Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis 39 (2005) 886–891
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

# Chemiluminometric determination of propranolol in an automated multicommutated flow system

Karine L. Marques, João L.M. Santos *, José L.F.C. Lima

*REQUIMTE, Departamento de Química-Física, Faculdade de Farmácia (UP), Rua Aníbal Cunha, 164, 4050 Porto, Portugal*

Received 3 April 2005
Available online 2 June 2005

**Abstract**

In this work, a fully automated analytical methodology for the chemiluminometric determination of propranolol hydrochloride in pharmaceutical preparations is proposed. The developed procedure was based on the oxidation of propranolol by potassium permanganate in acidic medium and was implemented in a multicommutated flow system. The combined automated actuation of syringe pumps and two-way solenoid valves, under computer control, assured a high versatility in terms of sample and reagent manipulation and an effective run-time control of all analytical parameters, including a time-based insertion that enabled a significant solutions saving and the exploitation of different strategies for sample/reagent mixing.

The calibration graph was linear over the range 20–150 mg $l^{-1}$ of propranolol hydrochloride with a relative standard deviation lower than 1.6% ($n = 7$). The results were in agreement with those obtained by the reference procedure with a relative deviation between −3.8 and 3.7% and a sampling rate of about 27 samples $h^{-1}$.


*Keywords:* Multicommutation; Chemiluminescence; Propranolol; Permanganate oxidation

## 1. Introduction

Propranolol hydrochloride, [(1-methylethyl)amino]-3-(1-naphthalenyloxy)-2-propanol hydrochloride, is a non-selective beta-adrenergic blocker belonging to class II of antiarrhythmics. Propranolol is commonly used in the management of hypertension, angina pectoris, cardiac dysrrhythmias, hypertrofic obstructive cardiomyopathy, myocardial infarction, anxiety, essential tremor and migraine. This therapeutic drug could also be misused in some sports requiring intense concentration, like golf, fencing, equestrian and aeronautic sports, being for this reason included in the list of forbidden substances. Due to its therapeutical and pharmacological relevance, several methods have been reported for propranolol hydrochloride determination including spectrophotometry [1,2], chromatography [3,4], AAS [2–5], fluorimetry [6], titrimetry [7] and colorimetry [8].

Chemiluminescence (CL) measurements are characterised by low detection limits, wide dynamic linear ranges, high speed of response and excellent sensitivity, which justifies the increasing number of analytical procedures applying CL methods and their extensive utilisation in the determination of several species in biochemical, toxicological, medical and environmental areas [9,10]. Given that a CL response is typically generated by fast reactions its efficient monitoring required that the sample/reagent mixing and the subsequent chemical reaction would have to take place in front of the light detector. In view of that, the emergence of flow injection analysis (FIA), nowadays, the most used developing tool for chemiluminometric methods, represented and enormous breakthrough in terms of analytical applicability of this technique [11,12]. However, despite its evident advantages FIA exhibits a few shortcomings that could confine the full potential of CL; limited versatility in terms of sample manipulation given that the inserted sample volume is determined by the internal volume of the sample loop; low automation level that restrained the run-time adjustment of important analytical pa-

* Corresponding author. Tel.: +351 222087132; fax: +351 222004427.
*E-mail address:* joaolms@ff.up.pt (J.L.M. Santos).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.04.027

rameters like sample volume and flow rate or the utilisation of distinct sampling approaches; high consumption of reagents since they are continuously added during analysis and not only focused on the sample zone; gradual deterioration of the peristaltic pump propulsion tubing, which demanded frequent system re-calibration.

Multicommutation is a recent approach for sample and reagent handling in automated continuous flow systems. These flow systems comprise discrete computer-controlled commutators (solenoid valves) that could be actuated individually or in combination permitting a great flow directionality which is particularly advantageous in terms of reaction zone formation. They are as well characterised by high simplicity, versatility and precision and easy of automation [13,14]. The association of multicommutation to chemiluminescence reduces reagent consumption, facilitates sample handling and sample/reagent mixing, improves manifold design, versatility and system performance, increases reproducibility and reduces the operator intervention.

