Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 5, pp. 635–641, 1994

0731–7085/94 $7.00 + 0.00

0731–7085(93)E0015-F

Automated flow-injection technique for use in dissolution studies of sustained-release formulations: application to iron(II) formulations

CONSTANTINOS A. GEORGIOU,† GEORGIA N. VALSAMI,‡ PANAYOTIS E. MACHERAS‡ and MICHAEL A. KOUPPARIS†*

† *Laboratory of Analytical Chemistry, Department of Chemistry, and* ‡ *Laboratory of Biopharmaceutics and Pharmacokinetics, Department of Pharmacy, University of Athens, Panepistimiopolis, Kouponia, Athens 15771, Greece*

Abstract: The application of flow-injection analysis (FIA) to automated dissolution studies of sustained-release formulations is described. The long-term stability of the dissolution–FIA analyser was checked during unattended operation for 42 h. The construction of multiple calibration curves with the so-called electronic dilution FIA procedure was used to extend the linear range of the determination. The computer-controlled FIA system and the principles of associated software are described and applied to dissolution studies of sustained-release formulations of iron(II) using its sensitive reaction with the colour reagent, ferrozine. The extended linear range of the determination is 1–130 ppm iron(II) and the precision (RSD) better than 3% ($n = 3$).

Keywords: *Iron determination in drug formulations; flow-injection analysis; ferrozine; dissolution testing automation; sustained-release formulations.*

Introduction

The clinical effectiveness of a drug formulation depends primarily on the bioavailability and not on the amount of active substance [1]. For solid dosage forms, particularly those with dissolution rate-limited bioavailability, dissolution testing can be a reliable, rapid and economical method for indication of the *in vivo* rate and the extent of drug release. Dissolution testing has become an important tool in the design of solid products [2–4]. Beyond the manual performance of the test described in the official monographs of pharmacopoeias, numerous dissolution systems with mechanized or automated sampling and analysis have been designed and used in order to accommodate the heavy load of analytical work associated with the test [5].

An attractive and very potential automated system for dissolution studies, based on the so-called flow-injection analysis (FIA) technique, was designed, tested and used for the first time by the authors' research group [6]. An FIA colorimetric analyser coupled to a dissolution basket apparatus and controlled by a microcomputer allowed the real time monitoring of the dissolution profiles of formulations of various drugs [6–12]. Dissolution studies in milk of formulations of various drugs were also achieved by introducing a dialysis cell in the FIA–dissolution system [13]. Because of its many advantages, the FIA technique has gained widespread recognition and usage in pharmaceutical analysis [14] including dissolution studies [15, 16].

During the last decade, much attention has been given to sustained-release formulations since these systems, for selected drugs, offer important advantages over the conventional immediate-release formulations, such as reduction of dosing frequency, improvement of therapeutic efficacy, compliance enhancement and extension of market exclusivity. The basic characteristics of sustained-release formulations are the controlled start, rate and extent of drug release, which are associated with the physicochemical properties of the formulation and not on the environmental conditions during the release process [17].

An ideal automated dissolution system for sustained-release formulations, capable of providing the entire dissolution profile over a prolonged period of time should have the

* Author to whom correspondence should be addressed.

following features: an analyser with a high degree of long-term stability; the reagent consumption (reagent cost) should be kept low and therefore a discontinuous mode of operation should be feasible; the expected concentration of the released drug in a wide range must be accurately determinable so that a calibration curve of wide range or multiple calibration curves must be available with the system used.

