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Multicommuted flow system employing pinch solenoid valves and micro-pumps Spectrophotometric determination of paracetamol in pharmaceutical formulations

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Abstract

A multicommuted spectrophotometric flow-based procedure for the determination of paracetamol in pharmaceutical formulations is proposed. The method is based on the reaction of paracetamol with sodium hypochlorite forming *N*-acetyl-*p*-benzoquinoneimine, which reacts with sodium salicylate in alkaline medium producing a blue indophenol dye that was measured at 640 nm. The flow system was designed employing four pinch solenoid valves and two solenoid micro-pumps, which were assembled aiming to obtain a compact module, resulting in minimization of reagents consumption and waste generation. Aiming to prove the usefulness of flow system an analytical procedure for paracetamol determination in pharmaceutical formulations was developed. To allow accuracy assessment samples were also analyzed using the AOAC reference method. Applying the paired *t*-test between results no significant difference at the 95% confidence level was observed. Other profitable features such as a linear response ranging from 5.0 to 125.0 mg l^{-1} ($R = 0.9992$, $n = 7$), a sampling rate of 60 determinations per hour, a detection limit of 0.4 mg l^{-1} paracetamol, a relative standard deviation of 1.5% ($n = 11$) for a typical sample solution containing 25.0 mg l^{-1} paracetamol, reagent consumption of 1.28 mg sodium hypochlorite and 6.4 mg sodium salicylate per determination were also achieved.

Keywords: Multicommutation; Flow injection analysis; Paracetamol; Pinch valve; Micro-pump; Spectrophotometry

1. Introduction

Paracetamol that is also known as acetaminophen is extensively used as an analgesic and antipyretic agents, which could be obtained in different pharmaceutical formulations. It is widely used as an alternative for patients susceptible to acetylsalicylic acid in treatment of pain and fever. Overdose of paracetamol could cause fatal hepatotoxicity and nephrotoxicity [1].

In this way, analytical methods have been reported for the determination of paracetamol in pharmaceutical formulations employing technique such as chromatography [2–4], voltammetry [5], spectrophotometry [6–8], chemiluminescence [9], spectrofluorimetry [10,11]. Procedures based on flow injection analysis (FIA) [12–17] and sequential injection analysis (SIA) [18,19] have been also proposed.

The continuous pumping of the reagents solutions is a feature due to classical FIA process [20], thus presenting as a consequence high reagent consumption and generating also large volume of waste. These disadvantages could be easily minimized employing flow manifolds based multicommutation [21,22]. The flow analysis module based on multicommutation approach involves the use of a set of solenoid valves nested to work as an independent commutation unit. This feature has been explored to design reliable and versatile flow system using low cost devices [23].

The solenoid micro-pumps controlled by microcomputer was introduced 3 years ago as a new device for fluid propelling in flow analysis system [24]. It was demonstrated that this device could replace peristaltic pump without sacrificing the overall performance of the procedure. The electronic hardware required

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to drive the micro-pump is similar to that usually employed in multicommuted flow system [21,22].

The solenoid micro-pump flow systems have been designed to employ one pumping device for each solution used in the analytical procedure [25,26]. In these cases, the pumping devices have installed near of the solution storing vessel and the flow system worked with inner pressure higher than the atmosphere. An interesting feature of the multicommutation flow process is its ability to handle two or more solutions using a single pumping channel [23,27]. In this case, the peristaltic pump had been installed between the detector and waste-storing vessel, sample and reagent solutions flowed through the flow network by suction, therefore the inner pressure was lower than the atmosphere.

Analytical procedures based on multicommutation process have been implement employing flow system manifold designed utilizing three-way solenoid valves as the core devices [23,27,28]. Pinch solenoid valves present a functioning structure more simple than that of the three-way solenoid valve, nevertheless it has been rarely employed to develop analytical procedures [29,30].

In this work we intended to design a flow network making use of pinch solenoid valves and solenoid micro-pumps to assemble a flow system based on multicommutation process, because, in our knowledge, it could provide an association not yet employed for analytical purpose. To prove the effectiveness of the flow system, the development of a spectrophotometric procedure for paracetamol determination in pharmaceutical formulations was selected. The method was based on the reaction of paracetamol with sodium hypochlorite forming *N*-acetyl-*p*-benzoquinoneimine, which reacts with sodium salicylate in alkaline medium producing a blue indophenol dye that presents maximum light absorption at $\lambda = 640$ nm [16,31].

