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Multi-pumping flow system for spectrophotometric determination of bromhexine

Ana C.B. Dias [b], João L.M. Santos [a], José L.F.C. Lima [a,*], Elias A.G. Zagatto [b]

[a] *REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Rua Anibal Cunha 164, Porto 4050, Portugal*
[b] *Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, P.O. Box 96, Piracicaba 13400-970, Brazil*

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Abstract

A novel flow-based procedure involving the multi-pumping approach was developed for the spectrophotometric determination of bromhexine in pharmaceutical preparations. The method is based on reaction with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and Ce(IV). Several solenoid micro-pumps are present in the manifold in order to provide improved system control. Critical tasks in continuous flow analysis, sample–reagent introduction and solution propelling, are then efficiently carried out. Moreover, the pulsed flow inherent to the mini-pumps ensures good mixing conditions, thus improving the reaction zone homogenisation. This aspect becomes particularly attractive for the implementation of analytical procedures involving several consecutive reactions as it happens with the proposed procedure.

Linear calibration plots ($r > 0.995$, $n = 6$) were obtained for bromhexine concentrations up to 400 mg l^{-1}. Detection limit was 2 mg l^{-1} and the sampling rate was about 45 samples h^{-1}. Results are precise (R.S.D. $< 1.5\%$; $n = 10$) and in agreement with those furnished by the reference procedure involving a potentiometric titration.

Keywords: Multi-pumping flow system; Pulsed flow; Bromhexine; 3-Methyl-2-benzothiazolinone hydrazone; Solenoid micro-pumps

1. Introduction

Bromhexine (2-amino-3,5-dibromo-*N*-cyclohexyl-*N*-methylbenzenemethanamine hydrochloride) is a mucolytic agent used in the treatment of respiratory disorders associated with productive cough. It is also used as an adjuvant to improve the response to antibiotics in the treatment of respiratory infections [1]. Several techniques have been proposed for bromhexine determination such as direct spectrophotometry upon diazotisation and coupling (Bratton–Marshall reaction) [2], spectrophotometry with chemometric techniques [3,4], liquid chromatography [5], capillary electrophoresis [6], etc. Most of them are, however, laborious and/or time-consuming, requiring a specific sample treatment or expensive equipment.

Flow-based techniques have been extensively applied to pharmaceutical analysis due to its flexibility, versatility and low reagent consumption [7]. In this way, a flow-injection procedure was already proposed for the determination of bromhexine [8] but it required a liquid–liquid extraction step that hindered the degree of automation.

Multi-pumping flow systems [9] were recently proposed as a novel flow strategy. The approach requires

* Corresponding author. Fax: +351-220037977.
E-mail address: limajlfc@ff.up.pt (J.L.F.C. Lima).

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several solenoid-actuated micro-pumps strategically positioned in the flow manifold that could be commuted individually or in combination allowing an effective and precise system control. Differing from the works of Weeks and Johnson [10] and Hanrahan et al. [11], that proposed conventional flow-injection approaches wherein the solenoid micro-pumps were just a means to propel the solutions substituting the usual peristaltic pump, in multi-pumping flow systems solenoid micro-pumps are the only active devices being accountable for solution propelling, sample–reagent introduction and manifold commutation. The sample volume defined through a time- or pulse-based scheme is then precisely selected, the introduction of reagents is efficiently accomplished, enabling also a versatile reagent random access selection, and the transportation of the sample zone towards detection is carried out under improved mixing conditions, thus making the reaction development easier. Moreover, the control of a multi-pumping flow system is supported by the discrete actuation of each flow stream, which enables the instantaneous adjustment of the flow rate of each solution and provided the means for the exploitation of distinct sampling strategies including variable sample volume, binary sampling and merging zones. As a consequence, multi-pumping flow systems exhibit high versatility and manifold simplicity allowing the implementation of fully automated analytical procedures. Establishment of a pulsed flow is inherent to the micro-pumps actuation, as a consequence of the chaotic movement of the solutions resulting from the sudden stepwise propelling. This yields an efficient sample–reagent–carrier interaction that leads to a better reaction zone homogenisation, thus improved analytical signals.

