A Simple Strategy for Simultaneous Determination of Paracetamol and Caffeine Using Flow Injection Analysis with Multiple Pulse Amperometric Detection

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Abstract
In this work, we report a simple and novel strategy for simultaneous analysis using flow injection analysis with multiple pulse amperometric (FIA-MPA) detection. The proposed strategy was successfully used for simultaneous determination of paracetamol and caffeine (model analytes) in pharmaceutical formulations. A sequence of potential pulses (waveform) was selected in such a way that PA is selectively oxidized at E_1 (+1.20 V/50 ms) and both compounds (PA + CA) are simultaneously oxidized at E_2 (+1.55 V/50 ms); hence, current subtraction (using a *correction factor*) can be used for the selective determination of CA. The proposed FIA method is simple, cheap, fast (140 injections h^{-1}), and present selectivity for the determination of both compounds in pharmaceutical samples, with results similar to those obtained by HPLC at a 95% confidence level.

Keywords: Simultaneous determination, Multiple-pulse amperometry, FIA, HPLC, Paracetamol, Caffeine, Pharmaceutical tablets, Urine.

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1 Introduction

Paracetamol (PA), or acetaminophen, is one of the most widely used analgesics in the world, and caffeine (CA) is one of the most common adjuvant analgesic drugs. Absorption of PA is accelerated when combined with CA and, the pain tolerance is increased [1,2]. Because of this synergism, PA and CA are frequently combined in pharmaceutical preparations. Thus, determination of both compounds is extremely critical for quality control purposes.

Several studies in the literature have reported about methods for the simultaneous determination of PA and CA. For some of these methods, determination of PA and CA requires a previous separation step, such as HPLC with UV detection [3–9]. Alternatively, capillary electrophoresis methods, such as capillary electrochromatography [10] and micellar electrokinetic capillary chromatography [11] have been used. However, there are several disadvantages associated with these methods, including relatively high cost (HPLC) and time consumption (HPLC and EC). Therefore, methods for simultaneous determination of PA and CA which do not require a previous separation step would be more attractive. In fact, several simultaneous determination methods have been proposed, based on direct UV-vis spectrophotometric detection [12–15], coupled with subsequent data treatment using chemometric methods (PCR, PLS) or multivariate calibration [16].

Other methods proposed for direct simultaneous determination of PA and CA are based on electrochemistry, such as voltammetry in steady-state batch systems. Some specific approaches based on differential pulse voltammetry [17,18] or square wave voltammetry [19,20] have been reported in literature. The principal advantages of voltammetric techniques are high sensitivity, low cost, simplicity and, in most cases, straightforward data treatment. Some drawbacks reported include fouling (passivation) of the working electrode surface, due to accumulation of the analyte or reaction products, which leads to a loss of electrode activity. However, the use of Multiple Pulse Amperometric (MPA) detection can prevent contamination of the working electrode surface. Besides the application of a potential pulse for analyte detection, this technique also enables constant application of a cleaning potential pulse. Furthermore, incorporation of FIA provides higher sensitivity and analytical frequency than batch systems. In fact, FIA systems coupled with MPA detection allowed for multi-component analysis [21–25] using just one working electrode, which was not possible using conventional amperometry.

Boron-doped diamond (BDD) thin film electrodes have been increasingly used in recent years. This material is becoming an alternative to traditional carbon or other

solid electrodes due to superior characteristics such as its wide potential window, low background currents, long term stability, low sensitivity to dissolved oxygen, and high resistance to fouling [26–29].

In the present work, the MPA detection coupled to FIA is proposed for simultaneous determination of PA and CA in pharmaceutical formulations. An appropriate potential pulse waveform was applied to a single boron-doped diamond (BDD) working electrode and simultaneous analysis was carried out without the use of chemometric techniques for data analysis. Results obtained from this novel FIA-MPA method were validated with respect to linearity, repeatability, recovery, detection and quantification limits, and by comparison with results from HPLC analysis.