2. Experimental

2.1. Reagents and samples

All solutions were prepared with analytical-grade chemicals. Purified water (resistivity > 18.2 MΩ cm^{-1}) was used throughout.

A 500.0 mg l^{-1} paracetamol ($C_8H_9NO_2$) stock solution was prepared by dissolving an appropriated amount of reagent (Labsynth, Brazil) in a 0.05 mol l^{-1} HCl solution. Working standard solutions with concentration ranging from 5.0 to 125.0 mg l^{-1} paracetamol were prepared daily by dilution with a 0.05 mol l^{-1} HCl solution. A 0.2% (w/v) sodium hypochlorite solution was prepared by dilution with water using a 2.0% (w/v) commercial bleach solution that was standardized using the iodometric titration method. A 5.0% (w/v) sodium salicylate solution was prepared by dissolving an appropriated amount of solid (Labsynth, Brazil) in a 0.5 mol l^{-1} NaOH solution. All solutions were stored in amber bottles.

The potential interfering such as sucrose, glucose, lactose, ethylene glycol, saccharin, caffeine, cyclamate, sorbitol, amide, benzoic acid, citric acid, acetylsalicylic acid and ascorbic acid were evaluated preparing a 25.0 mg l^{-1} paracetamol standard solutions plus the investigated substance. It was assured that its concentration in the solutions was 100-times higher than paracetamol concentration.

Tablet of samples were purchased from a local pharmacy and were prepared as described by Aniceto and Fatibello-Filho [16]. After solubilization, solutions were stored in amber bottles in order to prevent light oxidation.

2.2. Apparatus

Equipment set up comprised four pinch solenoid valves (three normally closed, Nresearch 161P011 and one normally open, Nresearch 161P021); two solenoid micro-pumps (Biochem, 090SP, nominal volume of 8 μl per stroke); a Pentium microcomputer; an optical-fiber CCD-array spectrophotometer (Ocean Optics, PC 1000) equipped with a Hellma flow cell (80-μl inner volume, 10-mm optical pathway); reaction coils and flow lines of PTFE tubing (0.8 mm i.d.); joint devices machined in acrylic; a 12 V power supply; an electronic control interface [26].

The power supply was employed to feed pinch solenoid valves and solenoid micro-pumps. The control interface was coupled to the output print port of the microcomputer to allow that the control of the flow system was performed by software, which was written in Visual Basic 3.0 with facilities to carry out also data acquisition.

2.3. Flow system manifold

The flow system manifold to implement the procedures based on multicommutation was designed employing pinch solenoid valves to handle solution and solenoid micro-pumps for pumping purpose and the flow diagram is depicted in Fig. 1. In this configuration valves and micro-pumps are switched OFF, thus no solution is flowing through the analytical path.

The system working sequence is described in Table 1 and when the software was started the microcomputer requested the values of the variables (pulse, cycle), which were supplied through the microcomputer keyboard. Afterwards, the analytical run was carried out by microcomputer sending electric pulses through the control interface to switch ON/OFF the micro-pumps P_1 and P_2 following the switching pattern showed in the pumps timing course (Fig. 1). The insertion of sample and reagent solutions into the reaction coils B_1 and B_2 was implemented exploiting the binary sampling approach [21]. In this sense, the pinch solenoid valves V_1, V_3 and V_4 were switched ON/OFF sequentially to insert slugs of sample (S) and reagents solutions (R_1, R_2) into the reaction coils B_1 and B_2. As it is depicted in the valves timing course (Fig. 1), V_2 was maintained switched ON, therefore the stream of the carrier solution (Cs) was maintained halt while the sampling step (St) was carried out. Because pinch solenoid valves V_1, V_3 and V_4 were switched ON/OFF sequentially, the reaction coil B_1 was loaded with a solution string comprising slugs of sample (S) in tandem with slugs of reagent solution (R_1). Under this condition, the mix comprising slugs of sample (S) and reagent solution (R_1) formed into the reaction coil B_1 was inserted into the reaction coil B_2 in tandem with slugs of reagent solution (R_2).