This work reports the design of an automated and easily operated flow system exploiting the analytical potential of multi-pumping and its application to the spectrophotometric determination of bromhexine in pharmaceutical preparations. The method is based on reaction with 3-methyl-2-benzothiazolinone hydrazone (MBTH) and Ce(IV). MBTH was originally proposed [12] as a spot test indicator for aromatic amines and heteroaromatic compounds. In the presence of oxidising agents, it forms strongly coloured compounds [13,14]. The two consecutive reactions of the method required fast sample–reagent mixing, which makes it particularly suitable for implementation in a multi-pumping flow system.

2. Experimental

2.1. Samples, standards, reagents

All solutions were prepared with deionised water and analytical grade chemicals. For liquid dosage forms (solution and syrup), the sample solutions were prepared by dissolving the required amounts in water and filling the volume up to 50 ml. The amounts were defined in accordance to the declared bromhexine contents of the assayed samples. For solid dosage forms, the sample solutions were prepared by weighing and powdering a representative number of tablets. Thereafter, the material was homogenised and an appropriate amount was sampled, dissolved in water and filtered.

The 500 mg l^{-1} bromhexine hydrochloride standard stock solution was weekly prepared by dissolving 50 mg of the drug (Sigma) in 100 ml of water. This solution was kept in the refrigerator at 5 °C. Working standards of bromhexine were daily prepared by water dilutions of the above solution.

A 7.5×10^{-3} mol l^{-1} Ce(IV) solution was daily prepared by dissolving 758 mg $Ce(SO_4)_2 \cdot 4H_2O$ in 250 ml of 0.36 mol l^{-1} H_2SO_4. A 4.0×10^{-3} mol l^{-1} MBTH solution was prepared by dissolving 86 mg in 100 ml of 0.36 mol l^{-1} H_2SO_4.

2.2. Apparatus

Measurements were performed with a LaboMed model Spectro 22RS spectrophotometer (at 550 nm) equipped with a 30 μl inner volume (10 mm optical path) flow cell. The manifold comprised three micro-pumps, a reaction coil made with 0.8 mm i.d. PTFE tubing and a home made confluence connector. The micro-pumps (Ref. 0905P, BIO-CHEM Valve Inc., Boonton, USA) were of the fixed displacement diaphragm type, being solenoid operated and dispensing 8.0 μl per stroke. The pumps were actuated by using a CoolDrive™ (NResearch Inc., NJ, USA). Control of the analytical system, as well as data acquisition and processing, was accomplished by means of a Pentium I based microcomputer with a

PC-LABCard model PCL-711B Advantech interface card. Software was developed using Quick-basic 4.5.

2.3. Flow manifold

The flow diagram of the system exploiting the multi-pumping approach for bromhexine determination is shown in Fig. 1 and included three solenoid micro-pumps (P_1, P_2 and P_3), each one responsible for the introduction a specific solution, positioned around the 'x' confluence point. P_1 inserted the Ce(IV) solution that acted also as the carrier stream, whereas P_2 and P_3 inserted the sample and the MBTH solutions, respectively. The pumps acted simultaneously as commuting and propelling devices, controlling at once the sample and reagents volumes, flow rate, sampling time and sampling sequence, which highly simplified manifold configuration.

The analytical cycle started by pumping the carrier reagent solution through P_1 at a fixed pulsed frequency in order to define baseline. Pulse frequency was defined in terms of pulse time that corresponded to the valve resting time between two consecutive pulses. The pulse frequency, in combination with the stroke volume, defined the flow rate. Sample was inserted by simultaneously actuating P_2 and P_3 for a pre-set number of pulses, which permitted to merge together small sample and MBTH aliquots. These aliquots underwent fast intermixing establishing the first-stage reaction zone. The number of sample aliquots defined the total sample volume and the pulse frequency established the flow rate. The sample zone was then carried out towards detection by repeated actuation of P_1 allowing the second-stage reaction to take place inside the coiled tubular reactor. Thereafter, the formed product was monitored at 550 nm. The height of the recorded peak constituted the measurement basis.