In this paper the development of FIA automated spectrophotometric analyser for use in dissolution studies of sustained-release formulations is described. The system developed utilizes all inherent advantages of the FIA technique, i.e. good long-term stability, capability of discontinuous mode of operation and multiple calibration curves using the so-called electronic dilution procedure [26]. Microcapsule formulations of iron(II) were chosen as model formulations in order to show that the two manual and time-consuming processes, namely filtration and complexation of iron(II) samples, can be accomplished reliably and automatically with the system proposed. To the best of the authors' knowledge, none of the numerous FIA spectrophotometric methods for iron determination (listed in Table 1) has been used in pharmaceutical analysis. From the various reagents available for iron(II), ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,3,4-triazine] was selected as a compromise in respect of reagent cost, water solubility of the complex, selectivity and sensitivity. Ferrozine was introduced in 1970 as a sensitive and inexpensive reagent for iron(II) [27]. The molar absorptivity of its complex with iron(II) is about 2.8×10^4 M^{-1} cm^{-1} and compares favourably with those of other common reagents for iron [about 2.2×10^4 M^{-1} cm^{-1} for bathophenanthroline, 1.1×10^4 M^{-1} cm^{-1} for 1,10-phenanthroline and 2.3×10^4 M^{-1} cm^{-1} for 2,4,6-tri(2-pyridyl)*S*-1,3,5-triazine, (TPTZ)].

Experimental

Materials

Microcapsule formulations of iron(II) sulphate were obtained from local commercial sources. The formulations used, namely Feofole and Microfer (Smith Kline and French, packed by Vianex S.A., Hellas, lot Nos 7J87 and 7G74, respectively) contained 150 mg $FeSO_4 \cdot H_2O$ ≡ 49.3 mg Fe/unit. All solutions were prepared in de-ionized water from analytical reagent grade materials.

Acetate buffer (0.50 M, pH 3.7) was prepared according to the usual practice. The acetate buffer was chosen because of its extremely low iron content.

Ferrozine [3-(2-pyridyl)-5,6-bis(4-phenylsulphonic acid)-1,2,4-triazine monosodium salt (Sigma)] solution 0.10% (w/v), was prepared by dissolving 0.10 g of ferrozine and 1.0 g of ascorbic acid in 100 ml of the acetate buffer.

Table 1
Some of the existing FIA spectrophotometric methods for iron

Reagent, reaction	ϵ_λ (M^{-1} cm^{-1})	Concentration range	Reference
Ferrozine (continuously circulated reagent)		5–45 ppm iron(II)	18
2-(3,5-Dibromo-2-pyridylazo)-5-[*N*-ethyl-*N*-(3-sulphopropyl)amino]phenol (research reagent)	$\epsilon_{568} = 8.8 \times 10^4$ $\epsilon_{748} = 3.9 \times 10^4$	20–440 ppb iron(II)	19
1,10-Phenanthroline		0.5–120 ppm iron(II)	20
Thiocyanate		0.5–180 ppm iron(III)	20
Catalysis of BrO_4^--I^- reaction		10–100 ppb iron(II) + iron(III)	21
Ammonium di-isopropyldithiophosphate (research reagent)	$\epsilon_{365} = 8.5 \times 10^3$	0.05–15 ppm iron(III)	22
2-Nitroso-5-(*N*-propyl-*N*-sulphopropylamino)phenol	$\epsilon_{753} = 4.3 \times 10^4$	4–100 ppb iron(II)	23
Tiron		8–40 ppm iron(III)	24
Ferrozine	$\epsilon_{562} = 2.62 \times 10^4$	39–1848 ppb iron(II)	25
Bathophenanthroline disulphonate	$\epsilon_{535} = 2.25 \times 10^4$		25

Iron(II) standard solutions: a 1000 ppm stock standard solution was prepared by dissolving 7.0217 g of $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in 0.10 M HCl and diluting to 1 l; working standard solutions in the range of 0.250–130 ppm iron(II) were prepared daily by dilution with 0.10 M HCl.

The dissolution medium was 0.10 M HCl.

All glassware was soaked in concentrated HCl before use since it was essential to avoid iron contamination.

Instrumentation

A fully automated home-made FIA analyser was constructed using an Ismatec MPN-8 peristaltic pump, a Rheodyne 5001 low pressure injection valve and a Bausch and Lomb spectronic 21 spectrophotometer and was automated using the AIM 65 microcomputer (Rockwell, USA). The analyser provides automatic delivery of aliquots of a small sample from the dissolution medium and injection to the analytical manifold (Fig. 1), control of the pump functions (start and stop), absorbance readout at various points of the peak curve and presentation of results in a tabular form of time, absorbance, concentration and percentage of dissolution. The interface and the data lodging routine used are described elsewhere [28], while the dissolution program was specifically developed for this application.

