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Multi-pumping flow system for the spectrophotometric determination of dipyrone in pharmaceutical preparations

José L.F.C. Lima [a], Sandra M. Oliveira Sá [b], João L.M. Santos [a,*], Elias A.G. Zagatto [b]

[a] *REQUIMTE/Departamento det Química-Fisica, Faculdade de Farmácia, Universidade do Porto, Rua Anibal Cunha 164, 4050 Porto, Portugal*

[b] *Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Caixa Postal 96, 13400-970 Piracicaba SP, Brazil*

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Abstract

A novel flow system for the spectrophotometric determination of dipyrone with *p*-dimethylaminobenzaldehyde exploiting the multi-pumping approach was developed. The proposed methodology utilises several micro-pumps for propelling the involved fluids under improved mixing conditions, introducing sample/reagent aliquots and providing commuting facilities. As a consequence the multi-pumping system presents high versatility and manifold simplicity, as well as a straightforward operational control and enhanced analytical capabilities. Linearity of the analytical curve was observed within 10 and 400 mg l^{-1} dipyrone ($r = 0.9997$; $n = 6$), results were precise (r.s.d. $< 0.12\%$; $n = 20$) and sampling rate was 50 h^{-1}. Detection limit was estimated as 1 mg l^{-1} dipyrone. The method was applied to pharmaceutical preparations and the results were in agreement with those obtained by the reference procedure with relative deviations within -1.7 and $+2.2\%$.

Keywords: Flow analysis; Multi-pumping flow system; Dipyrone; Spectrophotometry; *p*-dimethylaminobenzaldehyde; Pharmaceutical preparations

1. Introduction

The need for technological innovations for improvement of analytical procedures impels the search for new approaches and their efficient application. In this regard, multi-pumping flow systems were recently conceived as a new strategy for handling sample and reagent solutions and to transport them towards detection while efficient sample zone development is achieved [1]. The system comprises several computer-operated micro-pumps strategically positioned in the manifold that are commuted individually or in combination. Being the only active devices of the flow manifold, they are able to propel the involved fluids under improved mixing conditions, to introduce sample and reagent aliquots with different sampling

* Corresponding author. Tel.: +351-22-08-7132; fax: +351-22-00-4427.
E-mail address: joaolms@ff.up.pt (J.L.M. Santos).

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strategies (merging zones, tandem injection, sandwich injection), to implement stopped-flow procedures, to provide commuting facilities, etc. As a consequence, multi-pumping flow systems present high versatility and simple manifold configuration providing the means for the easy implementation of fully automated operation and control modes, which make them very attractive for the implementation of routine pharmaceutical analysis.

Dipyrone (metamizole) is a therapeutic agent commonly used as analgesic, antipyretic and antispasmodic in several pharmaceutical preparations. Being an effective painkiller in situations of severe pain its administration is sometimes associated with serious adverse effects like an increased risk of agranulocytosis and shock [2]. Its therapeutic relevance and the importance of the side effects have prompted the development of several methods for its determination both in pharmaceutical preparations and biological samples. Direct spectrophotometric methods were proposed for the determination of dipyrone in single or multicomponent formulations upon reaction with potassium iodate [3] or cobalt nitrate and KSCN [4]. Derivative spectrophotometry was used for the simultaneous determination of dipyrone in a binary mixture with ascorbic acid [5] and in ternary combinations with paracetamol and caffeine [6,7]. Other analytical methodologies include spectrofluorimetry [8], chemiluminescence [9] as well as chromatography [10] and amperometry [11]. The iodometric titration of dipyrone is recommended by the Pharmacopoeia [12] but it is slow and laborious thus less applicable to large-scale analysis.

The main purpose of this work was then to develop a fast, simple and low-cost procedure for spectrophotometric determination of dipyrone in pharmaceutical preparations using *p*-dimethylaminobenzaldehyde (PDAB) as the colour-forming reagent. Due to its analytical capabilities, easy of operation and versatility a multi-pumping flow system was designed and important analytical parameters such as pulse frequency, sample pulses, reactor length, number of pulses for transport, composition and concentration of the reagents were studied.

2. Experimental

2.1. Samples, standards and reagents

All solutions were prepared with deionised water and analytical grade chemicals were used.

A 1000 mg l^{-1} dipyrone standard solution was prepared by dissolving the appropriate amount of $C_{13}H_{16}N_3NaO_4S \cdot H_2O$ (Sigma) in water. This stock solution was maintained under refrigeration at 5 °C. Working standards within the 10–400 mg l^{-1} dipyrone range were daily prepared by water dilutions of the above stock.