2 Experimental

2.1 Reagents and Samples

Paracetamol (PA) and caffeine (CA) were obtained from Synth (Diadema - Brazil). All reagents were of analytical grade and were used without further purification. Solutions were prepared using deionized water from a Gehaka–Master System, with a resistivity no less than 18 MΩ cm. Acetic acid/acetate buffer solution (0.1 mol L^{-1}; pH 4.7) was used as the supporting electrolyte. Pharmaceutical formulations (tablets) containing PA and CA were obtained from local drugstore. All samples and standards stock solutions were prepared in water and diluted in buffer solution before experiments. Acetonitrile was purchased from Merck (Darmstadt, Germany).

2.2 Instrumentation, Electrochemical Cell and Electrodes

All electrochemical measurements were performed using a μAutolab Type III potentiostat (EcoChemie - Metrohm). A single-line flow injection analysis system was employed using 1.0 mm internal diameter polyethylene tubing. The flow rate was controlled by pressure generated from a water column and aquarium air pump system [30]. HPLC measurements were performed using a Hitachi pump L-2130, Hitachi LC-4250 UV-vis detector and, a Shim- pack CLC-ODS column (25 mm × 4.6 mm; Shimadzu). The mobile phase was composed of acetonitrile and phosphate buffer 0.05 mol L^{-1} at pH 2.1 (8 : 92, v/v).

A home-made electrochemical wall-jet cell was constructed from a glass cylinder and two Teflon covers, which were firmly fitted on the top and bottom of the cylinder (internal diameter = 2.3 cm; depth = 2.5 cm; total volume = 9.5 mL), based on a similarly designed cell previously reported (Figure 1) [31].

The top cover contains 3 holes for the counter (CE) and reference (RE) electrodes and syringe needle. The syringe needle was positioned into the hole in the center of the cover (~2 mm from the electrode surface). The bottom of the cell contained a single hole (which was also precisely located at the center of the cell) in which the

