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Reversed Flow Injection System for the Spectrophotometric Determination of Cetylpyridinium Chloride in Pharmaceutical Products with Eriochrome Black T in Triton X-100 Medium

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Abstract

This paper reports the development of a reversed FIA system for the spectrophotometric determination of cetylpyridinium chloride (CPC) in pharmaceutical products. The method is based on the reaction between CPC and Eriochrome Black T (EBT) with the formation of a product with strong absorption at 645 nm. At optimized conditions the reversed FIA system was able to process 40 samples per hour with a detection limit of 0.24 μg mL^{-1} and a quantification limit of 0.80 μg mL^{-1}. Recovery rates between 92 and 107% were obtained in the analysis of pharmaceutical products using the proposed methodology.

Keywords Flow injection analysis, cetylpyridinium chloride, spectrophotometry.

1. Introduction

The use of cationic surfactants is widespread in several classes of manufactured products including disinfectants, textile softeners, cosmetics and pharmaceuticals. One of the most important cationic surfactants is the cetylpyridinium chloride (CPC), which is largely employed as antibacterial agent in the treatment and prevention of human infections due to action of Gram-negative bacteria [1]. Also, it has been demonstrated that CPC is effective in reducing of bacteria in several food products like beef [1], poultry [2] and apples [3].

In the pharmaceutical industry, the CPC is principally employed in the formulation of mouthwash solutions used for daily disinfection of oral cavity and in medicines employed for the treatment of soft throat infections. In most countries, such pharmaceuticals can be easily purchased without medical indication, which has leaded to an indiscriminate use of these products by the population. Once some problems can be observed after abusive usage of products containing CPC like teeth darkening and irritation of throat and oral mucosa [4], the monitoring of CPC concentration in these pharmaceuticals is a very important task in terms of public health.

Several methodologies were already reported for the determination of cationic surfactants, in a general way, in different kinds of samples [5-9]. Among these methodologies, spectrophotometric ones were preferred probably due to the simplicity and the low-cost associated to this technique. In turn, only few papers described the development of methods for specific determination of CPC, which was performed by different analytical techniques like liquid chromatography [10-12], gas chromatography [13], potentiometry [14-16], voltammetry [17] and spectrophotometry [18].

Since its creation, flow injection analysis has improved several applications in all branches of analytical chemistry. Due to its inherent characteristics, FIA has been used to automate batch analytical procedures with some advantages, such as high analytical throughput, better precision and low reagents and sample consumption [19-20]. Even with these various advantages, no other work reporting the spectrophotometric flow injection analysis of CPC could be found in the current literature.

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Also, the use of Eriochrome Black T (EBT) was never reported for spectrophotometric determination of CPC in any kind of sample. So, the aim of this work was to optimize a reversed FIA system for the quantification of CPC in pharmaceutical products. The reaction between cetylpyridinium ion and Erichrome Black T (EBT) in an organized medium was explored for spectrophotometric determination and the system was optimized in relation to chemical and flow variables. A reversed FIA approach was employed in order to avoid the increase of baseline noise due to absorbance verified by EBT in the wavelength chosen for measurements.

2. Experimental

2.1. Apparatus

Spectra were recorded with Femto 800 XI (São Paulo, Brazil) scanning UV-Vis spectrophotometer and a conventional quartz cuvette with 10-mm optical path.

The FIA set-up consisted of a Femto 700 Plus (São Paulo, Brazil) UV–vis spectrophotometer equipped with a Hellma (Jamaica, NY, USA) standard glass flow-cell of 80 μL internal volume and 10 mm optical path. The instrument was set at 645 nm for all absorbance measurements. A Gilson Minipuls 3 (Villies-le-Bel, France) peristaltic pump, furnished with flexible PVC tubes (Tygon®), was used to propel all solutions and an Upchurch V 452 six-port valve (Oak Harbor, WA, USA) was employed to inject the reagents mixture into the system. The manifold was built up with PTFE tubes with 0.8 mm bore and PEEK plastic connections.

All pH measurements were carried out with a Digimed pHmeter (São Paulo, Brazil), model DM-22, equipped with a combined glass electrode (Ag/AgCl as reference), model CV-1, also supplied by Digimed.

2.2. Reagents and solutions

All solutions were prepared with analytical grade reagents and high purity deionized water, obtained in a Simplicity Milli-Q(Millipore, Saint Quentin Yvelines, France) water purification system.