The FIA analyser was coupled with a rotating basket apparatus. A small filter attachment (filter for pipette tips Centraur Chemical Co., USA) was attached to the sample probe so that undissolved fragments were not inserted in the manifold.

Procedure

The home-made dissolution program was loaded in the RAM of the microcomputer, and calibration curves were constructed using the working iron(II) standard solutions. Measurement of one standard or sample involved: injection of the standard; measurement/calculation of the baseline absorbance as the mean of 20 absorbance readings; measurement/calculation of the peak height absorbance as the maximum of 40 absorbance readings in the vicinity of the peak maximum; and measurement of the absorbance in the descending part of the FIA peak curve by taking one absorbance reading after a preselected time interval from the injection of the sample. Timing as shown in Fig. 2 was input to the dissolution program as soon as the system was started. This timing sequence was based on the flow manifold used (flow rates and coil lengths) and was optimized by preliminary experiments. The calibration curves were constructed by linear regression of absorbance vs iron(II) concentration. Two calibration curves were calculated, one from the absorbance at maximum peak height (1–70 ppm) and the other from the absorbance at the descending part of the FIA peak (2–130 ppm). This technique of preparing calibration curves of various linear ranges using a single set of standards is the so-called electronic dilution procedure [26] and permits analysis of samples in a wide concentration range without manual

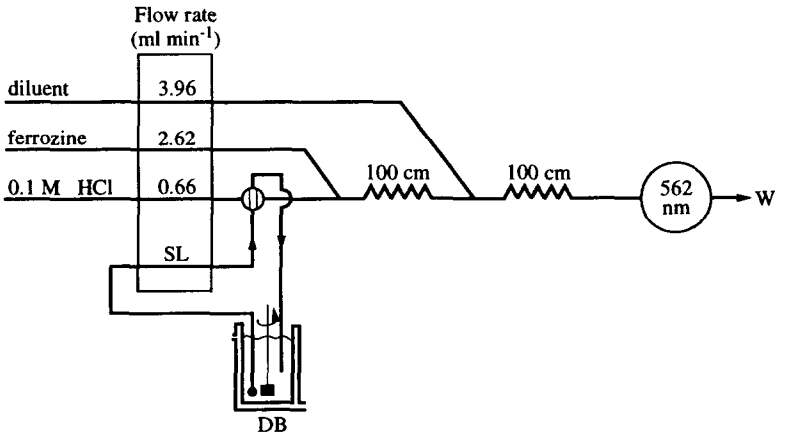

Figure 1
FIA analytical manifold developed for the dissolution studies of iron microcapsules. Diluent, acetate buffer. DB, dissolution basket apparatus; SL, sampling line; W, waste. A single pump is used for the analytical manifold and for sampling the dissolution medium.

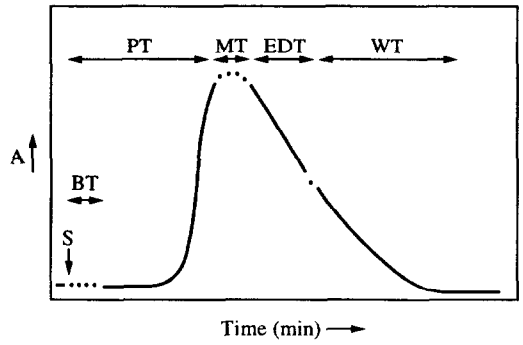

Figure 2
Typical timing of a single FIA measurement. Baseline time BT = 5 s; peak time PT = 31 s; measurement time MT = 10 s; electronic dilution time EDT = 8 s; and wash time WT = 20 s. Absorbance readings are indicated by dots and injection of the analysed solution by S.

dilution of the concentrated ones. This technique is very useful for automated spectrophotometric methods in which the Beer–Lambert law is valid in a limited concentration range. The principle of this technique is shown in Fig. 3.

Subsequently, the solid dosage form was placed into a 250-mesh screen basket and the whole was immersed and rotated at 100 rpm in a double-wall beaker containing 500 ml of the dissolution medium, and kept at 25 ± 0.5°C. A cover was required to diminish evaporation of the dissolution medium since the dissolution experiments were prolonged (~7 h).