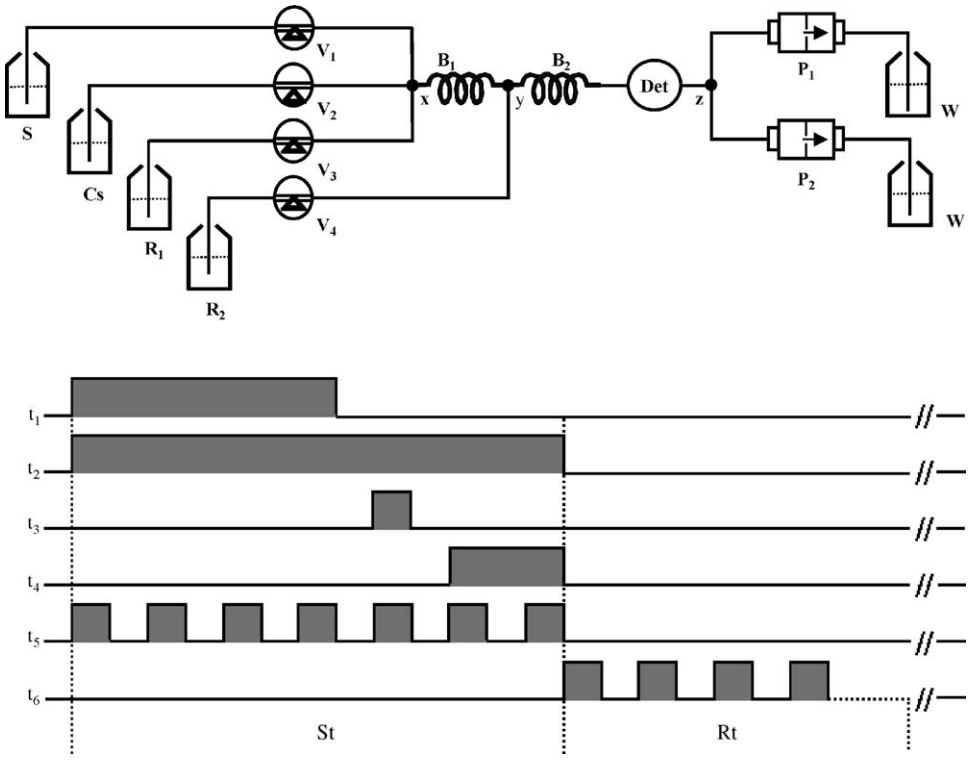

Fig. 1. Flow diagram of the multicommuted flow system. S: sample; Cs: carrier solution, HCl 0.05 mol l^{-1}; R_1 = 0.2% (w/v) sodium hypochlorite solution; R_2 = 5.0% (w/v) sodium salicylate solution; V_1, V_3 and V_4: pinch solenoid valve, normally closed; V_2: pinch solenoid valve, normally open; P_1 and P_2: solenoid micro-pumps; *x*, *y* and *z*: joint devices machined in acrylic; B_1 and B_2: polyethylene reactor coils, 4.0 and 75.0 cm length, respectively, 0.8 mm i.d.; W: waste; Det: spectrophotometric detector at 640 and 780 nm; t_1, t_2, t_3, t_4, t_5 and t_6: timing course diagram for switching ON/OFF pinch valves V_1, V_2, V_3 and V_4 and micro-pumps P_1 and P_2, respectively; St and Rt: sampling step and signal reading step, respectively. The shadow areas beneath of lines indicate that the corresponding pinch solenoid valve or solenoid micro-pumps were switched ON.

The reaction between the paracetamol and hypochlorite occurred while the sample zone was displaced through the reaction coil B_1. Reaction with salicylate to form the compound monitored at 640 nm occurred while sample zone was displaced trough the reaction coil B_2 towards the detector (Det). As it is depicted in the devices switching timing course diagram (t_1, t_2, t_3, t_4, t_5 and t_6), the steps concerning to insertion of sample and reagents solutions were carried out switching at the same time the corresponding pinch valves and the micro-pump P_1.