A 20.0 g l^{-1} PDAB (Sigma) solution was daily prepared in 2.5% (v/v) HCl. It was kept in amber bottle and protected from light.

Pharmaceutical dosage forms of dipyrone analysed were those commercially available in Portugal and Brazil: Nolotil capsules 575 mg, Nolotil injection 2 g/5 ml, Dolocalma capsules 575 mg, Dolocalma injection 2 g/5 ml and Nevraldor solution 500 mg ml^{-1}. For the analysis of Nolotil and Dolocalma capsules 575 mg, 20 capsules of the drug were used. The material from each capsule was removed and accurately weighed, allowing the mean weight of the contents of a single capsule to be estimated. Thereafter, the material was homogenised and an appropriate amount was sampled, dissolved in water and filtered. For analyses of Nolotil and Dolocalma (injections 2 g/5 ml), and Nevraldor (solution, 500 mg ml^{-1}), five samples were used and the solution contents were mixed for representative purposes. Thereafter the required volume was sampled and diluted with water.

2.2. Apparatus

The proposed flow system comprised two micro-pumps, a spectrophotometer, a reaction coil and flow lines made from 0.8-mm i.d. PTFE tubing, a home made confluence connector and accessories. The micro-pumps (Ref. 090SP-BIOCHEM Valve Inc., Boonton, USA) were of the fixed displacement diaphragm type, being solenoid operated and dispensing 8 μl per stroke. The LaboMed model Spectro 22RS spectrophotometer was equipped with a 70 μl inner volume (10-mm

optical path) flow-cell. For data acquisition and system control, a microcomputer with a PC-LABCard model PCL-711B interface card from Advantech was used. The software was developed in Quick-basic 4.5 and permitted the control of the micro-pumps and the real-time data processing. Solenoid pumps were operated by means of a CoolDrive™ (NResearch Inc., New Jersey, USA) power drive.

2.3. Manifold

The flow diagram is shown in Fig. 1 and presents the solenoid micro-pumps for sample and reagent streams positioned near the x confluence point. In the developed procedure the reagent solution acted also as sample carrier stream. After sample/reagent convergence the main chemical reaction took place inside the coiled tubular reactor and the formed product was monitored at 430 nm during passage of the processed sample through the detector. The height of the recorded peak constituted the measurement basis. When micro-pumps P_1 or P_2 were actuated the sample or reagent solutions were directed to fill the analytical path. The pumps were sequentially operated establishing a tandem stream inside the main channel consisting on the intercalation of neighbouring slugs of sample and reagent solutions. These slugs underwent fast intermixing as the entire processed sample was transported towards detection.

The analytical cycle was started when the reagent carrier stream was pumped by P_2 at a fixed pulse frequency (which defined the flow rate for the 8 μl stroke volume) in order to establish the baseline. The sample was inserted by means of P_1 as a pre-set number of pulses (defining the sample volume), which were intercalated with a pre-set number of reagent slugs in order to establish the sample zone in a tandem fashion. The pulse frequency determined the flow rate during sample insertion. The sample zone was then pushed towards detection by repeated actuations of P_2 and by using a pre-set number of transport pulses.

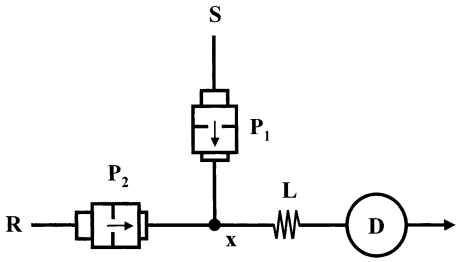

Fig. 1. Flow diagram. S = sample; R = reagent solution (2.0% PDAB in 2.5% v/v HCl); L = reactor (50 cm); P_1 and P_2 = solenoid micro-pumps; x = confluence point; D = detector (430 nm).

2.4. Reference method

For accuracy assessment of the results obtained by the proposed procedure dipyrone bulk drug and dipyrone pharmaceutical formulations were analysed by iodometric titration according to the reference procedure [12]. A given amount of the pharmaceutical preparation was transferred into an Erlenmeyer flask and titrated with a 0.05 mol l^{-1} iodine solution in acetic acid using the blue iodine starch complex as indicator.