Analytical recovery studies were performed after grinding the microcapsules contained in 10 capsules and dissolving the equivalent of one capsule in 500 ml of 0.10 M HCl. After filtration of the undissolved excipients, 10.0 ml of this solution was spiked with the appropriate volume of the 100 ppm standard solution, the volume was adjusted to 100 ml with 0.10 M HCl and the resulting solution was analysed.

Results and Discussion

A three-line FIA manifold was used in the proposed method (Fig. 1). The sampling line had a dead volume of only 0.5 ml. Dissolution medium flowing through this line is filtered in the dissolution cell overcoming the problem of the out-of-the-cell filtration which leads to errors due to reduction of the active formulation surface. To minimize the consumption of the ferrozine reagent, the pump of the analyser was automatically stopped during the dissolution sampling interval (30 min) starting only 60 s before measurement. Then a 100-μl aliquot of the dissolution medium was injected in the 0.10 M HCl carrier (same concentration as the dissolution medium). The sample was then mixed at the first confluence point with

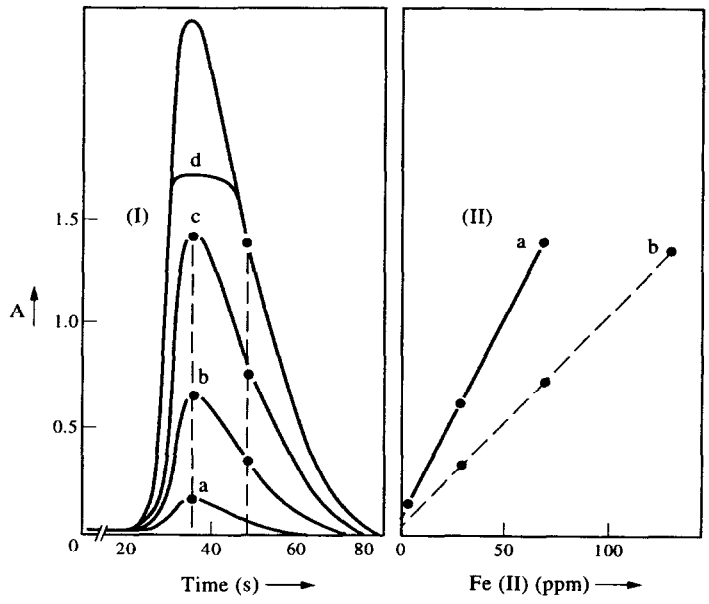

Figure 3
Principle of electronic dilution. I: Overlaid FIA absorbance peaks for 5.00 (a), 30.0 (b), 70.0 (c) and 130 (d) ppm iron(II) standards. II: (a) Calibration curve resulting from peak height; (b) calibration curve from electronic dilution (absorbance measurements on the descending part of the curves at points shown by the dashed line).

the ferrozine reagent. The ascorbic acid contained in the reagent reduced any iron(III) present to iron(II) and the ferrozine iron complex was formed. The pH of the ferrozine solution after sample injection dropped to 3.3 owing to the HCl contained in the dissolution medium. Nevertheless, this pH value is in the optimum range for complex formation [29]. After mixing the sample with ferrozine reagent, the reaction mixture was merged with a diluent stream (acetate buffer) to lower the sensitivity of measurement.

The calibration curve range was 1–70 ppm for peak maximum measurements and 2–130 ppm for absorbance readings taken in the descending part of the peaks. These ranges can be shifted to 0.25–20 and 0.5–36 ppm, respectively, by injecting 200 μl of sample, cancelling the diluent stream and using a single 50-cm coil. The linearity and long-term stability of the calibration curves are shown in Table 2. The electronic dilution calibration curve showed half the sensitivity of the normal calibration curve, extending the upper concentration limit to 130 ppm iron(II). This feature is useful for experiments giving concentrations ranging from low values during the early stages to high values at the end of the dissolution. Typical FIA peaks are shown overlaid in Fig. 3, together with the two corresponding calibration curves. The excellent reproducibility of FIA allowed the construction of the electronic dilution calibration curve taking readings in the slope of the FIA peaks without significant loss of precision as indicated by the RSD (%) values in Table 2. A small drift (increase) in the slope of the electronic dilution calibration curve is due to the shift of FIA peaks towards longer residence times. This shift can be attributed to a small decrease in flow rates caused by the ageing of the pump tubes. The above-mentioned drift is approximately 0.5% h^{-1} or ~3.5% per dissolution experiment that is tolerable in dissolution experiments. Once set up, the method requires calibration using only a few standard solutions namely 1.00, 5.00, 30.0, 70.0 and 130 ppm iron(II).