A 1000 mg L^{-1} stock standard solution of cetylpyridinium

chloride (CPC) was prepared by dissolution of 0.2740 g of the reagent ($C_{21}H_{38}Cl.H_2O$, Acros Organics, St. Loius, USA) in exactly 250 mL water. This solution was stable for one week, at least. All standard CPC solutions were prepared by adequate dilution of stock solution with water just before use.

A 1% m/v Triton X-100 solution was prepared by simple dissolution of 1 g of the reagent (Vetec, Rio de Janeiro, Brazil) in exactly 100 mL water.

A reagent solution (R_1) was prepared by dissolution of 0.0172 g of Eriochrome Black T (EBT) reagent, furnished by Vetec (Rio de Janeiro, Brazil,) in around 100 mL water. Afterwards, 25 mL of the 1% m/v Triton X-100 solution and 5 mL of the 1000 mg L^{-1} stock solution of CPC were added, the mixture was transferred to a 500 mL volumetric flask and the volume was filled up to the mark. The final concentrations of EBT, Triton X-100 and CPC in the final solution were 7.5 x 10^{-5} mol L^{-1}, 0.05% m/v and 10 mg L^{-1}, respectively.

The acetate buffer solution (R_2) with total concentration of 0.10 mol L^{-1} was prepared by dissolving 3,40 g of solid sodium acetate ($C_2H_3O_2Na.3H_2O$) (Vetec, Rio de Janeiro, Brazil) in around 200 mL water. After this, a 2 mol L^{-1} HCl solution was added until pH achieved 5.5. Then, the solution was transferred to a 250 mL volumetric flask and the volume was completed to the mark with water. The final pH of the solution was then measured and did not vary more than 0.1 pH unity.

2.3. Reversed FIA system operation

A schematic diagram of the reversed FIA system is depicted in Fig. 1. In the set-up, reagent solution of EBT containing Triton X-100 and CPC is directly pumped to the injection valve, loading the loop (375 µL). The excess of reagent mixture is discarded, flowing directly to the waste flask. At the same time, the carrier stream (water, 1.4 mL min^{-1}) flows through other way in the valve, reaching the confluence point, where it merges with the mixture of sample (1.9 mL min^{-1}) and acetate buffer solution (pH 5.5 and 1.0 mL min^{-1}). The solution obtained is then driven to the reaction coil (50 cm, 250 µL) and, after this, to the flow-cell positioned in the optical path of the spectrophotometer (645 nm). At this time, the baseline is established. Afterwards, the injection valve is switched and the carrier stream drifts the reagent plug to the confluence point, where it merges with the sample plus buffer stream. The mixture flows to the reaction coil, where reaction between CPC and EBT takes place. Then, the analytical zone (sample plus reagents) reaches the spectrophotometer, yielding a transient signal of absorbance, which increases with the increase of CPC concentration in the sample. Peak height was always used as quantitative variable. It is important to remark that this assembly was used in order to avoid the establishment of baseline when EBT solution was passing through detector, which caused instability in the signals due to the absorption of EBT alone in the wavelength chosen for the measurements.

2.4. Sample preparation

Mouthwash solution samples were purchased in the local market. They did not receive any special treatment and were used after proper dilution to fit linear portion of the analytical curve.

Two tablets for throat infection treatment were also analyzed. They were also purchased in the local market. These samples were prepared by simple dissolution of the tablet in around 100 mL water, under agitation. After total dissolution of the sample, the resultant solution was transferred to a 250 mL volumetric flask and the volume was completed to the mark. The final solution was then injected directly into the FIA system.