After sampling step (St), the signal reading step (Rt) was carried out, which was done by switching ON/OFF the micro-pump P_2. Under this condition, the carrier solution (Cs) was propelled through the flow system, thus displacing the sample zone through the reaction coil (B_2) towards the detector (Det), where the indophenol dye compound was monitored. The software was designed to carry out data acquisition at two wavelengths (640 and 780 nm) aiming to compensate some noise that could occur. The micro-pumps pulsation frequency was maintained at 5 Hz and it was settled equal time interval (0.1 s) to maintain it switched ON and OFF. While data acquisition was performed a plot of the results was displayed on the microcomputer screen as a time function to allow its visualization in real time. The results were saved as an ASCII file to permit further treatment.

The sampling step (St) depicted in Fig. 1 comprised a single sampling cycle and its solutions inserting pattern was constituted of four pump pulses for sample solution (S), one for reagent solution (R_1) and two for reagent solution (R_2). In this sense, the volumes of the solutions inserted into the analytical path were 32 μl (sample), 8 μl (sodium hypochlorite) and 16 μl (sodium salicylate). The number of the sampling cycles per sampling step (St) and also the pumping pulse pattern were varied in order to find the better operational condition of the proposed analytical procedure.

Once the better operational conditions were established a set of paracetamol samples was analyzed in order prove the useful-

Table 1
Operational sequence of the multicommuted flow system for paracetamol determination

Step	Event	V_1	V_2	V_3	V_4	P_1	P_2	Pulses	Cycles
	Inserting sample (S)	1	1	0	0	1/0	0	4	
1	and reagent solutions	0	1	1	0	1/0	0	1	8
	(R_1, R_2)	0	1	0	1	1/0	0	2	
2	Sample zone displacing and signal reading	0	0	0	0	0	1/0	180	–

ness of the proposed procedure. To allow accuracy assessment samples were also analyzed employing the AOAC reference method [32].

3. Results and discussion

3.1. Micro-pump performance

Preliminary experiments were carried out in order to evaluate the micro-pump capacity to displace solution by suction through the analytical path. This assay was delineated considering that this arrangement was not yet used. In this case, the micro-pump was installed between the detector (Det) and waste-storing vessel (W) as it is shown in Fig. 1. Initially, it was verified that the micro-pump delivered 8 μl of solution per stroke as it was indicated in the manufacturing data sheet. Nevertheless, after working continuously during 1 h, a decrease of the delivered solution volume occurred, thus affecting unfavorably the precision of the measurements. We think that the micro-pump warming could cause this effect. Aiming to overcome this drawback a second micro-pump (P_2) was incorporated in the flow system module and a pumping shared strategy was implemented. Under the settled conditions, the micro-pump P_1 was drove to insert sample and reagent solutions aliquots into the reactions coils B_1 and B_2. Afterwards, this micro-pump was maintained OFF and the micro-pump P_2 was switched ON/OFF several times to displace the sample zone through the reaction coil (B_2) towards waste (W). After the signal reading step (Rt), other analytical run was started switching the micro-pump P_1. Operating the system continuously during 4 h using a paracetamol standard solution, no significant variation in signal magnitude and precision were observed, thus indicating that the trouble was solved.

3.2. Effect of the manifold operational parameters

The micro-pump delivered per stroke a solution volume of 8 μl, thus considering that this value was a constant parameter the better ratio between volumes of sample and hypochlorite solution to generate better signal was investigated. In the first case, it was done by varying the number of pumping pulse (P_1) from 1 up to 6 maintaining valve V_1 switched ON and the second one, the number of the pumping pulses varied from 1 up to 4 maintaining valve V_3 switched ON. While these assays were performed, a single pumping pulse was settled to insert a slug of the reagent solution (R_2). To find the appropriated volume of the reagent solution R_2, the pulses number of micro-pump P_1 was varied from 1 to 10 maintaining valves V_4 switched ON. Under the condition, aliquots of sample and hypochlorite solution were mixed into the reaction coil B_1 where paracetamol was oxidized. Because the reagent amount in this step could affect the sensitivity of the procedure, experiments were carried out varying the ratio between aliquots of sample and reagent solution yielding the results showed in Fig. 2. Considering the signal magnitude as the evaluated parameter we can observe that better result was achieved when the ratio between sample and hypochlorite solution was 4:1 (curve a). Since each micro-