The ferrozine reagent concentration was optimized by obtaining calibration curves in the range 1–70 ppm iron(II) using 0.025, 0.050, 0.10 and 0.15% (w/v) ferrozine reagent concentrations. The 0.10% concentration was selected as optimum as a compromise in respect of high sensitivity (full colour development), wide linear range and low reagent consumption. The concentration of ascorbic acid used, 1.0% (w/v), was sufficient to reduce traces of iron(III) to iron(II) and calibration data remained the same when iron(III) standard solutions were used.

The developed analytical method was evaluated by recovery experiments from solutions prepared from the formulations used. Table 3 shows recoveries ranging from 99.2 to 103.2%

Table 2
Long-term stability of the calibration curves during unattended operation of the FIA analyzer for 42 h

Time (h)	Slope ($A \times ppm^{-1} \times 10^3$)	Intercept ($A \times 10^2$)	r	RSD (%) ($n = 3$)
	Peak measurement (range 1–70 ppm)			
0	19.4 ± 0.7	5.0 ± 3.0	0.9994	0.8–1.1
7	19.7 ± 0.5	6.0 ± 2.0	0.9997	0.4–0.9
14	19.5 ± 0.6	7.0 ± 3.0	0.9995	0.6–0.8
21	19.2 ± 0.6	7.0 ± 3.0	0.9995	0.5–1.2
28	19.4 ± 0.9	8.0 ± 4.0	0.999	0.7–0.9
35	19.7 ± 0.7	7.0 ± 3.0	0.9994	0.4–1.1
42	19.8 ± 0.5	7.0 ± 2.0	0.9996	0.6–1.5
Mean	19.5 ± 0.2	6.7 ± 0.9		
	Electronic dilution (range 2–130 ppm)			
0	9.07 ± 0.04	3.8 ± 0.2	0.99999	0.1–3.0
7	9.8 ± 0.1	4.4 ± 0.4	0.99995	0.2–1.0
14	10.1 ± 0.2	5.1 ± 0.9	0.9998	0.4–1.5
21	10.48 ± 0.06	5.0 ± 0.3	0.99998	0.2–1.0
28	10.95 ± 0.02	4.77 ± 0.08	0.99999	0.3–2.5
35	10.85 ± 0.06	5.1 ± 0.3	0.99998	0.1–1.9
42	11.21 ± 0.06	5.4 ± 0.3	0.99998	0.4–2.1
Mean	10.3 ± 0.7	4.8 ± 0.5		

Table 3
Recovery* of iron(II) from the two formulations

Formulation	Fe (mg unit^{-1}) Found	Added	Recovered	Recovery (%)
Feofole	48.2	50	51.6	103.2
		100	99.2	99.2
Microfer	47.8	50	51.1	102.2
		100	102.1	102.1
Mean				101.7

*n = 3; RSD = 0.5–1.0%.