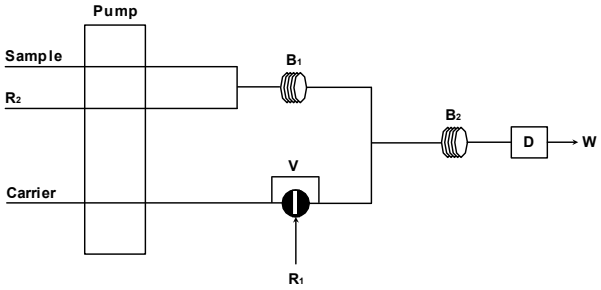

Fig. 1 Reversed flow injection analysis system for the spectrophotometric determination of CPC in pharmaceutical products with EBT. S = sample (1.9 mL min^{-1}), R_2 = buffer solution (pH 5.5, acetate system, 0.10 mol L^{-1} total concentration) containing 10 mg L^{-1} CPC (1.0 mL min^{-1}), R_1 = reagent solution (7.5 x 10^{-5} mol L^{-1} EBT containing 0.05% m/v Triton X-100), C = carrier fluid (water, 1.4 mL min^{-1}), B_1 = mixing coil (100 cm, 500 µL), B_2 = reaction coil (50 cm, 250 µL), V = injection valve with a 375 µL loop, P = peristaltic pump, D = detector (spectrophotometer set at 645 nm) and W = waste.

3. Results and discussion

This work was carried out taking into consideration two steps: (1) initial evaluation of reaction in batch mode and, (2) assembling and optimization of the flow injection system for fast and selective determination of CPC in mouthwash solutions and tablets employed in the treatment of soft throat infections.

The first step comprised the evaluation of the reaction between EBT and CPC in terms of chemical conditions and its characterization by using Job's method. The second step was completed by optimizing chemical and flow variables of the FIA system employing an univariate method. Also, a study about possible interferents was performed and the analytical characteristics of the system were determined.

3.1. Initial evaluation of the reaction

The first experiment performed in this work was related to the evaluation of the reaction between EBT and CPC. For this purpose, spectra of solutions of EBT in the presence and absence of CPC, without pH adjustment, were recorded in the range of 420 – 800 nm. The spectra obtained are shown in Fig. 2.

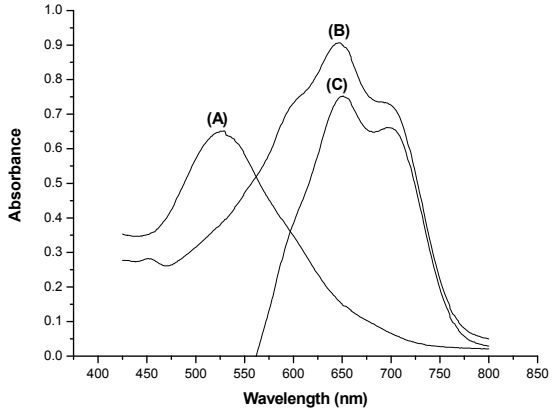

Fig. 2 Spectra of EBT solutions (1.0 x 10^{-4} mol L^{-1}) in the absence (A) and presence (B) of CPC (25 mg L^{-1}). It is also shown the spectrum obtained by difference (C). No adjustment of the pH was performed.

As it can be seen in Fig. 2, the addition of CPC to the EBT solution caused a strong modification in the spectrum obtained. The spectrum of EBT solution, as expected, presented a characteristic band with maximum located at 525 nm, which corresponds to the absorbance of the predominant specie of EBT in solution. After addition of CPC to EBT solution, the spectrum changed and then presented a strong absorption band with maximum at 645 nm. At same time, a strong reduction of the band at 525 nm was noted, indicating that the EBT was consumed and another specie, derived from association between EBT and CPC, was formed. It is important to remark that the pH of solutions did not varied significantly, changing from 6.1 (EBT alone) to 5.9 (EBT in the presence of CPC).

The reaction product observed in the solution probably was an ion-association specie yielded from the interaction between the cationic surfactant CPC and the monodissociated anionic EBT specie (H_2L^-). According to Reeves et al. [21] this association is common as the electrostatic attraction between the oppositely charged ions brings them into close proximity. Similar association between other cationic surfactants and anionic dyes were already reported in the literature [22-24].

In order to verify the stoichiometry of the reaction between CPC and EBT, the Job's method [22] was applied. In this experiment, the sum of the molar concentrations of CPC and EBT were kept constant, the ratio of the concentrations was varied and the absorbances of the mixtures were measured at 645 nm against a convenient blank solution prepared for each point of the experiment. All solutions were buffered at pH 6 using acetate buffer with 0.10 mol L^{-1} total concentration, a pH close to those observed in the previous experiment where no adjustment of pH was performed. As can be seen in Fig. 3, the data obtained showed that CPC molar ratio which gave maximum absorbance was 0.5, indicating that it reacts with EBT (in fact with a monodissociated specie, H_2L^-) in a proportion of 1:1 and reinforcing the assumption raised before that an ionic-pair is formed between cationic CPC and anionic EBT specie.