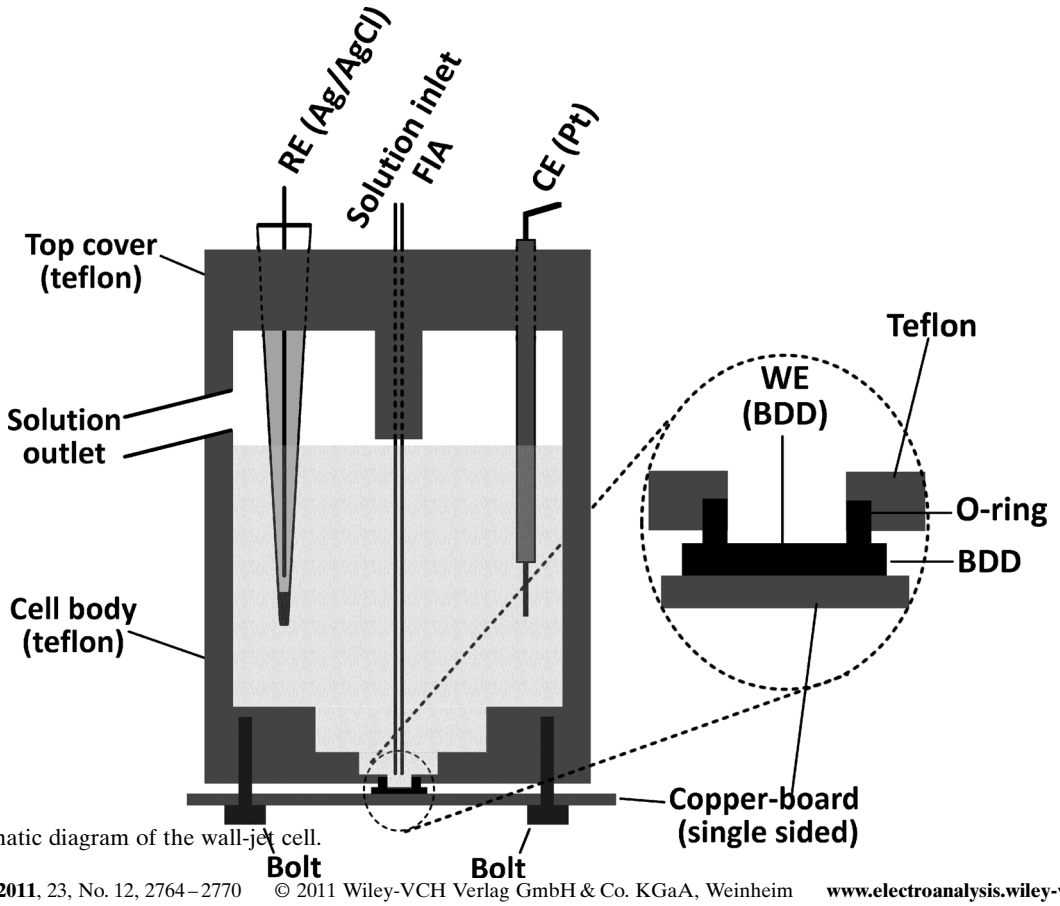

Fig. 1. Schematic diagram of the wall-jet cell.

working BDD electrode was inserted (WE). An O-ring was used for definition of the electrode area (0.50 cm^2). The electric contact was made with the copper-board positioned under the BDD electrode. It is important to emphasize that the BDD working electrode does not require manual cleaning procedures (tedious and time consuming) as other solid electrodes (platinum, gold, glassy carbon). The electrochemical pretreatment of the working electrode can be easily performed with the wall-jet cell positioned (without disassembling) in the FIA system, which would not be possible with a thin-layer cell arrangement (due to generation of bubbles and loss of electrical contact between the electrodes). Other advantage of the proposed wall-jet cell is its operation at atmospheric pressure which decreases the possibility of leaks in the flow cell.

A thin film (~1.2 μm) of boron-doped diamond (BDD; ~8000 ppm doping level) on a polycrystalline silicon wafer was used as the working electrode (Adamant Technologies SA, La Chaux-de-Fonds, Switzerland). Prior to use, the BDD electrode was cathodically pretreated with 0.5 mol L^{-1} H_2SO_4 by applying −3.0 V for 900 s [32]. The counter and mini-reference electrodes were respectively, platinum wire and Ag/AgCl ($KCl_{sat.}$) [33].

3 Results and Discussion

Initially, the electrochemical behavior of PA and CA in the presence of acetic acid/acetate buffer (0.1 mol L^{-1}) was investigated using a single-line flow injection system with MPA detection. Figure 2A presents the potential pulse scheme used for the study of the electrochemical behavior of PA and CA using FIA-MPA. Figure 2B shows amperograms obtained from application of 10 fast potential pulses (each at 100 ms/cyclic form) as a function of time (one single measurement), for sequential injections of two solutions onto FIA system: (solution 1) a 100 μL aliquot of a solution containing 34.3 mg L^{-1} PA; and (solution 2) a 100 μL aliquot of 4.9 mg L^{-1} CA. Hydrodynamic voltammograms were easily obtained by plotting the peak current values as a function of the corresponding applied potential pulses (Figure 2C).

The oxidation current of PA starts to increase at approximately +0.70 V and reaches a maximum near +1.00 V, while the oxidation current of CA starts to increased at approximately +1.30 V, and reaches a maximum near +1.50 V. As previously described [20,34,35], electrooxidation of PA involves the transfer of two electrons and two protons, generating *N*-acetyl-*p*-quinoneimine, while the electro-oxidation of CA involves the transfer of four electrons and four protons, generating 4,5-diol (uric acid analogue), which then rapidly fragments.