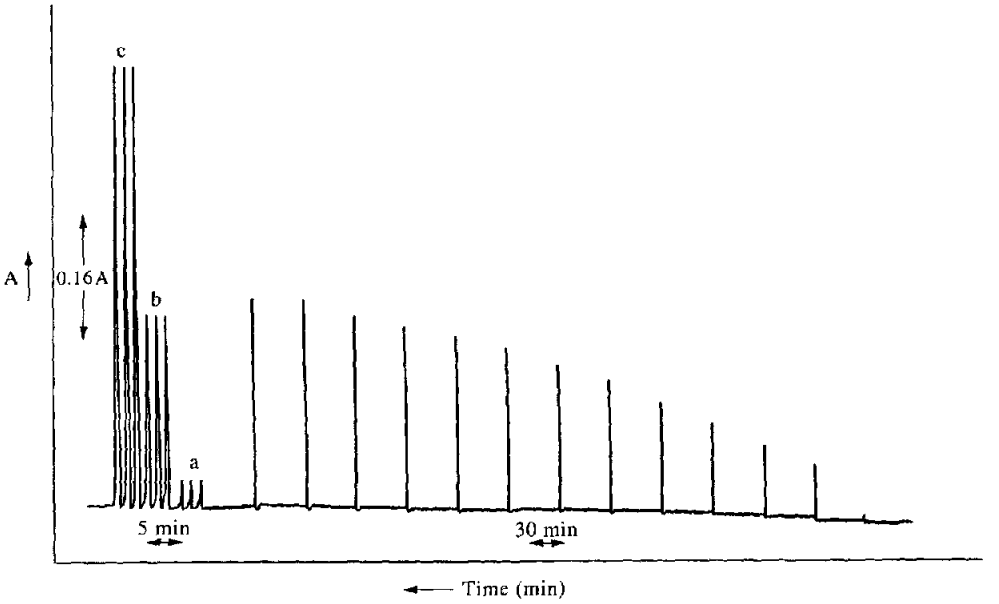

Figure 4
Typical absorbance peaks for the calibration curve [5.00 (a), 30.0 (b) and 70.0 (c) ppm iron(II)] and the dissolution profile for Microfer.

with a mean value of 101.7%. Excipients used in the manufacturing of conventional tablets and capsules (sodium lauryl sulphate, lactose, sucrose, cellulose acetate hydrogen phthalate, magnesium stearate and starch) did not interfere; recoveries from synthetic solutions containing 1 g l^{-1} of the excipients ranged from 98–102.5%. Therefore the method can also be used for determination of the content of any iron(II) formulation.

Results for dissolution experiments done in triplicate are shown in Table 4. From consideration of the tablet-to-tablet variation, the precision of the proposed automated method is excellent; dissolution experiments were carried out in triplicate owing to their prolonged time. A three-point calibration curve together with the dissolution profile as a series of absorbance peaks are presented in Fig. 4 for Microfer. As

Table 4
Results for the dissolution study of Feofole and Microfer

Time (h)	% Dissolved ± SD (n = 3) Feofole	Microfer
0.5	0.8 ± 0.4	0.7 ± 0.5
1.0	5.1 ± 1.7	7.2 ± 2.5
1.5	10.8 ± 2.7	11.2 ± 3.3
2.0	17.4 ± 3.0	14.7 ± 2.9
2.5	23.2 ± 3.1	18.0 ± 2.7
3.0	28.3 ± 2.5	20.9 ± 2.7
3.5	33.4 ± 1.8	23.5 ± 2.7
4.0	37.4 ± 1.1	25.7 ± 2.5
4.5	40.7 ± 0.8	28.0 ± 1.9
5.0	43.1 ± 1.0	30.2 ± 1.4
5.5	44.5 ± 0.9	32.2 ± 0.9
6.0	46.0 ± 0.7	34.0 ± 1.0
6.5	47.3 ± 1.0	35.8 ± 0.9
7.0	48.1 ± 1.1	37.9 ± 1.0
7.5	49.0 ± 0.8	38.8 ± 1.0

shown (Table 4) after 7.0 h the extent of dissolution was 48% for Feofole and 38% for Microfer formulations. No official requirements are available for comparison.

Conclusions

This new automated method for iron(II) determination features long-term stability of the calibration curve, extended dynamic range, excellent accuracy and precision and low reagent consumption. The real-time monitoring of dissolution enables one to evaluate the process in the early stages and to react accordingly (e.g. discontinue the experiment). The stability of the constructed FIA system allows the unattended use of this method for dissolution studies of sustained-release formulations. The method can also be used for assay and dissolution studies of other conventional iron formulations. This method can also be used for the simultaneous monitoring of multiple dissolution experiments provided that a flow selection valve is added to the automated FIA–dissolution system. Of course, classical dissolution studies for sustained-release formulations not requiring the derivatization step can also be performed with the system developed if appropriate modifications of the analytical manifold are applied.