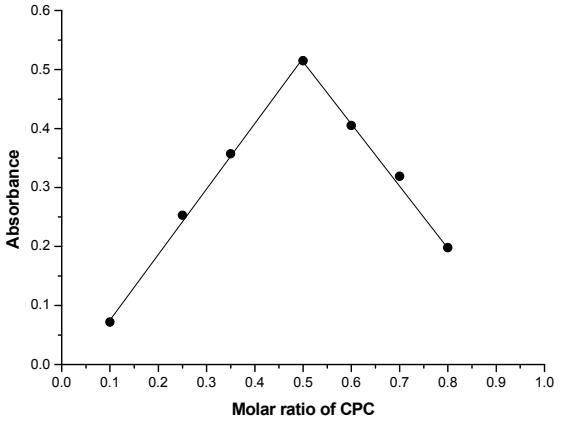

Fig. 3 Application of the Job's method to the reaction between CPC and EBT.

3.2 Optimization of the FIA system

Once verified the reaction between CPC and EBT, with a consequent formation of a product with strong absorption in the visible range (with maximum at 645 nm), a reversed flow injection system was assembled and optimized to perform the determination of CPC in pharmaceutical products. As mentioned previously, a reversed FIA system approach was chosen in order to avoid excessive noise in the baseline due to the absorbance of the own reagent (EBT) in the wavelength used for the measurements.

Optimization strategy of the FIA system was based on an univariate strategy, taking into account chemical and physical variables that could affect the performance of the system in terms of sensitivity. Also, an evaluation of possible interferents was carried out.

3.3. Optimization of the Chemical Variables

The first variable optimized was the pH of the reaction medium. This parameter assumes fundamental importance in the present case because the reagent, EBT, behaves as a weak acid in aqueous solutions. In order to evaluate the influence of the pH on the analytical signal, buffer solutions with different pH were utilized in R_2 (see Fig. 1) line. The pH was studied in the range of 2 – 12 utilizing different buffer systems (always with 0.10 mol L^{-1} total concentration) according to the range under investigation. A phosphoric acid buffer was employed for pH 2.0 and acetate buffers were employed between 4.0 and 6.0. In the neutral and slightly alkaline ranges (pH between 7.0 and 8.0) phosphate buffers were employed and in the alkaline range (pH 9.0 to 12) borate buffers were used. All experiments were carried out using a CPC standard solution and an EBT solution with 10 mg L^{-1} and 5.0 x 10^{-5} mol^{-1}, respectively. The results obtained in this experiment are shown in Fig. 4.

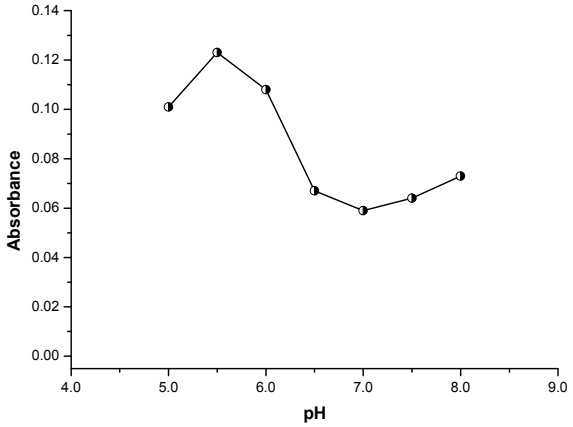

Fig. 4 Influence of pH on the analytical signal of CPC in the reversed flow injection system. CPC = 10 mg L^{-1}; EBT = 5.0 x 10^{-5} mol L^{-1}.

As it can be seen, higher analytical signal (difference between blank and 10 mg L^{-1} CPC solution) was observed for pH values around 5.5, in which there is predominance of the monodissociated EBT specie. This result reinforced the hypothesis that an ion-association specie is formed by simple electrostatic interaction between large CPC ions (cationic) and the monodissociated EBT (anionic). The specie formed was not totally soluble in water medium, forming a colloidal system even in flow conditions.

The effect of total concentration of acetate in buffer solution was also investigated. In this experiment, the total concentration of acetate was tested in the range of 0.01 to 0.20 mol L^{-1}. No significant variation of the signal was observed in the whole range tested. Therefore, the buffer concentration was kept at 0.10 mol^{-1} in order to ensure good buffer capacity and minimum pH variation even for complex samples.