The use of potassium permanganate as a reagent to generate chemiluminescence has been widely investigated [15]. Most of the applications involve the determination of organic molecules with phenolic or amine functional groups. Since propranolol has an amine group it would be predictable that its reaction with potassium permanganate would yield a CL reaction. The reduction of potassium permanganate in acidic medium yielding a CL response require fast sample/reagent mixing and the immediate presentation of the developing reaction zone to detection, which is straightforwardly accomplished in a multicommutated flow system increasing sensitivity and reproducibility.

In this work, a multicommutated flow system for the chemiluminometric determination of propranolol based on its oxidation by potassium permanganate was developed and evaluated in the analysis of pharmaceutical preparations.

## 2. Experimental

### 2.1. Reagents

All chemicals were of analytical reagent grade and doubly deionised water was used throughout.

A $1.0\,g\,l^{-1}$ propranolol hydrochloride (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) standard solution was daily prepared by dissolving 100 mg in 100 ml of $1.5\,mol\,l^{-1}$ sulphuric acid and kept in the refrigerator. Working standard solutions were prepared by appropriate dilution of the stock solution with $1.5\,mol\,l^{-1}$ sulphuric acid.

A $5.0 \times 10^{-3}$ $mol\,l^{-1}$ potassium permanganate (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) solution was prepared by dissolving 197.55 mg in $0.1\,mol\,l^{-1}$ sulphuric acid (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and completing the volume to 250 ml with the same acid solution.

Sample solutions of commercially available pharmaceutical preparations were prepared either by dissolving required amounts of powdered tablets or the required volume of injectable solution with $1.5\,mol\,l^{-1}$ sulphuric acid.

### 2.2. Equipment

For chemiluminescence measurements, a Camspec CL-2 chemiluminescence detector (Campsec Ltd., Cambridge, UK) equipped with a three-port 60 μl inner volume quartz flow cell with internal mixing, was used. The luminometer had a wavelength response range of 320–600 nm and a flow cell working pathlength of 5 mm.

The flow manifold consisted of a set of two 161 T 031 (NResearch, West Caldwell, USA) two-way solenoid valves. Flow lines and reaction coils were made from 0.8 mm i.d. PTFE tubing. End-fittings, connectors and confluence points were also used.

Two Crison Micro BU 2031 (Crison Instruments SA, Barcelona, Spain) automatic burettes equipped with 5.0- and 2.5-ml syringes, respectively and controlled by a microcomputer through serial protocol (RS-232C) were used to propel the solutions. A home-made power driver based on a ULN 2003 integrated circuit was used to operate the solenoid valves.

Data acquisition and control of the analytical system was accomplished through a PC-LABCard model PCL–711B interface card from Advantech (Taipei, Taiwan) and a 486DX-based microcomputer. The software was developed in QuickBASIC 4.5 and permitted to control the automatic burettes, solenoid valves, data acquisition and processing.

### 2.3. Flow manifold and procedure

The flow network comprised two two-way (normally closed) solenoid valves ($V_1$ and $V_2$) and two automatic burettes ($P_1$ and $P_2$) (Fig. 1). $V_1$ and $V_2$ were used to establish two distinct analytical pathways either for sample insertion or for sample detection; $P_1$ was operated in aspiration and propulsion modes and was accountable for the insertion of both the sample solution (in aspiration mode) and the sulphuric acid carrier solution (in propulsion mode). $P_2$ was responsible for the introduction of the potassium permanganate reagent solution.