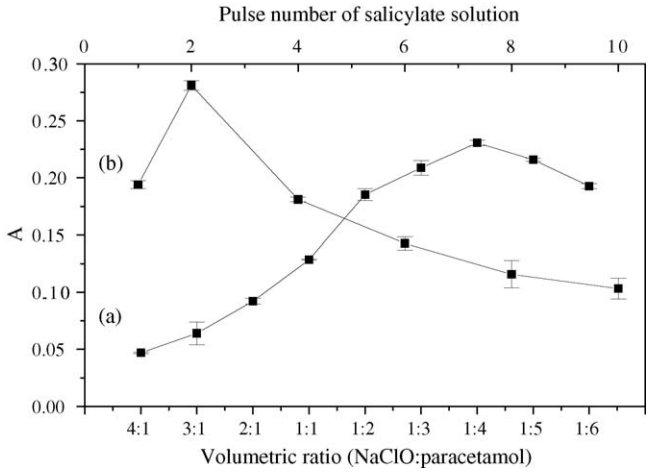

Fig. 2. Effects concerning to the ratios between volumes of hypochlorite, paracetamol and salicylate solutions: (a) hypochlorite/paracetamol volume ratio; (b) pulses number of salicylate solution (upper scale).

pump delivered a solution volume of 8 μl per stroke, the solution volumes per sampling cycle were 32 μl and 8 μl of sample and hypochlorite solutions, respectively. In this assays the sampling step (St) comprised 10 sampling cycles.

In the assays commented above, salicylate solution was inserted into the reaction coil B_2 settling one pulse per sampling cycle, therefore its total volume into the sample bulk was 80 μl. Experiments to find the better volume of sodium salicylate solution to improve sensitivity were performed varying the pulse number of the micro-pump P_1 from 1 to 10 maintaining valve V_4 switched ON. In Fig. 2 (curve b) it is shown that better result was achieved when micro-pump P_1 was switched ON/OFF two times per sampling cycle. The decrease in signal observed when sodium salicylate solution volume was higher than 16 μl (two micro-pump pulse) could be attributed to dilution. In these assays, sample and hypochlorite slugs inserted into the reaction coil B_2 per sampling cycles was maintained at 32 μl and 8 μl, respectively, while the volume of the sodium salicylate solution aliquot varied from 8 to 80 μl. Considering that sensitivity was the subject, the ratio 4:1:2 (sample:hypochlorite:salicylate) per sampling cycles between the slugs of the solutions was selected.

Because the length of the reactor coils could affect the reaction development and sensitivity experiments to define their dimensions were performed. The lengths of the reaction coils B_1 and B_2 were varied from 0 to 10 cm and from 10 to 150 cm, respectively, thus the inner volumes of coils B_1 and B_2 varied from 0 to 50 μl and from 50 to 750 μl, respectively. The volume of the sample zone was maintained at 560 μl. The signal magnitude related to the dimension of the reaction coil B_1 presented similar value when its length was 4, 6 or 10 cm, therefore indicating that mixing between solutions was efficient and the involved reaction was very fast. The results related to the length of the reaction coil B_2 showed that analytical signal increased up to 75 cm. Considering these results and aiming to save time the lengths of coils B_1 and B_2 were fixed at 4 and 75 cm, respectively.

The aforementioned assays were performed settling 10 sampling cycles, then to verify the effect of the sample volume on the analytical signal, experiments were carried out varying the

number of sampling cycles from 1 to 16. It was observed that signal increased up to 8 sampling cycles and no significant increment was observed when the number of sampling cycles was higher than 8. This effect could be explained considering that the inner volume of the reaction coil B_2 was 375 μl and the volume of sample zone was 448 μl (256 μl of sample solution, 64 μl of sodium hypochlorite and 128 μl sodium salicylate solution), therefore dispersion effect was minimized. Considering the results, the best operational conditions selected are showed in Table 1. The sequence of events was controlled according to the pinch solenoid valves and micro-pump switching pattern depicted in Fig. 1.

