugs, were simultaneously determined through oxidation with the tris(2,2′-bipyridine)–iron(III) complex in an acid medium [11]. The reactions were monitored by measuring the rate of appearance of the absorbance of the ferroin complex (λ_{max} 490 nm). Taking into account the pseudo-first-order constant ratio found between oxyphenbutazone and paracetamol (k_o/k_p = 2.44), a simplified version of the linear-graph Worthington and Pardue method [12] was used for the resolution of these mixtures. Regarding selectivity, the proposed method was practically free from interferences.

Mixtures of epinephrine and norepinephrine, two hormones which play a particularly important rôle in the regulation of physiological processes in living systems, were also assayed by using this type of reaction. Thus, these compounds were oxidized by the tris(1,10-phenanthroline)–iron(III) complex in a weakly acidic medium, and the reaction rate was monitored spectrophotometrically at 510 nm (absorption maximum of the ferroin complex formed) [13]. By applying the proportional-equation method on the basis of two measurement parameters such as the initial rate and the absorbance reached after 5 min of the reaction development the simultaneous determination of both catecholamines can be performed over a wider ratio range.

Applications

The methods for the analysis of drugs individually and in mixtures described above were applied to several pharmaceutical formulations. Table 3 shows some of the results obtained in the determination of drugs in this type of sample, which were consistent with the nominal values given by the manufacturers for the pharmaceuticals, thereby confirming suitability of the proposed methods for pharmaceutical analyses.

Conclusions

The implementation of straightforward techniques for the automation of kinetic methods based on open systems (e.g. the stopped-flow technique) offers great potential in the analytical control of drugs. The main advantages of this technique can be summarized as follows: (a) It improves analytical features such as the dynamic range and

Table 3
Some applications of the stopped-flow technique to the pharmaceutical analysis

Drug	Sample (Laboratory)	Content	
		Nominal	Found*
Iodide	Elixifilin (Morrith)	8.66 mg ml^{-1}	8.64 mg ml^{-1}
	Angiofiline (Seid)	10.0 mg ml^{-1}	10.5 mg ml^{-1}
T_4 and T_3	Tiroides Leo (Leo)	100.0 μg/tablet	103.1 μg/tablet
	Triyodotironina Leo (Leo)	25.0 μg/tablet	25.0 μg/tablet
Theophylline	Tedral (Parke-Davis)	130.0 mg/tablet	129.0 mg/tablet
	Teo-lanirapid (Beohringer Manheim)	75.0 mg/tablet	74.3 mg/tablet
Paracetamol	Algomen (Menarini)	200.0 mg/tablet	198.0 mg/tablet
	Actron (Miles Martin)	133.3 mg/tablet	133.0 mg/tablet
Procaine · HCl	Otosedol (Pensa)	12.6 mg ml^{-1}	12.8 mg ml^{-1}
	Sulmetin inyectable (Semar)	4.0 mg ml^{-1}	4.1 mg ml^{-1}
Perphenazine	Mutabase 2–10 (Essex España)	2.0 mg/tablet	2.0 mg/tablet
	Deprelio (Estedi)	2.0 mg/tablet	2.0 mg/tablet
Chlorpromazine	Largatrex (Rhone-Poulenc Farma)	40.0 mg ml^{-1}	40.3 mg ml^{-1}
	Clorpromazine Bama (Bama Geve)	25.0 mg ml^{-1}	25.2 mg ml^{-1}
Epinephrine + Norepinephrine	Stoma anestesia dental (Bucca)	10.0 μg/2 ml	9.97 μg/2 ml
		10.0 μg/2 ml	9.89 μg/2 ml

* Average of three or four determinations.

the precision (see Table 1), minimizes interferences and requires small sample amounts; (b) it increases the reaction rate, probably because the rapid and thorough mixing of the reactants favours collisions between their molecules, thus exerting a sort of "instrumental catalysis". This results in very high sampling rates (Table 1) and simplifies analytical procedures as in the above-mentioned case of the determination of perphenazine and chlorpromazine; (c) it improves on the performance of methods based on slow reactions as reactant manipulation is minimized, thus increasing the precision and throughput of determinations compared with the conventional kinetic approach.

Acknowledgement — The authors thank the CICYT (Project No. PB87-0821) for financial support.

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[*Received for review 2 May 1989*]