Only PA can be selectively quantified if FIA with amperometric detection at constant potential is used. In fact, under these conditions, selective quantification of CA is not possible, because PA is also electroactive in the same potential region (higher than +1.30 V) and thus a potential interferent. In the present work, we propose an alter-

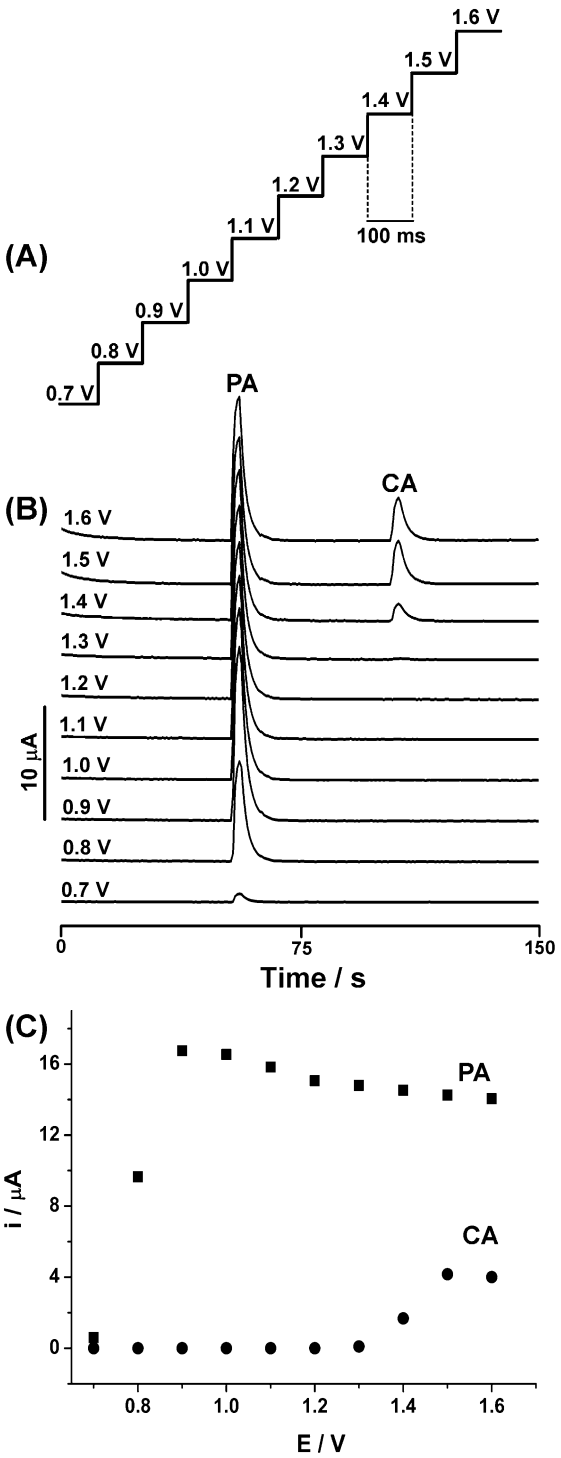

Fig. 2. (A) MPA waveform (cyclic form) applied to the BDD working electrode as a function of time; (B) FIA-MPA amperograms obtained from injection of 100 μL of two solutions: PA (34.3 mg L^{-1}) and CA (4.9 mg L^{-1}); (C) Hydrodynamic voltammograms obtained by plotting peak current values as function of the corresponding applied potential pulses. Potential pulse times: 100 ms each: Supporting electrolyte: Acetic acid/acetate buffer (0.1 mol L^{-1}; pH 4.7); Flow rate: 4.5 mL min^{-1}; Sample injection volume: 100 μL.