During the analytical cycle (Fig. 2), $V_1$ was initially closed while $V_2$ was opened allowing the carrier solution to flow through the detector ($P_1$ in propulsion mode) to establish baseline ($t_b$). For sample insertion, $V_1$ was switched on and the sample was aspirated by $P_1$ into holding coil L at a preset sampling time ($t_s$), which, depending on the flow rate, established the sampling volume. During sample insertion $V_2$ was switched off (closed) in order to prevent the reflux of the solution inside the detector cell during sample aspiration. Subsequently, $V_1$ was moved back to position closed while $V_2$ was opened and the sample zone was carried towards the detector ($P_1$ in propulsion mode) during a pre-defined trans-

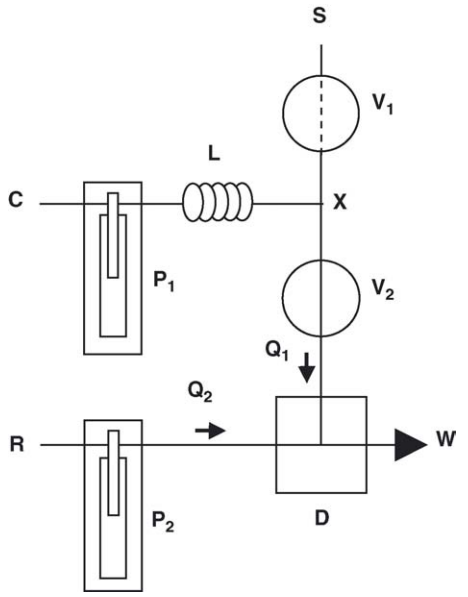


Fig. 1. Diagram of the flow manifold for propranolol hydrochloride determination: $V_1$ and $V_2$, two-way solenoid valves; dashed lines inside the valve symbols correspond to the closed position and solid lines to opened position; D, detector; $P_1$ and $P_2$, automatic burettes; L, holding coil (1-m long); S, sample (150 μl); C, carrier solution (0.1 mol $l^{-1}$ $H_2SO_4$); R, reagent solution ($2.5 \times 10^{-3}$ mol $l^{-1}$ $KMnO_4$ in 0.1 mol $l^{-1}$ $H_2SO_4$); $Q_1$ and $Q_2$, flow rates 0.3 ml $min^{-1}$; X, confluence point.

port time ($t_t$). When the front section of the sample zone was approaching the detector flow cell the potassium permanganate solution was inserted through $P_2$ and merged with the sample stream during a reagent insertion time ($t_r$) that coincided with the passage of sample zone. This way and since CL reactions occur very rapidly, the reagent and the sample were only mixed within the detector where the reaction took place.

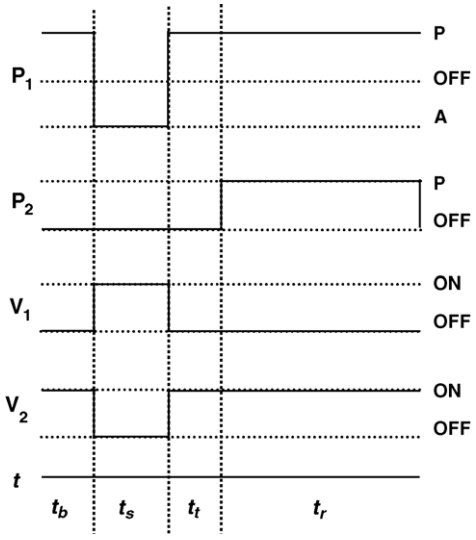


Fig. 2. Diagram of burettes and solenoid valves status during the analytical cycle. $P_1$ and $P_2$, automatic burettes; P, propulsion mode; A, aspiration mode; OFF, stopped; $V_1$ and $V_2$, solenoid valves, upper horizontal lines, ON, valve activated; lower horizontal lines, OFF, valve deactivated; *t*, timeline; $t_b$, baseline definition; $t_s$, sampling time (30 s); $t_t$, transport time (20 s); $t_r$, reagent time (80 s).

Following sample detection $V_1$ and $V_2$ returned to the initial positions and a new sample insertion took place starting a new cycle. A clean-up phase was not required because during the transport phase ($t_t$ in Fig. 2), the holding coil L was filled with the carrier solution, which makes it ready for the insertion of a new sample.