Following the optimization of chemical variables, the influence of the EBT concentration in R_1 solution (injected into the flow system) was investigate. The EBT concentration in this solution directly affects the sensitivity of the methodology since it must provide enough EBT to consume maximum CPC

as possible and then yield highest analytical signals. All tests were performed with a 10 mg L^{-1} standard solution of CPC and the pH was maintained at 5.5 by using an acetate buffer solution with 0.10 mol L^{-1} total concentration in the R_2 line. Also, it is important to notice that a loop with 250 µL was used in all experiments. The EBT concentration in solution was varied in the range of 1.0 x 10^{-5} – 1.0 x 10^{-4} mol L^{-1}.

The results found in this experiment showed that maximum sensitivity can be achieved by injecting an EBT solution with concentration between 5.0 x 10^{-5} and 7.5 x10^{-5} mol L^{-1}. Therefore, in order to save reagent and generate a lower amount of residue due to FIA system operation, at this moment, a solution containing 5.0 x 10^{-5} mol $^{-1}$ EBT was employed. Afterwards, an analytical curve was constructed in order to verify if, in the conditions already established, there was a linear relationship between CPC concentration and absorbance. It was verified that a linear relationship only could be observed for CPC concentrations in the range of 5 – 10 mg L^{-1}. For CPC concentrations lower than 5 mg L^{-1}, a practically constant absorbance signal was observed (Fig. 5A). In order to overcome this drawback, it was decided to add some CPC to buffer solution and optimize again the concentration of EBT in the solution injected into the FIA system. In this case, only EBT solutions with 5.0 x 10^{-5} and 7.5 x 10^{-5} mol^{-1} were tested and CPC concentration added to buffer solution was varied from 5 to 20 mg L^{-1}. The addition of CPC to buffer solution solved the problem of lack of linearity of analytical curves, which always presented correlation coefficients higher than 0.99. However, the slope of the curve varied with the addition of different concentrations of CPC and better sensitivity was observed when an EBT concentration of 7.5 x 10^{-5} mol L^{-1} was employed along with a CPC concentration of 10 mg L^{-1} in the buffer solution. The typical analytical curve obtained after addition of CPC to buffer solution is shown in the Fig. 5B.

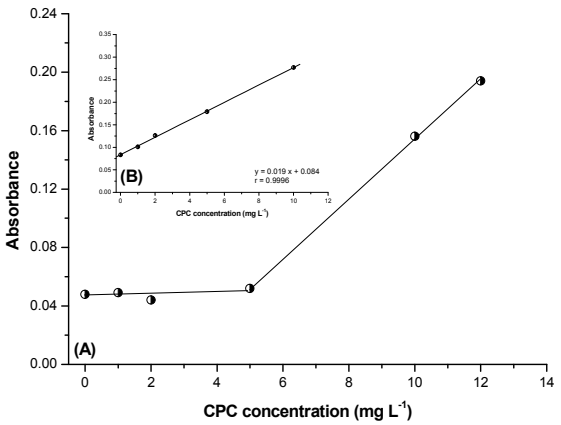

Fig 5 Analytical curves constructed by using reagent solutions without (A) and with CPC (B). EBT = 7.5 x 10^{-5} mol L^{-1}.

The next parameter evaluated was the influence of the concentration of Triton X-100 added to the EBT solution. The addition of this surfactant was tested taking into consideration that an ion-association with low solubility in water was formed from reaction between EBT and CPC. In this case, the presence of the Triton X-100 could improve the water solubility of the reaction product, due to its inclusion inside the micelles, causing an increase in the absorbance and, consequently, improving method sensitivity. Current literature reports some cases in which this approach was employed successfully [25-27]. Triton X-100 was chosen for the procedure once it does not present effective charge in aqueous medium and thus would not participate of the considered reaction. The influence of Triton X-100 concentration was evaluated in the range of 0 (absence) – 1.0% m/v. The results are shown in Fig. 6.