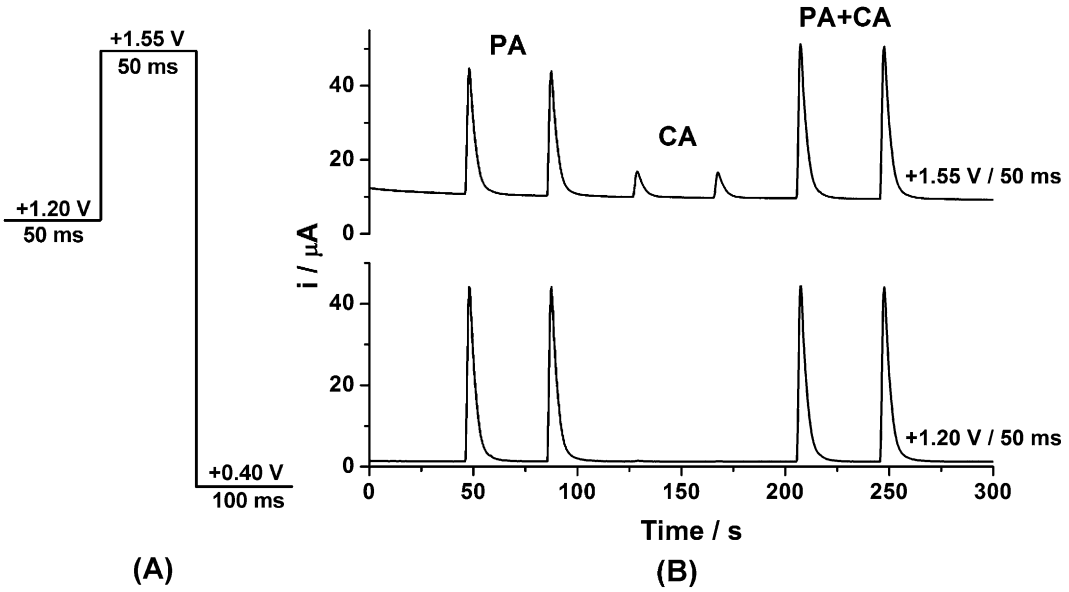

Fig. 3. (A) MPA waveform (cyclic form) applied to the BDD working electrode as a function of time; (B) FIA-MPA responses ($n=2$) for solutions containing only PA (60.0 mg L^{-1}), only CA (8.0 mg L^{-1}) and PA + CA (60.0 + 8.0 mg L^{-1}). Cleaning potential pulse: +0.40 V (amperogram not shown); Supporting electrolyte: Acetic acid/acetate buffer (0.1 mol L^{-1}; pH 4.7); Flow rate: 4.5 mL min^{-1}; Sample injection volume: 100 μL.

native method for the simultaneous determination of PA and CA, based on the use of the MPA technique in a flow injection analysis system. Using this novel method, PA can be selectively detected at a potential pulse lower than +1.30 V, while both PA and CA can be detected at a more positive potential pulse (+1.55 V). The oxidation current of CA can be then obtained by subtraction of the currents detected at the two potential pulses.

Figure 3A shows a potential pulse scheme for the simultaneous determination of PA and CA by FIA-MPA. As outlined below, each optimized potential pulse was designed for a specific purpose:

(1) +1.20 V/50 ms: PA oxidation and selective quantification;
(2) +1.55 V/50 ms: Oxidation of both PA and CA compounds. CA can then be quantified by subtraction of the currents obtained from both potential pulses (using a *correction factor*);
(3) +0.40 V/100 ms: applied to avoid contamination of the working electrode surface.

Figure 3B shows amperograms obtained at potential pulses of +1.20 V and +1.55 V for injections of three different test solutions: (1) only PA (60.0 mg L^{-1}); (2) only CA (8.0 mg L^{-1}); and (3) both PA and CA analytes at the same initial concentration. As can be seen, only PA is oxidized at +1.20 V, while at +1.55 V both PA and CA are oxidized. In addition, although we observed good results without applying the third potential pulse (+0.40 V/100 ms); better reproducibility was observed after long-term experiments if the third potential pulse was applied.