3. Results and discussion

Preliminary experiments revealed that reaction of dipyrone with PDAB in acidic medium was relatively slow, therefore the analytical signals were improved as the reaction time increased. Implementation of a flow-based methodology for the routine determination of dipyrone would have then to deal with two distinct and complementary aspects. Firstly, sample and reagent mixing would have to be promptly achieved in order to guarantee an efficient sample zone homogenisation and thus a higher degree of reaction completion prior to detection. In addition, sample dispersion would have to be restrained aiming at the attainment a low detection limit. A third feature was also aimed: the proposed system should be able to provide a wide dynamic concentration range without requiring manifold reconfigurations, which would be easily accomplished by means of a versatile manipulation of the sampled volume.

Multi-pumping flow systems, a recently proposed flow strategy [1], exhibited operational characteristics that are ideal for this task. Utilisa-

tion of solenoid micro-pumps for sample and reagent introduction and for their transportation towards detection enables the implementation of very simple analytical systems, as this two fundamental operations, usually carried out by independent units, are combined in a single process which is easily controlled and not subject to cumulative errors. Considering that the micro-pumps could be individually or jointly actuated it is possible to exploit distinct approaches for sample insertion, consisting either on the intercalation of sample or reagent aliquots, merging of sample and reagent zones, or the introduction of a single variable-volume sample slug. Selection and insertion of a pre-selected sample volume into a multi-pumping flow system is an operation that markedly differs from other flow methodologies. In contrast to flow injection systems [13] where the sampled volume is determined by the internal volume of a sampling loop (which has to be replaced whenever a new sample volume is required) or to sequential injection [14] or multi-commuted [15] systems where the sampled volume is determined by the sampling time (for a given flow rate), the sample volume in multi-pumping systems is defined by the number of pulses and the pulse (stroke) volume. In fact, considering that for each pump actuation a solution volume corresponding to the pump stroke volume is inserted into the analytical path, the number of pulses or pump actuations establish the total sampled volume. A third variable, pulse frequency, determine the time required for sample or reagent insertion, and consequently, the flow rate. In this way, the flow rate of a given solution is easily adjusted by setting appropriate pulse frequency, which makes it very easy to control the residence time of the sample zone prior to detection, or the implementation of stopped-flow strategies.

Another important difference between multi-pumping and other flow systems concerns the characteristics of the flowing stream. Whereas the flow is typically laminar and sample zone homogenisation is accomplished by radial and axial dispersion in flow injection or sequential injection systems, a pulsed flow pattern caused by the micro-pump actuation is produced in the multi-pumping systems. During sample zone transportation this pulsed flow, which is strictly reproducible, permits the attainment of very stable flow rates and—at the same time—leads to a chaotic movement of the involved solutions in all directions producing a fast and effective sample/reagent mixing that results in an enhanced reaction zone and thus improved analytical signals. This aspect is of relevance for dipyrone determination, since it requires an increased reaction time associated with low axial dispersion and good mixing conditions.

The influence of the number of sample pulses, which established the sample volume inserted into the analytical path, was investigated by taking into account several premises: low detection limits, wide dynamic concentration range and high sample throughput. If priority was given to lower detection limits then a higher number of sample pulses would have to be used in order to restrain dispersion and to enhance sensitivity. However, excessive sample pulses resulted in broader peaks and thus in a sampling rate lessening. Moreover, the increased analytical signal obtained with additional sample pulses could restrict the working concentration range as deviations from the Beer's law would be more likely to occur. Development of two peaks, at the front and trailing edges of the sample zone has been often reported in flow injection analysis when a too large sample volume is used. It was avoided by inserting the sample not as a single plug but by intercalating very small sample in tandem with reagent aliquots, thus exploiting the binary sampling approach [15]. In this way, instead of establishing only two reaction interfaces at the sample zone boundaries, which are strictly dependent of solutions mutual mixing, the produced tandem stream enabled multiple reaction sites, even when large whole sample volumes are considered. This promoted a fast mixing.

After inserting a 100 mg l^{-1} dipyrone standard solution into the flow system of Fig. 1 with a 50-cm reactor (250 μl internal volume), it was verified that increasing the number of sample pulses increased analytical signals. Even when a very large sample zone was created by using 30 sample pulses intercalated with 30 reagent pulses, which established a 480 μl sample zone exceeding the 250

μl reactor volume, only a single but broad peak was obtained. However the time required to resolve such a peak (baseline to baseline return) was also increased. Moreover, the reproducibility was affected due to the inappropriate mixing conditions. As a compromise between sampling rate and sensitivity, 10 sample pulses were selected for the posterior experiments. As they were intercalated with 10 reagent pulses, a 160 μl reaction zone was produced. It should be noted that sensitivity could be easily enhanced by increasing the number of sample pulses without changing the manifold configuration.