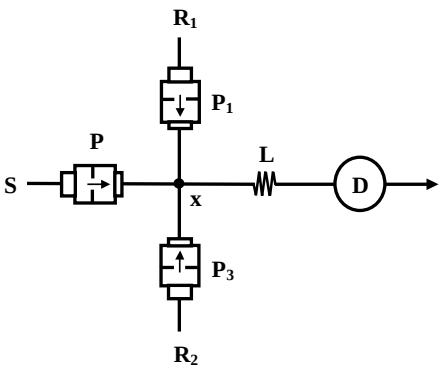

Fig. 1. Flow diagram. S: sample; R_1: 7.5×10^{-3} mol l^{-1} Ce(IV) solution in 250 ml of 0.36 mol l^{-1} H_2SO_4; R_2: 4.0×10^{-3} mol l^{-1} MBTH solution in 0.36 mol l^{-1} H_2SO_4; L: reactor (50 cm); P_1, P_2 and P_3: solenoid micro-pumps; x: confluence point; D: detector (550 nm).

2.4. Reference method

For accuracy assessment of the results obtained by the proposed procedure, bromhexine bulk drug and bromhexine pharmaceutical formulations were analysed by potentiometric titration [15]. A given amount of the pharmaceutical preparations was diluted with 96% (v/v) ethanol and titrated with 0.1 mol l^{-1} standardised sodium hydroxide solution.

3. Results and discussion

Preliminary experiments revealed that the analytical sensitivity was strongly influenced by both reagents addition order and acidity of the reaction medium. Moreover, the reaction rate was relatively low, which would eventually demand a long-term reaction development to ensure adequate sensitivity.

Design of the automated procedure for bromhexine determination based on this reaction—thus involving the consecutive introduction of two reagents—would have to establish a compromise between two fundamental aspects. The sample–reagent intermixing would have to be achieved rapidly in order to provide a suitable reaction time prior to detection and the reaction zone homogenisation would have to be, to a great extent, independent of the coil length since better sensitivity is usually attained with a short reaction/dispersion coil.

The characteristics of the recently proposed multi-pumping approach [9] seemed ideal to fulfil these requirements. The generated pulsed flow exhibited a more effective mixing capacity comparatively to the typical laminar flow of conventional continuous flow systems and a lower axial dispersion. The solenoid micro-pumps, used as self-propelling monocommuting devices, provided the means for an effective and versatile control of the sample and reagent

insertion processes, which could be advantageously exploited in different sampling strategies, such as single sample volume, binary sampling (intercalation of consecutive sample and reagent aliquots), merging zones (simultaneous insertion of sample and reagent zones), etc. In this way, it would be possible to manipulate the formation of the reaction zone, thus directly controlling its development.

3.1. Sampling strategy, sample volume and pulse frequency

Performance of the binary sampling process was evaluated by alternatively actuating the sample and the MBTH micro-pumps (P_2 and P_3) at a defined pulse frequency that was similar for both pumps. The established reaction zone was then pushed towards detection by the carrier solution Ce(IV) by repeated actuation of P_1. The number of pulses plus the pulse volume determined the inserted sample volume. After varying the number of sample and reagent pulses within 2 and 22 (corresponding to 16–176 μl volume for a 8 μl pulse volume) at a frequency of 120 min^{-1}, it was observed that the analytical signal increased until 14 sample pulses and then very slightly decreased due to the appearance of double peaks emphasising the poor reaction zone homogenisation (Fig. 2). The 50 cm reaction coil (corresponding to an inner volume of ca. 250 μl) did not provide adequate sample–reagent intermixing for sample volumes higher than 112 μl. Considering that the intercalated reagent volume contributed to the total volume of the sample zone, a sample volume of 112 μl corresponded effectively to a reaction zone of 224 μl.

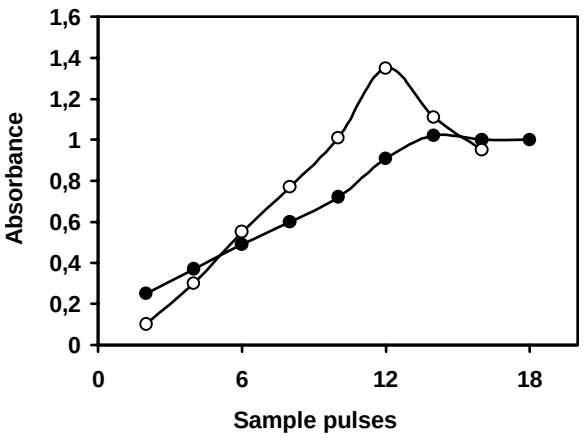

Fig. 2. Influence of the number of sample pulses for distinct sampling strategies: (●) binary sampling; (○) merging zones. Figure refers to the intercalation (binary sampling) or merging (merging zones) of sample and MBTH pulses.