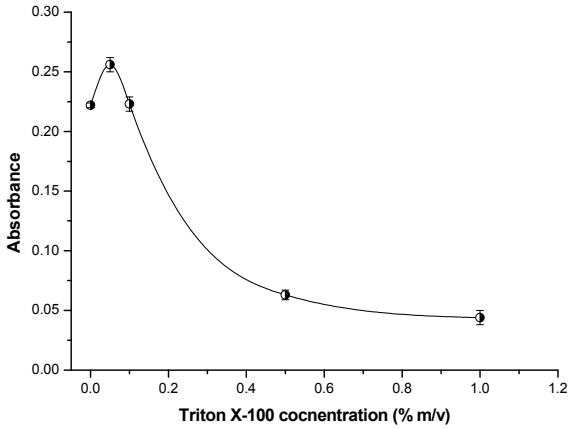

Fig 6 Influence of Triton X-100 concentration in the reagent solution (R_1) on the analytical signal of CPC in the reversed flow injection system. CPC = 10 mg L^{-1}; EBT = 7.5 x 10^{-5} mol L^{-1}.

The absorbance signal increased 16% when Triton X-100 concentration changed from 0 (absence) to 0.05% m/v. This increase can be credited to the inclusion of the ion-association specie inside the micelles formed in the Triton X-100 medium with consequent increase of the absorptivity of the colored specie due to its higher solubility in the aqueous medium [28]. Nevertheless, after this point, the absorbance signal showed an abrupt decrease, probably due to the occurrence of strong interactions between Triton X-100 and the EBT molecule (Fig. 7), which could lead to dissociation of the ion-association complex [29]. In view of these results, a concentration of Triton X-100 of 0.05% m/v was chosen for the method and used in all further experiments.

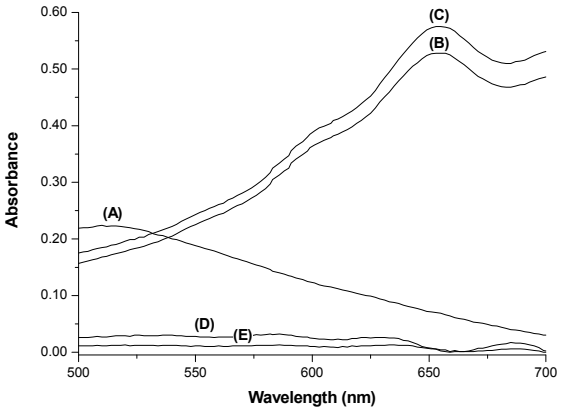

Fig 7 Spectra of CPC (25 mg L^{-1}) and EBT (5.0 x 10^{-5} mol L^{-1}) solutions in the presence of different concentrations of Triton X-100. (A) EBT; (B) EBT+CPC; (C) EBT + CPC + Triton X-100 (0.050% w/v); (D) EBT+CPC+ Triton X-100 (0.15% w/v); (E) EBT+CPC+Triton X-100 (0.25% w/v)

3.4. Optimization of physical variables

After optimizing all chemical parameters related to the FIA system, a detailed study about the influence of physical variables was performed. The parameters evaluated in this study were the injection volume, carrier flow-rate and the

volume of reaction coil. It is important to remark that sample and buffer solution flow rates were not evaluated, being always maintained at 1.9 and 1.0 mL min^{-1}, respectively, values that were employed during optimization of chemical parameters.

The first physical parameter evaluated was the volume of EBT solution (containing Triton X-100) injected into the system. In the present case it controls the amount of reagent inserted for reaction of sample, being very important in the control of method sensitivity. The experiment was carried out by varying the volume of the loop between 50 and 500 µL. The analytical signal increased with the increase of loop volume up to 375 µL and, after this point, it was not observed any gain in the sensitivity but only the increase of the time required to complete the signal. This result indicated that no dispersion of the central part of the sampling zone was observed for volumes higher than 375 µL. Therefore, a volume of 375 µL was established for the method.

Next physical variable optimized was the length of the reaction coil, which is the part of the system that establishes the residence time of the sample zone inside the system thus controlling the reaction time between reagent (carrier) and analyte (sample) inside the FIA system and also the mixture between such streams. Longest reactors enhance the contact time between EBT and CPC, allowing that reaction could achieve equilibrium before sample zone passing through the flow cell thus providing increased sensitivity. Also, in this situation the mixture between carrier (reagent) and sample (analyte) streams is improved, minimizing baseline noise. On the other hand, excessive increase of the reactor length can lead to high dispersion of the sample plug, which causes signal decreasing. In the present work, coiled reactors with lengths ranging from 10 to 100 cm (50 to 500 µL) were tested. The absorbance signal increased with the increase of reactor length up to 50 cm (250 µL). After this, the signal was practically constant, just suffering a soft decreasing probably due to sample zone dispersion. In order to attain best sensitivity for the system a reactor with 50 cm was then used.