Interestingly, as shown in Figure 3B, PA oxidation currents are not the same at both potential pulses (+1.20 V and +1.55 V). The PA oxidation current detected at +1.20 V is greater than the PA current detected at +1.55 V, probably due to the fact that electrochemical oxidation of PA occurs faster than hydrodynamic or diffusion replacement of material near the surface of the working electrode. Because of this PA oxidation current difference, simple subtraction between currents detected at the two potential pulses does not directly yield the CA oxidation current. To circumvent this problem, we applied a *correction factor* that corresponds to the exact difference between the current detected for PA at +1.20 V and +1.55 V. This *correction factor* was obtained by injecting a solution containing only PA onto FIA-MPA system and was determined using the following equation:

$$correction\ factor = i_{PA+1.55\,V}/i_{PA+1.20\,V}$$

Then, the current originating from CA oxidation detected at +1.55 V can be calculated using the following equation:

$$i_{CA} = i_{+1.55\,V} - (factor \times i_{+1.20\,V})$$

The influence of parameters such as flow rate and potential pulse time on the FIA-MPA response was also evaluated. The influence of flow rate (0.50–6.0 mL min^{-1}) was studied with the application of the three potential pulses, using a solution containing both PA (60.0 mg L^{-1}) and CA (8.0 mg L^{-1}). At flow rates up to 4.5 mL min^{-1}, the current was observed to increase at both potential pulses (+1.20 and +1.55 V) and remained constant at higher values. Based on these results, a flow rate of 4.5 mL min^{-1} was used for all subsequent experiments. A range of potential pulse times (50, 100, 200 and, 300 ms)

Table 1. Influence of variation in potential pulse times in the FIA-MPA system response.

Pulse time	Current (μA) +1.20 V			Current (μA) +1.55 V			*Correction factor*
	PA	CA	PA + CA	PA	CA	PA + CA	$i_{PA+1.55V}/i_{PA+1.20V}$
50 ms	43.06	0	43.05	34.18	7.01	41.97	0.794
100 ms	31.61	0	31.33	27.45	5.08	32.81	0.868
200 ms	28.13	0	28.75	26.42	4.15	31.59	0.939
300 ms	25.50	0	25.18	25.16	3.80	30.46	0.987

was also evaluated using the same solutions. For these experiments, potential pulse times were simultaneously varied at +1.20 and +1.55 V, while a constant potential pulse time (100 ms) was used at +0.40 V. In addition, the flow rate (4.5 mL min^{-1}) and injection volume (100 μL) were constant. The effect of potential pulse times on resulting peak currents at both potential pulses are shown in Table 1.

With increasing potential pulse time, the amperometric signal decreased and the *correction factor* ($i_{PA+1.55V}/i_{PA+1.20V}$) increased due to the current sampling time changes associated with variations in the potential pulse time. Specifically, using the MPA software (GPES – Autolab/Eco Chemie), the current is acquired once, close to the end of the potential pulse time. In fact, using the above defined conditions (flow rate = 4.5 mL min^{-1}; electrode area = 22.9 mm^2), the current decreased for small time periods (< 300 ms), while for greater times (> 300 ms), the current reached a steady state value due to the constant flow rate used in these experiments.

Based on these experiments, a potential pulse time of 50 ms was adopted at +1.20 and +1.55 V and 100 ms at +0.40 V. Using these conditions, the current sampling time in each amperogram was 200 ms (total time of the waveform), allowing acquisition of rapid transient signals without loss of reproducibility.

The stability of the proposed method was assessed by successive injections ($n = 12$) of solutions containing known concentrations of PA and CA (60.0 and 8.0 mg L^{-1}, respectively), at a flow rate of 4.5 mL min^{-1} and with increasing sample injection volumes (50, 100, 200 and, 300 μL). The results of these experiments are shown in Table 2.

Good repeatability was obtained for peak current responses at both potential pulses, independently of the injected sample volume (*RSD* < 1.23 %). However, sample injection volumes directly influenced the performance of the FIA-MPA system, where increased sample volumes were associated with improved *RSD* values and reduced the sampling rates. Therefore, based on these experiments, an intermediate sample volume (100 μL) was adopted.

Table 2. Influence of increasing sample injection volumes on electrode response stability and sampling rates.