Regarding length of the reaction coil, it was evaluated by using 10 sample and reagent pulses. A slight decrease in analytical signal was noted as the coil length increased, meaning that the mixing provided by the 50 cm coil was adequate, as it was the related sampling rate.

In flow analysis, sensitivity is often hindered due to the reduced sample residence time inside the main reaction coil, and stopping the main stream when the sample zone was inside it may circumvent this effect. In the present procedure, this possibility was not exploited, as preliminary experiments pointed out that increasing the stop period (0–120 s) did not affect the peak height. This confirmed that, although relatively slow, the involved chemical equilibria were already reached during the sample mean residence time. This is probably a beneficial consequence of the pulsed flow.

Another strategy for sample insertion is the utilisation of single sample volumes, as it happens for instance in flow-injection systems when the sample inside the sampling loop is introduced in a single-channel flow manifold. In the proposed multipumping system, this approach was accomplished simply by actuating the sample micro-pump for a defined number of consecutive pulses. In this situation, the sample was introduced between two identical plugs of MBTH that were delimited by the Ce(IV) solution, the carrier stream. A binary string with the configuration: Ce(IV)/MBTH/sample/MBTH/Ce(IV) was then formed. Analogously to the experiments related to the binary sampling process, influence of the number of sample and MBTH pulses was evaluated. The analytical signal increased with the number of sample pulses but it was lower than that one obtained with the binary sampling for identical sample volume. Moreover, double peaks appeared for lower sample volumes (10 pulses, ca. 80 μl) and baseline became unstable. These results would be improved for longer reaction coil longer but sampling rate would be abridged.

Another strategy for sample insertion, merging zones, could also be accomplished with the proposed

system through the simultaneous actuation of the sample and reagent pumps allowing the combined introduction of sample and reagent aliquots. Similarly to what happened with binary sampling and single sample volumes, increasing the number of sample–reagent pulses improved sensitivity, baseline stability and analytical repeatability. The improvement in sensitivity was noted up to 12 sample pulses (96 μl of sample, 192 μl for reaction zone) and further increase led to the appearance of double peaks (Fig. 2). For higher sample concentrations (>200 mg l^{-1} bromhexine) the double-peak effect appeared for even lower number of pulses. In order to permit the analysis of an extended concentration range, the number of sample pulses was selected as 10. Even considering that double peaks were evident at sample volumes lower than those obtained with binary sampling, what could eventually prefigure a poorer mixing, the peak height was comparatively higher. In a different perspective, it is possible to consider that the dispersion attained with binary sampling was excessive. As the merging zones approach enables lower reagent consumption and higher sample throughput, this sampling strategy was selected.

Pulse frequency related to the reagent carrier stream is the main parameter determining the total flow rate, which could affect the analytical signal. However, only a slight enhancement in sensitivity was noted by decreasing pulse frequency (thus flow rate) until a frequency of about 100 min^{-1}. Beyond this value, sensitivity was almost unaffected by variations in flow rate. As a compromise between sensitivity and sampling rate, a pulse frequency of 120 min^{-1} (corresponding to a pulse time of 0.5 s and a flow rate of about 0.96 ml min^{-1}) was selected for the subsequent experiments.

3.2. MBTH and Ce(IV) concentration

Parameters that markedly affect the magnitude of the analytical signal were MBTH and Ce(IV) concentrations, as well as acidity of the reaction medium. Influence of MBTH concentration was evaluated within 5.0×10^{-4} and 8.0×10^{-3} mol l^{-1}. The analytical signal increased with the MBTH concentration until 4.0×10^{-3} mol l^{-1}, and then markedly decreased. Apparently, an increase in MBTH concentration caused side reactions, which decreased the yield. Consequently, a concentration value of 4.0×10^{-3} mol l^{-1} was selected for posterior experiments. Ce(IV) concentration was tested within 1.2×10^{-3} and 1.0×10^{-2} mol l^{-1}. For lower Ce(IV) concentrations, no analytical signal was obtained whereas for the upper concentration limit, a precipitation occurred. Best analytical signals (without precipitation) were obtained with 7.5×10^{-3} mol l^{-1} Ce(IV). Influence of acidity was evaluated within 0.09 and 1.44 mol l^{-1} H_2SO_4 concentrations in the MBTH and Ce(IV) reagent solutions. With 0.09–0.18 mol l^{-1} H_2SO_4, Ce(IV) solution precipitated. Beyond 0.18 mol l^{-1}, the analytical signal increased with the acidity until 0.36 mol l^{-1} and then approached stabilisation. For concentrations higher than 0.5 mol l^{-1}, the analytical signal was pronouncedly affected by the Schlieren effect [16].