3.3. Effect of the reagents concentration

The assays described above were performed using a 25.0 mg l^{-1} paracetamol standard solution, a 0.3% (w/v) sodium hypochlorite solution (R_1) and a 8.0% (w/v) sodium salicylate solution (R_2). Additional assays concerning to reagent concentration were carried out varying the sodium hypochlorite concentration from 0.5 to 5.0% (v/v) and sodium salicylate from 3.0 to 10.0% (w/v). Since alkalinity of the medium could effect sensitivity experiments were performed varying the sodium hydroxide concentration from 0.1 up to 1.0 mol l^{-1}. The experiments were performed using a set of paracetamol standard solutions with concentration ranging from 5.0 to 125.0 mg l^{-1}. Considering linearity ($R = 0.9992$) and sensitivity as the main parameters better result was obtained when a 0.2% (w/v) sodium hypochlorite solution and a 5.0% (w/v) sodium salicylate solution were used.

The reaction occurred in alkaline medium, thus aiming to evaluate its effect on sensitivity, assays varying sodium hydroxide concentration from 0.1 to 1.0 mol l^{-1} were carried out. The best results were achieved when concentration was within the range of 0.4–0.6 mol l^{-1} sodium hydroxide, thus a 0.5 mol l^{-1} sodium hydroxide solution was selected for the further experiments. Similar effect was also reported by Aniceto and Fatibello-Filho [16].

3.4. Potential interfering

Compounds such as glucose, lactose, ethylene glycol, saccharin, caffeine, cyclamate, sorbitol, amide, benzoic acid, citric acid, acetylsalicylic acid and ascorbic acid are usually found in the paracetamol formulations. Considering that these substances could cause interference, a set of assays was performed in order to establish the tolerance limit of the propose method. It was considered as a tolerable limit the substance concentration that could cause an absorbance variation around 5% comparing with measurement obtained using a paracetamol standard solution without addition of the assayed substance. Analyzing the results showed in Table 2 we can observe that the least tolerance occurred for caffeine and ascorbic acid. In these cases, tolerance limit was 10 times higher than the paracetamol concentration, which could be considered enough to use the proposed procedure for paracetamol determination in pharmaceutical formulations. The results commented in this section are in accordance with results reported in the consulted references [12,16,17].

Table 2
Tolerance limit for different chemical species obtained using a 25.0 mg l^{-1} paracetamol standard solution

Foreign species	Tolerance limit
Sucrose, glucose, lactose, ethylene glycol, benzoic acid	100[a]
Citric acid, sorbitol, amide, acetylsalicylic acid	50
Saccharin, cyclamate	25
Ascorbic acid, caffeine	10

Data are interferent/analyte concentration ratios, in w/w. A foreign species was considered as non-interferent when its effect on the analytical signal is lower than 5%.
[a] Maximum tested ratio.

3.5. Figures of merit and accuracy

The analytical curve was linear within the range of 5.0–125.0 mg l^{-1} paracetamol presenting a typical equation $y = (0.018 \pm 0.005) + (0.0093 \pm 0.0002)x$, ($R = 0.9992$, $n = 7$), where y = absorbance and x = mg l^{-1} paracetamol. The relative standard deviation of 1.5% ($n = 11$) that was estimated processing a typical sample containing 25.0 mg l^{-1} paracetamol. Other favorable characteristics such as reagents consumption of 1.28 mg sodium hypochlorite and 6.4 mg of sodium salicylate per determination, a sampling throughput of 60 determinations per hour, a detection limit of 0.4 mg l^{-1} paracetamol estimated as it was suggested by IUPAC [33] (three times of the blanck standard deviation divided by the slope of the linear equation), and a quantification limit of 1.3 mg l^{-1} paracetamol estimated considering the 10 σ criterion. Recoveries ranging from 92 to 111% were verified for samples spiked with 25.0 and 50.0 mg l^{-1} paracetamol.