Acknowledgements — Support from the Greek National Drug Organization through a research grant is gratefully acknowledged. We are also grateful to IBM Hellas for the donation of an IBM/PS 2 PC.

References

[1] V.F. Smolen, *Controlled Drug Bioavailability*. Wiley, New York (1984).
[2] H.M. Abdou, in *Remington's Pharmaceutical Sciences* (R.A. Gennaro, Ed.), pp. 653–666, Mack, Easton Pensylvania (1990).
[3] J.L. Choen, B.B. Hubert, L.J. Leeson, C.T. Rhodes, J.R. Robinson, T.J. Roseman and E. Shefter, *Pharm. Res.* **7**, 938–987 (1990).
[4] *U.S. Pharmacopeia*, XXII rev. U.S. Pharmacopeial Convention, Rockville, MD (1990).
[5] B.J. Compton and O.N. Hinsvark, *Pharm. Technol.* **10**, 28–36 (1986).
[6] M. Koupparis, P. Macheras and C. Reppas, *Int. J. Pharm.* **20**, 325–333 (1984).
[7] M. Koupparis, P. Macheras and C. Tsaprounis, *Int. J. Pharm.* **27**, 349–359 (1985).
[8] M. Koupparis and E. Sarantonis, *J. Pharm. Sci.* **75**, 800–804 (1986).
[9] P. Anagnostopoulou and M. Koupparis, *Anal. Chem.* **58**, 322–326 (1986).
[10] M. Koupparis and A. Barcuchova, *Analyst* **111**, 313–318 (1986).
[11] M. Koupparis and P. Anagnostopoulou, *Anal. Chim. Acta* **204**, 271–283 (1988).
[12] M. Koupparis and P. Anagnostopoulou, *J. Pharm. Biomed. Anal.* **6**, 35–46 (1988).
[13] P. Macheras, M. Koupparis and C. Tsaprounis, *Int. J. Pharm.* **33**, 125–136 (1986).
[14] A. Rios, M.D. Luque de Castro and M. Valcarcel, *J. Pharm. Biomed. Anal.* **3**, 105–121 (1985).
[15] A.K. Ahmad, E.M. Abdel-Moety, A.A. Moustafa and A.E. El-Gendy, *Arch. Pharm. Chemi. Sci. Ed.* **14**, 113–118 (1986).
[16] R.A. Kenley, S.E. Jackson, G.C. Visor and J.S. Winterle, *Drug Dev. Ind. Pharm.* **13**, 39–56 (1987).
[17] A. Yacobi and E. Halperin-Walega (Eds), *Oral Sustained Release Formulations. Design and Evaluation*, Pergamon Press, New York (1988).
[18] E. Dutt, A. Eskander-Hanna and H. Mottola, *Anal. Chem.* **48**, 1207–1211 (1976).
[19] H. Wada and G. Nakagawa, *Anal. Chim. Acta* **153**, 199–206 (1983).
[20] T. Lynch, N. Kernoghan and J. Wilson, *Analyst* **109**, 843–846 (1984).
[21] T. Yerian, T. Hadjiioannou and G. Christian, *Talanta* **33**, 547–549 (1986).
[22] T. Cardwell, D. Caridi, R. Cattrall and I. Hamilton, *Anal. Chim. Acta* **192**, 129–132 (1987).
[23] N. Ohno and T. Sakai, *Analyst* **112**, 1127–1130 (1987).
[24] R. Kuroda, T. Nara and K. Oguma, *Analyst* **113**, 1557–1560 (1988).
[25] V. Kuban, D. Gladilovich, L. Sommer and P. Popov, *Talanta* **36**, 463–467 (1989).
[26] S. Olsen, J. Ruzicka and E.H. Hansen, *Anal. Chim. Acta* **136**, 101–112 (1982).
[27] L. Stookey, *Anal. Chem.* **42**, 779–781 (1970).
[28] C.A. Georgiou and M.A. Koupparis, *J. Autom. Chem.* **13**, 199–207 (1991).
[29] F. Ceriotti and G. Ceriotti, *Clin. Chem.* **26**, 327–331 (1980).

[Received for review 25 July 1993; revised manuscript received 18 October 1993]