Influence of temperature on the reaction development was investigated by immersing the reaction coil inside a water-bath with the temperature adjusted within 18 and 35 °C. The analytical signal increased with temperature up to 25 °C with no further increase being observed for higher temperatures. It was then decided to operate the system at room temperature (about 25 °C) maintained through air conditioning facilities.

3.3. Analysis of pharmaceutical preparations

The proposed system was then applied to analysis of pharmaceutical preparations with the following operating conditions: 10 pulses for sample insertion (80 μl), simultaneous merging with 10 pulses of 4 mmol l^{-1} MBTH solution, pulse frequency of 120 min^{-1} (corresponding to a flow rate of approximately 0.96 ml min^{-1}) for the 7.5 mmol l^{-1} Ce(IV) carrier solution, 50 cm reaction coil, 25 °C temperature. Under these conditions, a linear working range up to 400 mg l^{-1} bromhexine was obtained. The detection limit based on a 3σ interval was 2.0 mg l^{-1} bromhexine. The analytical curve was represented as $A = 0.0676C + 2.2023$, where A is the peak height in absorbance and C the bromhexine concentration in mg l^{-1}, with a correlation coefficient of >0.995 ($n = 6$). The relative standard deviation of results was <1.5% ($n = 10$).The interference effect of several compounds commonly used as excipients, like sucrose, lactose, galactose, glucose, starch, talc, sodium benzoate and magnesium stearate was assessed.

Table 1
Results obtained in the determination of bromhexine in pharmaceuticals preparations

Sample	Amount declared	Amount found	
		Proposed methodology	Reference methodology
Lisomucin (solution) (mg ml^{-1})	2.0	2.09 ± 0.01	2.03 ± 0.01
Lisomucin (syrup) (mg ml^{-1})	1.6	1.65 ± 0.05	1.59 ± 0.04
Bisolvon (tablets) (mg)	8.0	8.30 ± 0.18	7.98 ± 0.22

Samples containing a fixed amount of bromhexine and increasing concentrations of the excipients (up a 100-fold molar ratio) were analysed by the developed methodology. A compound was considering as non-interfering if the analytical signal variation was ±3% when compared to the one obtained in its absence. No interfering effect was observed under the system operating conditions.

The proposed multi-pumping flow system was evaluated in the analysis of several pharmaceutical dosage forms of commercially available formulations. Sample were analysed in triplicate and results are summarised in Table 1. The results were in agreement with those furnished by the reference procedure, as confirmed by the Student's *t*-test: the estimated *t*-value (1.382) was lower than the tabulated one (4.303) for a confidence level of 95% ($n = 3$). The analytical flow system was robust and stable, with a baseline drift lower than 0.01 absorbance h^{-1}. Sampling rate was about 45 samples h^{-1}. Micro-pumps functioning remained unaltered over a continuous 6-month operation period. Moreover, some of the micro-pumps were already used in previous experiments [9], which confirmed their extended lifetime even considering that they are usually actuated at pulse frequencies higher than 120 min^{-1}.

4. Conclusions

The multi-pumping flow system described constitutes a valuable alternative to other already available methodologies for bromhexine determination. It assures a high degree of automation being as well a simple, low cost and fast analytical procedure. The long-term undisturbed solenoid micro-pumps functioning along with a high operational versatility resulting from the multiple tasks they could be assigned to, like solutions insertion, propelling and mixing, enable the implementation of robust, reliable easily controlled flow networks. Fundamental parameters like sample volume, reagents volume and flow rate are easily adjusted guaranteeing an effective control of reaction zone homogenisation and reaction development. This permits their efficient utilisation as multipurpose compact analytical systems, with low consumptions of both sample and reagents, without requiring physical reconfiguration of the flow manifold.

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