Influence of the reactor length was evaluated by inserting a 100 mg l^{-1} dipyrone standard solution by using 10 sampling pulses, equivalent to a whole sample volume of 160 μl, which was carried towards detection through different length reactors (10, 25, 50, 75 and 100 cm, inner volumes of about 50, 125, 250, 375 and 500 μl). The results showed that the analytical signal increased slightly with the reactor length until 50 cm and then markedly decreased (Fig. 2). This confirms that homogenisation is rapidly achieved and the sample dispersion prevails after 50 cm, contributing for attenuation of the analytical signal.

Another relevant parameter in system design was the flow rate. For a given micro-pump stroke, the flow rate is determined by the pulse frequency corresponding to the number of pump actuations for a given period of time. The micro-pumps are characterised by an upper limiting value for the frequency of actuation of about 250 cycles min^{-1}. Considering the pump stroke volume of 8 μl, the maximum flow rate that could be achieved was 2.0 ml min^{-1}. If higher flow rates were required then micro-pumps of larger stroke volumes would have to be used. Influence of the flow rate was evaluated by setting pulse intervals (resting time between pulses) of 0.25, 0.40, 0.60, 0.80 and 1.0 s corresponding to pulse frequencies of 240, 150, 100, 75 and 60 min^{-1}, which determined flow rates of about 1.9, 1.2, 0.8, 0.6 and 0.48 ml min^{-1}. It was verified that for low pulse frequencies (60–75 min^{-1}) and low flow rates, the analytical signal increased with the pulse frequency (Fig. 3), probably due to a decrease in dispersion. Thereafter, it reached a maximum, exhibiting a slight decrease for very high pulse frequencies, which could be a result of the reduced residence time. The flow rate affected not only the analytical signal but also the sample throughput. Lower pulse frequencies or lower flow rates were associated with higher residence times with a concomitant increase of the time required for the analysis. On the other hand, very high pulse frequencies, near the upper operational limit of the micro-pumps were more susceptible to errors, which could affect the analytical repeatability. Aiming a compromise between sampling rate and sensitivity, a pulse frequency of 150 min^{-1} (resting time between pulses of 0.4 s) equivalent to a flow rate of 1.2 ml min^{-1} was selected. A related aspect to be considered is that the number (or volume) of pulses to carry the sample zone towards detection is mainly dependent of the inner volume of the

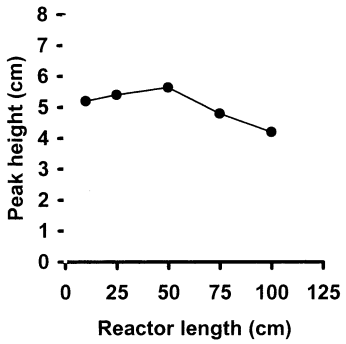

Fig. 2. Influence of the reactor length. Figure refers to the insertion of a 100 mg l^{-1} dipyrone solution at sample volume of 80 μl (10 pulses).

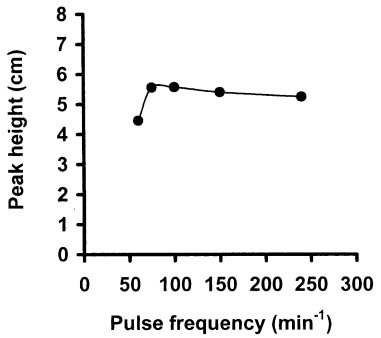

Fig. 3. Influence of the pulse frequency. Figure refers to the frequency of actuation of the 8 μl micro-pump for insertion and transport of an 80 μl sample volume.

analytical path (reactor plus flow cell) and less affected by the flow rate. In this regard, it was verified that 80 carrier pulses (640 μl) were suitable for proper transportation of the 160 μl sample zone through the 250 μl reactor and the 70 μl flow cell at a pulse frequency of 150 pulses min^{-1}, which permits to attain a sampling rate of about 50 h^{-1}.

Influence of PDAB concentration was investigated within the 10.0–25.0 g l^{-1} range. The analytical signal increased markedly with the PDAB concentration until 20.0 g l^{-1} and then decreased (Fig. 4). In view of the higher peaks obtained, 20.0 g l^{-1} PDAB was used in all further experiments.