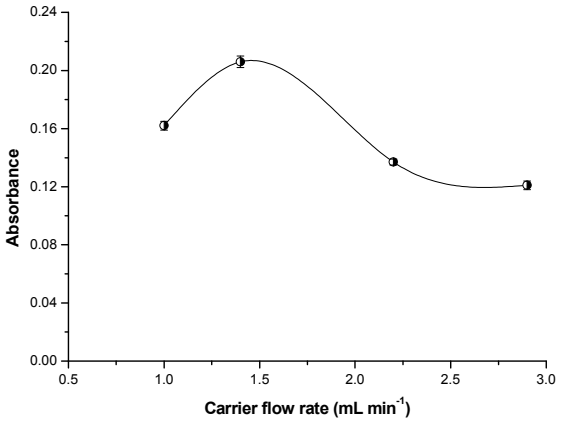

Fig 8 Influence of carrier flow rate on the analytical signal obtained for CPC with the reversed flow injection system. CPC = 10 mg L^{-1}; EBT = 7.5 x 10^{-5} mol L^{-1}; Triton X-100 = 0.05% w/v.

As the same way of the reactor length, the carrier flow rate controls the reaction time. Also, this parameter influences the dilution of sample plug inside the system, affecting the magnitude of analytical signal. In order to evaluate the effect of carrier flow rate on sensitivity, it was tested between 1.0 and 2.8 mL min^{-1}. As can be seen in Fig. 8, highest absorbance signals were recorded for lowest carrier flow rates. This behavior can be credited to the lower dilution imposed to sample zone when carrier flow rate decreases. Again, in order to achieve better sensitivity for the system, a carrier-flow rate of 1.4 mL min^{-1} was set.

3.5. Interference study

A study was performed in order to evaluate the effect of possible interferent species on CPC reaction with EBT in presence of Triton X-100. Substances usually found in the pharmaceutical products of CPC like ethanol, fluoride, sucrose and glucose were tested as possible interferents. The interferences due to the presence of glucose and sucrose were tested in the range of 5 - 1000 mg L^{-1}. In both cases no significant variation of the signal (± 5%) was observed. The interference due to fluoride ion, which is added in almost all mouthwash solutions, was investigated in the range 5 – 250 mg L^{-1}. No noticeable interference was noted up to 50 mg L^{-1} and, after this concentration, a soft increase of the signal was verified. However, since concentrations of fluoride in such products are around 250 – 300 mg L^{-1} and samples had to be diluted 100 times before injection into the system, this component was not considered an important interferent. In turn, the influence of ethanol on analytical signals was studied in the range of 0.5 to 20% v/v. The signals were practically constants from 0.5 to 10% v/v ethanol, being noted only the increase of the baseline noise at 10% v/v ethanol concentration. This increase probably occurred due to the incomplete mixture of sample with carrier fluid (and reagent) inside the system thus promoting the Schlieren effect. At 20% v/v ethanol, concentration, the Schlieren effect was more pronounced and a remarkable decrease of signal was verified (31%). However, as the same way of fluoride, the concentration of ethanol in final solution injected into the system were always much lower than 10% v/v because of dilution necessary to samples fit the linear portion of the analytical curves.

4. Method evaluation

4.1. Analytical characteristics

The proposed FIA system was critically evaluated with regard to accuracy, precision, detection limit, quantification limit, linear range and measurement frequency. Operating the system under optimized conditions a linear fit was derived between 1.0 and 10 mg L^{-1}, with a typical equation A = 0.024 [CPC (mg L^{-1})] + 0.070 with a correlation coefficient of 0.999, where A is the absorbance signal measured as peak height. The detection limit concentration, estimated as three times the standard deviation of 10 measurements of blank solution, was 0.24 mg L^{-1}. In the same way, the quantification limit concentration, derived from 10 times the standard deviation of the blank, was 0.80 mg L^{-1} [30-31]. The RSD observed was 2.8% at the 1.0 mg L^{-1} level. The robustness of the method was estimated by comparing the slopes of ten analytical curves constructed in ten different (and straight) days. The slopes just varied between 0.023 and 0.027, with an average value of 0.025 and a relative standard deviation of 4%. A measurement frequency of 40 h^{-1} was derived for the proposed FIA system, already considering the flow interruption for sample (or standard solutions) changing.