To obtain the analytical curve, a calibration procedure was performed involving the insertion of a set of standard solutions employing the same analytical parameters used for the sample.

### 2.4. Reference method

The accuracy of the developed procedure was evaluated by analysis of either propranolol hydrochloride bulk drug and propranolol hydrochloride pharmaceutical formulations according to the Portuguese Pharmacopoeia [16] by potentiometric titration in alcohol with NaOH 0.1 M.

## 3. Results and discussion

Preliminary studies revealed that the reaction between propranolol hydrochloride and potassium permanganate in acidic medium yielded a fast kinetic CL response, being the emitting specie not an oxidation product of propranolol but rather a reduction product of permanganate, probably excited Mn(II) ions [15]. The emitted light intensity was directly related to the amount of propranolol hydrochloride in the sample and depended on potassium permanganate and sulphuric acid concentrations. Bearing in mind that the chemical reaction had to take place in front of the detector and that dispersion was limited, analytical system performance would have to guarantee a prompt and efficient sample/reagent mixing. In these sense, analytical parameters such as sample volume, reagent volume, flow rate, etc., had a paramount importance.

### 3.1. Sample volume, transport time, reagent time and sampling strategy

In a multicommutated flow system, the inserted sample volume is not physically defined, as it happens with the internal volume of the sampling loop of a conventional FIA manifold, but is determined in terms of a sampling time ($t_s$). This is established by a time-based routine that controls, for a given flow rate, the selection of the almost unlimited range of sample volumes that could be introduced in the analytical system. Consequently, simply by adjusting the sampling time and without the need for re-configuring the flow manifold, it is possible to manipulate sample dispersion and thus the attainable dynamic linear range of the methodology. This prospect is particularly attractive when analysing samples of assorted concentration values, as it happens for instance when carry-

ing out dissolution studies where the analyte concentration could diverge between very dissimilar values.

By using increasing sampling times (at a flow rate of 0.3 ml $min^{-1}$), it was observed an increase in the analytical signal up to $t_s$ of 30 s (corresponding to a sample volume of about 150 μl) and a subsequent stabilisation.

Following insertion, the sample plug was carried out towards the detector's flow cell, where it met the permanganate reagent solution. Given that this solution was not continuously added, but only introduced when the sample plug arrived at the detector, it was necessary to establish a sample transport time ($t_t$) with the purpose of assuring a proper and reproducible synchronisation of the two solutions merging. This way, sample/reagent mixing and reaction development would not be compromised and a significant reagent saving would be achieved. The definition of an adequate $t_t$ had to take into account not only flow rate but also the length of the connecting tubing and the extent of the merging zone. It was verified that, since there was no reaction between carrier solution and sample, the flow rate during the transport phase did not interfere significantly with the analytical signal, as its influence on sample dispersion was negligible, though it could affect sample throughput. A somewhat dissimilar behaviour was ascribed for variations in the length of the connecting tubing. The chemiluminescence intensity was initially low for very short tubing; it increased as the length increased up to 70 cm and decreased once again for longer coils, as dispersion prevails. However, the selection of a $t_t$ with respect to the starting point of the merging of the two solutions (that coincide with the beginning of permanganate insertion) revealed to be crucial for the attainment of high chemiluminescence intensity. A similar importance was given to the selection of the reagent time ($t_r$), which corresponded to the conclusion of permanganate insertion and thus the merging ending point, the whole reagent volume consumed in the analysis being determined by $t_r$ and flow rate. By using a 0.3 ml $min^{-1}$ flow rate, the reagent time was evaluated in the range 40–100 s. It was observed that by using a $t_s$ = 30 s and $t_t$ values between 0 and 20 s, the maximum signal was reached with a $t_r$ = 100 s. When a shorter reagent time was used (40–60 s), the analytical signals obtained with consecutive sample insertions displayed two peaks (except for the 1st sample signal that consisted on only one peak). This was caused by the fact that, due to the reduced $t_t$, only the front portion of each sample plug was detected while its tailing edge remained in the dispersion coil. At the next analysis, both this tail and the front of the succeeding sample plug reacted with potassium permanganate generating two peaks per detection. The increment of the reagent time to 80 s allowed for the analysis of the whole sample plug and the attainment of a single peak.