HCl concentration of the reagent/carrier solution was also a very important parameter since it affected the reaction rate and thus the sensitivity of the determination and was studied at concentrations values of up to 4.0% (v/v). It was verified that for low sample volumes (32 μl equivalent to 4 pulses) the analytical signal decreased as the acid concentration increased. This result can be explained by an increment in the reaction rate that results in a faster reaction development beyond which sample dispersion prevails. For higher sample volumes (120 μl, 15 pulses) the analytical signal increased with the acid concentration which confirms the increase in the reaction extent. However as the acid concentration raised, and despite the efficient mixing, a pronounced concentration gradient was originated between the sample zone and the carrier solution, establishing a refractive index gradient that caused perturbations in the analytical signal. The effect (Schlieren) was more pronounced at the front and in a lesser extent at the trailing edge of the sample zone. It affected the profile and intensity of the recorded signal (which is further emphasised by the pulsed nature of the flowing stream) particularly at low dipyrone concentrations due to the occurrence of a significant blank reading. Aiming at reducing the intensity of the Schlieren effect while maintaining a good sensitivity an HCl concentration of 2.5% (v/v) was selected for the analysis.

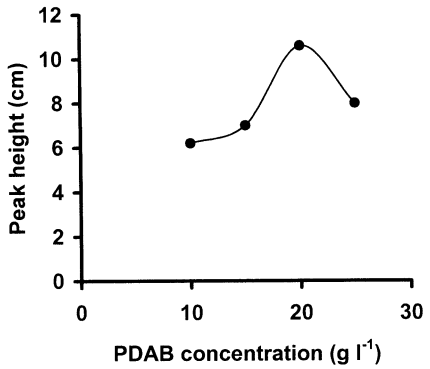

Fig. 4. Influence of the PDAB reagent concentration in analytical signal.

After system dimensioning, linear calibration plots over a dipyrone concentration range from 10 to 400 mg l^{-1} were obtained. The analytical equation was:

$$A = 0.0487C + 1.65$$

where A = peak height; C = dipyrone concentration. The regression coefficient was estimated as 0.9997 ($n = 6$).

The detection limit based on a 3σ interval, and the lower quantifiable value, were estimated as 1 and 4 mg l^{-1} dipyrone, respectively.

3.1. Analysis of pharmaceutical preparations

The proposed procedure was evaluated in the determination of dipyrone in commercially available pharmaceutical preparations. The results were in good agreement with those provided by the reference procedure [12] with relative deviations from −1.7 to +2.2%. Precise results were obtained, the relative standard deviation being estimated as less than 0.12% after 20-fold consecutive determinations of typical samples. No interference was found from the excipients used to manufacture the analysed pharmaceutical forms. The proposed flow system is very stable and robust, and baseline drift has not been verified during extended working periods. The sampling throughput is about 50 samples per hour. Analysis of the dipyrone determinations summarised in Table 1 permits one to conclude that there is no statistic differences between methods at the 95% probability level.

Table 1
Comparative results obtained in the determination of dipyrone in pharmaceutical preparations, by the proposed and the reference procedure [12]

Sample	(Dosage) mg/formulation	Amount found (mg)		
		Developed methodology	Reference methodology	RD[a]
Nolotil (capsule)	575	562.9±0.7	570.4	−1.4
Nolotil (injection-5 ml)	2000	2010.9±2.4	2004.8	0.3
Dolocalma (capsule)	575	555.6±0.7	565.1	−1.7
Dolocalma (injection-5 ml)	2000	2034.4±2.4	1990.3	2.2
Nevraldor (solution-1 ml)	500	506.0±0.6	501.8	0.8

[a] Relative deviation, expressed in percentage, of the developed methodology regarding the reference procedure.

4. Conclusions

The developed methodology is a valuable strategy for the determination of dipyrone in pharmaceutical preparations and could be an advantageous alternative to other available procedures, because it exhibits an high degree of automation, it is simple, fast, precise, accurate, requires low reagent consumption and minor operator intervention. The multi-pumping flow system exhibited fast homogeneous mixing even in situations of limited dispersion, which contributes to improve the reaction development. This assumption was further reinforced by the intercalation of small sample and reagent slugs, which permits to reduce the time required for the analysis as well as reagent consumption. Therefore it is a suitable analytical approach for application whenever sensitivity enhancement is required, as it usually happened in procedures based on relatively slow reactions, without compromising the sampling throughput. Furthermore, the proposed methodology presents high versatility, enabling the analysis of an extended range of concentrations without requiring physical manifold re-configurations. The utilisation of a single active component (micro-pump) with several functions (solution insertion, mixing, transport, etc.) revealed to be an attractive strategy for the implementation of fast, low-cost, reliable and easily automated analytical procedures.

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