4.2. Application

Five samples (three mouthwash solutions and two tablets for soft throat infections) of pharmaceutical products

containing CPC were analyzed by the developed methodology in order to prove its applicability. All samples were prepared as described in the experimental section and analyzed in triplicate. The results are shown in Table 1.

Table 1 Results obtained in the analysis of commercial samples of mouthwash solutions (S_n) and tablets (T_n) containing CPC. All values are expressed as an average ± standard deviation of three independent assays.

Sample	CPC declared	CPC found
S_1	520 mg L^{-1}	519 ± 23 mg L^{-1}
S_2	520 mg L^{-1}	520 ± 10 mg L^{-1}
S_3	520 mg L^{-1}	556 ± 18 mg L^{-1}
T_1	1.466 mg per tablet	1.00 ± 0.06 mg per tablet
T_2	1.00 mg per tablet	0.98 ± 0.05 mg per tablet

As it can be seen in Table 1, the concentrations of CPC found in the samples of mouthwash solutions and in the sample T_2 (tablet) were in good agreement with those labeled in the pharmaceutical products, especially if a tolerance limit of 10% is considered. On the other hand, the result found for the tablet T_1 was 30% lower than that declared by the manufacturer. Including, in this case, it was noted inconsistence between the mass of each tablet declared by manufacturer (2.5 g) and the actual mass of each tablet present in the package (2.20 ± 0.05 g). These results are not sufficient to attest or discard the accuracy of the procedure since the values declared in the packages cannot be considered as good references for the amounts of CPC in the samples. Therefore, a recovery test was performed in order to evaluate the accuracy of the developed procedure (Table 2).

Table 2 Results obtained in the recovery test. All values are expressed as an average ± standard deviation of three independent assays.

Sample	CPC added (mg L^{-1})	CPC found (mg L^{-1})
S_1	100	107 ± 10
	250	92 ± 7
S_2	100	94 ± 5
	250	96 ± 7
S_3	100	92 ± 3
	250	96 ± 6
T_1*	2	97 ± 3
	4	104 ± 5
T_2*	2	99 ± 3
	4	101 ± 4

* In these samples, the addition was done on the solution obtained after dissolution of the tablet.

For the mouthwash solution samples, the recovery test was carried out by adding CPC directly in the original samples in a proportion of around 20 and 50% of those declared in the labels. How all samples contained a declared concentration of 520 mg L^{-1} CPC, they were spiked with 100 and 250 mg L^{-1} of CPC. Then, they were diluted and analyzed as the same way of non-spiked samples. In the case of tablets, the additions were of 2 and 4 mg L^{-1} of CPC directly in the solutions resulted from tablets dissolution. As it can be seen in Table 2, all recoveries were between 92 and 107%, indicating that the method is not subjected to matrix interferences during analysis of the pharmaceutical products tested. Once no spectral interferences were observed, in this condition, the recovery test could be employed to attest the accuracy of the methodology for CPC determination in such products.

5. Conclusion

The FIA system developed in this work showed to be suitable, in terms of sensitivity and selectivity, for quantifying CPC in solution with EBT, being possible applies it in the determination of this component in pharmaceutical products. Also, it presented high analytical throughput allowing its use in a routine system with excellent productivity.

In the experimental context, it is important to remark that the addition of CPC to the EBT reagent solution was necessary to improve the linearity of the analytical curves and the use of Triton X-100 increased the sensitivity of the FIA methodology, probably due to the higher solubility in water of the product obtained from reaction of CPC with EBT in a medium containing this surfactant. The method was applied to the analysis of several pharmaceutical products that contained CPC as active ingredient. Its accuracy was tested by applying a recovery test, being obtained recovery ratios that varied from 92 to 107%.

The sensitivity achieved with the developed methodology was better than that reported by Benamor et al. [19], which explored the reaction of CPC with bromopirogallol red in the presence of Sr(II), for the spectrophotometric determination of the analyte in pharmaceutical formulations. Due to the good sensitivity observed in this work, the use of the reaction of CPC with EBT can be explored for the determination of CPC in other types of samples.

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