An advantageous feature of multicommutated flow systems, which is closely related to their versatility, is the possibility of utilisation of distinct strategies for sample/reagent mixing and reaction zone formation, as is the case of merging zones, single sample volumes and binary sampling. The utilisation of single sample volumes was not practical because the too short residence time would not provide sufficient sample/reagent intermixing for adequate reaction development. Therefore, besides the merging of sample and reagent zones we have also evaluated the binary sampling approach consisting on the intercalation (by alternate actuation of pumps $P_1$ and $P_2$) of very small aliquots of sample and potassium permanganate, which, due to their reduced volume, rapidly undergo homogenisation. The obtained results were similar to those furnished by the merging zones approach and the later one was, most likely, of simpler implementation.

### 3.2. Flow rate

Flow rate was a fundamental parameter affecting the magnitude of the analytical signal in two distinct perspectives; first of all, it determined the transport of the reaction zone and thus its residence time in front of the detector, conditioning the light intensity measured; on the other hand, and in combination with the reaction coil length, it influenced the exact location within the flow manifold where the CL phenomena (light emission) occurred. In this sense, a too low or a too high flow rate could either result in a maximum of emission preceding or after the detector's flow cell. As it was previously referred, the flow rate during the sample transport phase was not determining in terms of reaction development but only regarding sampling rate. On the other hand, the effective flow rate during detection was the sum of the flow rates of the two merging streams (sample and reagent). This way, the utilisation of too high flow rates for both streams would result in a much reduced residence time of the reaction zone within the detector's flow cell affecting not only sensitivity but also reproducibility. Upon evaluation of distinct flow rates and aiming at establishing a compromise between sensitivity and sample throughput, a flow rate of 0.3 ml $min^{-1}$ was selected for both flowing streams.

### 3.3. Potassium permanganate and acid concentration

Concentration of potassium permanganate was an important parameter due to the inner filter effect, which means that at increased concentrations the solution becomes less transparent. By using potassium permanganate solutions at concentrations ranging from $0.5 \times 10^{-3}$ to $10 \times 10^{-3}$ mol $l^{-1}$, it was verified that the analytical signal increased up to a concentration value of $2.5 \times 10^{-3}$ mol $l^{-1}$ and then slightly decreased (Fig. 3). A $2.5 \times 10^{-3}$ mol $l^{-1}$ value was selected for the posterior experiments.

Considering that the chemiluminescence reaction was enhanced in acidic medium the concentration of the $H_2SO_4$ solution, used as carrier and in the preparation of the sample and the permanganate reagent solutions, was also an important factor. For the carrier solution, the acid concentration was assessed at values ranging from 0.1 to 2 mol $l^{-1}$. It was observed that the CL intensity remained stable until 0.5 mol $l^{-1}$ $H_2SO_4$ and then decreased as the acid concentration increased (Fig. 4). A 0.1 mol $l^{-1}$ sulphuric acid solution

Table 1
Results obtained in the determination of propranolol hydrochloride in pharmaceutical preparations

| Pharmaceutical preparation | Amount declared (mg/formulation) | Amount found (mg/formulation) | | RD[a] (%) |
|---|---|---|---|---|
| | | Reference methodology | Developed methodology[b] | |
| Inderal (injection) | 1 | 1.09 ± 0.01 | 1.1 ± 0.04 | 0.92 |
| Inderal 10 (tablets) | 10 | 10.10 ± 0.14 | 9.87 ± 0.26 | −2.23 |
| Inderal 40 (tablets) | 40 | 40.60 ± 0.54 | 39.49 ± 0.81 | −2.73 |
| Inderal 80 (tablets) | 80 | 79.63 ± 0.30 | 76.60 ± 2.39 | −3.8 |
| Inderal LA 80 (delayed-release tablets) | 80 | 81.21 ± 0.43 | 79.63 ± 1.25 | −1.95 |
| Inderal LA (delayed-release tablets) | 160 | 161.85 ± 1.24 | 167.9 ± 3.07 | 3.74 |
| Propranolol ratiopharm 40 | 40 | 42.40 ± 1.14 | 43.42 ± 1.88 | 2.4 |
| Propranolol ratiopharm 80 | 80 | 79.20 ± 1.68 | 80.92 ± 1.10 | 2.17 |