Table 3
Analytical performance comparison

Parameters	Proposed procedure	Ref. [12]	Ref. [13]	Ref. [14]	Ref. [15]	Ref. [16]	Ref. [17]	Ref. [19]
Linear range (mg l^{-1})	5.0–125.0	0.25–30.0	0.8–100.0	0–24.0	0.6–20.0	1.0–100.0	180.0–300.0	Up to 60.0
R.S.D. (%)	1.5	0.4	0.5	1.9	1.8	1.0	0.7	1.2
Limit of detection (mg l^{-1})	0.4	–	0.8	0.2	0.2	0.5	–	0.25
Sample consumption (ml)[a]	0.256	0.150	0.05	0.5	0.1	0.25	0.05	0.185
Reagents consumption (ml)[a]	0.192	5.55	0.23	1.1	1.8	1.64	3.6	0.247
Waste generation (ml)[a]	1.9	5.7	0.28	1.6	5.5	3.45	3.65	4.1
Throughput (h^{-1})	60	26	360	70	20	80	120	27

[a] Sample and reagents consumptions and waste generation corresponding to one analysis.

Table 4
Paracetamol concentration in pharmaceutical formulations as determined by the proposed and reference (AOAC) [32] methods

Samples[a]	Nominal amount	Amount found (mg tablet^{-1})[b]	
		Proposed method	AOAC [32]
Torsilax®	300	312 ± 11	305 ± 6
Cibalena a®	150	132 ± 2	137 ± 5
Excedrin®	500	515 ± 4	506 ± 7
Resprin®	400	434 ± 9	411 ± 10
Tylenol®	750	757 ± 6	730 ± 9
Saridon®	250	255 ± 2	245 ± 5
Vick pyrena®	500	520 ± 5	510 ± 6
Resfenol®	400	388 ± 7	398 ± 5
Sonridor®	500	515 ± 8	505 ± 4

[a] Other compounds present. Torsilax®: caffeine, sodium dichlorophenol, carisoprodol. Cibalena a®: acetylsalicylic acid, caffeine. Excedrin®: caffeine. Resprin®: phenilefrina chloridrate, pentoxyverine citrate, carbinoxamine maleate. Saridon®: caffeine, propyphenazone. Vick pyrena®: ascorbic acid, citric acid, sugar, sodium saccharin, aspartame, D&C no. 10 yellow. Resfenol®: chlorphenamine maleate, phenilefrina chloridrate. Sonridor®: sorbitol, caffeine, sodium saccharin, sodium bicarbonate, povidone, sodium sulphate lauryl, dimeticonae, citric acid, sodium carbonate, caffeine.

[b] Results average of three consecutive determination ± the corresponding standard deviation.

The main parameters of the proposed analytical procedure and other flow-based procedures for paracetamol spectrophotometric determination are summarized in Table 3, where we can see that the overall performance of the proposed procedure could be considered favorable comparing with the cited procedures, therefore indicating that the proposed flow system configuration is reliable.

After settling the best operational condition and intending to prove the feasibility of the proposed system, a set of pharmaceutical formulations was analyzed yielding the results showed in Table 4. Comparison of data obtained employing the proposed procedure with those obtained using the AOAC reference method [32] yielded the linear equation $y = (3 \pm 7) + (0.98 \pm 0.02)x$ with regression coefficient of 0.997. The t-values calculated comparing intercept and slope with 0 and 1 were 0.257 and 1.567, respectively. These values were lower than the theoretical t-value (2.000) for 95% probability and 52 degrees of freedom the comparability of the two populations, indicating that both procedures provide statistically comparable results.

4. Conclusions

The flow system network designed associating solenoid pinch valve and solenoid micro-pumps is simple, ease for operation, very versatile and robust providing facilities for automation.

The system provided long-term stability that was proved by working continuously during 4 h. No significant variation in response range and precision of measurements was observed. This assay was repeated several days and similar performance was always observed.

The solenoid pinch valve and solenoid micro-pump could be view as a commuting device and in this sense a set of them can be easily assembled to comprise an active hardware to permit the handling of reagent solutions controlled by software. Its could became an alternative to the use of peristaltic pump with favorable impact in the cost.

The low reagent consumption resulting also in low waste generation could be considered as an additional advantage afforded by the proposed flow system.

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