[a] Relative deviation of the developed methodology with respect to the reference procedure.
[b] Mean ± S.D.

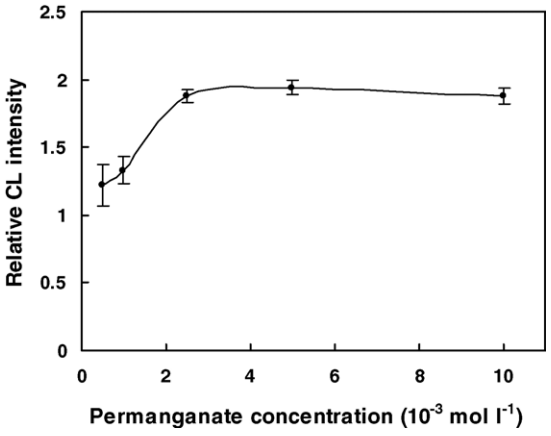


Fig. 3. Influence of potassium permanganate concentration. Sample volume and flow rates as in Fig. 1.

was selected for the subsequent experiments. A similar result was obtained for the acid concentration used in the preparation of the reagent solution. In contrast, the highest CL emission was verified when the sample solution was prepared in 1.5 mol $l^{-1}$ sulphuric acid.

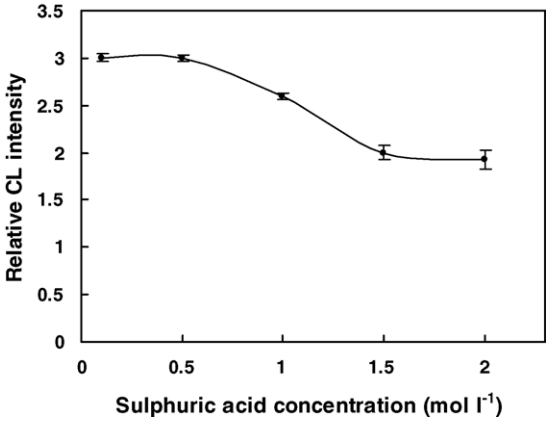


Fig. 4. Influence of sulphuric acid concentration. Sample volume and flow rates as in Fig. 1.

### 3.4. Analysis of pharmaceutical preparations

After system optimisation and by using a sampling time of 30 s and a flow rate of 0.6 ml $min^{-1}$, linear calibration plots for propranolol hydrochloride concentrations between 20 and 150 mg $l^{-1}$ were obtained. The analytical curve was represented as $A = 0.0199C - 0.0306$, where $A$ was the relative chemiluminescence intensity and $C$ was the concentration of propranolol hydrochloride expressed in mg $l^{-1}$ with a correlation coefficient of 0.998.

The developed methodology was evaluated in the determination of propranolol hydrochloride in commercially available pharmaceutical formulations. The obtained results where comparable with those furnished by the reference method with a relative deviation between −3.8 and 3.74%. The precision was good, with a relative standard deviation lower than 1.6% ($n = 7$). Results are summarised in Table 1.

The analytical system was stable, and no baseline drift was verified throughout the experiments. The sampling rate was about 27 sample $h^{-1}$.

### 3.5. Interferences

In order to apply the developed methodology to the determination of propranolol hydrochloride in pharmaceutical formulations, the influence effect of several compounds commonly used as excipients was assessed. Samples containing propranolol hydrochloride at a fixed concentration

Table 2
Interfering effect of excipients on the developed methodology

| Interference | Tolerance molar ratio[a] |
|---|---|
| Citric acid | 10 |
| Sucrose | 10 |
| Talc | 100[b] |
| Lactose | 100[b] |
| Magnesium stearate | 100[b] |
| Starch | 100[b] |

[a] $3.38 \times 10^{-3}$ mol $l^{-1}$ propranolol hydrochloride added.
[b] The highest value tested.

of 100 mg $l^{-1}$ and increasing concentrations of the excipient were analysed by the developed methodology. A compound was considered as non-interfering, if the analytical signal variation was ±3% when compared to the analytical signal obtained in the absence of the referred compound. The obtained results (Table 2) showed that under the used reaction parameters, most of the studied excipients did not interfere.

## 4. Conclusions

The obtained results showed that chemiluminescence is a valuable detection procedure for the determination of propranolol hydrochloride in bulk drug and pharmaceutical preparations, which is reinforced by the limited interference of the substances commonly used as excipients. The proposed method is sensitive and accurate, requires no sample pretreatment, provides a wide working concentration range and is more simple than most of the reported methods.

Regardless of its simplicity and easy of operation, the proposed multicommutated flow methodology assured a fast reaction zone homogenisation and an flexible control of dispersion, by means of the adaptable time-based sample insertion. However, probably its most remarkable attribute is the significant solutions saving that it provides, when compared to batch methods or the habitually used multichannel FIA systems. On the other hand, owing to its modular structure and versatility, the developed flow system can be easily applied in the routine analysis of other compounds without the need for physical re-configuration.

## Acknowledgements

The authors are grateful to the consortium agreement GRICES/CAPES for financial support in the researchers exchange. One of us (KLM) thanks FCT for a Ph.D. grant.

## References

[1] H. Salem, J. Pharm. Biomed. Anal. 29 (2002) 527–538.
[2] M.A. El-Ries, F.M. Abou Attia, S.A. Ibrahim, J. Pharm. Biomed. Anal. 24 (2000) 179–187.
[3] F. Geiser, M. Schultz, L. Betz, M. Shaimi, J. Lee, W. Champion Jr., J. Chromatogr. A 865 (1999) 227–233.
[4] M.E. Abdel-Hamid, Il. Farmaco. 55 (2000) 136–145.
[5] S. Khalil, N. Borham, J. Pharm. Biomed. Anal. 22 (2000) 235–240.
[6] J.A.M. Pulgarín, A.A. Molina, P.F. López, Anal. Chim. Acta 370 (1998) 9–18.
[7] U.M. Abbasi, F. Chand, M.I. Bhanger, S.A. Memon, Talanta 33 (1986) 173–175.
[8] Lj. Živanović, D. Radulović, M. Jovanović, Pharm. Acta Helv. 63 (1988) 350–352.
[9] P. Fletcher, K.N. Andrew, A.C. Calokerinos, S. Forbes, P.J. Worsfold, Luminescence 16 (2001) 1–23.
[10] A.M. García-Campaña, W.R.G. Baeyens, Analusis 28 (2000) 686–698.
[11] R.W. Abbott, A. Townshend, R. Gill, Analyst 111 (1986) 635–640.
[12] S.A. Al-Tamrah, A. Townshend, A.R. Wheatley, Analyst 112 (1987) 883–886.
[13] R.A.S. Lapa, J.L.F.C. Lima, B.F. Reis, J.L.M. Santos, E.A.G. Zagatto, Anal. Chim. Acta 366 (1998) 209–215.
[14] F.R.P. Rocha, B.F. Reis, E.A.G. Zagatto, J.L.F.C. Lima, R.A.S. Lapa, J.L.M. Santos, Anal. Chim. Acta 468 (2002) 119–131.
[15] B.J. Hindson, N.W. Barnett, Anal. Chim. Acta 445 (2001) 1–19.
[16] Portuguese Pharmacopoeia VII, vol. I, Lisboa, 2002, pp. 1258–1259.