Sample volume (μL)	*RSD* (%)		Sampling rate (h^{-1})
	+1.20 V	+1.55 V	
50 ($n = 12$)	1.23	1.21	160
100 ($n = 12$)	0.65	0.69	140
200 ($n = 12$)	0.48	0.57	115
300 ($n = 12$)	0.25	0.24	105

In order to determine the linear working range of the FIA-MPA system, a series of experiments were performed using standard solutions containing only PA or only CA. Both compounds displayed good linearity over a large concentration range (1.0–755 mg L^{-1} for PA and 1.0–315 mg L^{-1} for CA), with excellent correlation coefficients for both analytes ($R = 0.999$). Evaluation of the linear working range for PA in the FIA-MPA system was also used to determine the concentration range for which the *correction factor* ($i_{PA+1.55V}/i_{PA+1.20V}$) is constant. These experiments demonstrated that the *correction factor* is constant over a large PA concentration range (9.9–177.0 mg L^{-1}; *correction factor* = 0.771 ± 0.012; $n = 13$). However, the *correction factor* should still be determined for each calibration procedure (by injection of a solution containing only PA), because small variations may occur between analyses conducted on different days.

Analytical curves for the simultaneous quantification of PA and CA were determined by taking into consideration the concentration range for which the *correction factor* is constant and the concentration of PA and CA in pharmaceutical formulations available in the Brazilian market (approximately 7.7-fold more PA than CA). For all standard solutions, the ration between PA and CA concentration was the kept constant. Figure 4 presents transient signals obtained from injection of 2 solutions containing only PA (a, b) which were used for calculation of the *correction factor*, 5 solutions containing simultaneously increasing concentrations of PA (c–g: 7.6 to 196 mg L^{-1}) and CA (c–g: 1.0 to 25.7 mg L^{-1}); and 4 appropriately diluted pharmaceutical samples (h–l).

The above described analytical curves yielded the following calibration equations:

$$i\ (\mu A) = 0.478 + 0.691\ PA\ (mg\,L^{-1}) \qquad r = 0.999$$

$$i_{PA} = i_{+1.20\,V}$$

$$i\ (\mu A) = 0.072 + 0.693\ CA\ (mg\,L^{-1}) \qquad r = 0.999$$

$$i_{CA} = i_{+1.55\,V} - (factor \times i_{+1.20\,V})$$

The limits of detection (*LOD*; 3*S*/*N*) were 0.10 mg L^{-1} (0.66 μmol L^{-1}) for PA and 0.17 mg L^{-1} (0.87 μmol L^{-1}) for

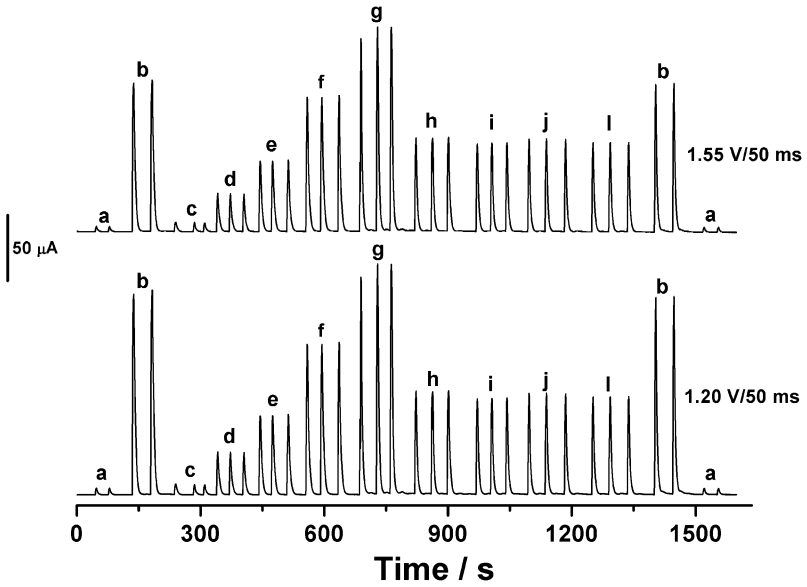

Fig. 4. FIA-MPA amperograms obtained after injections of 2 solutions containing only PA (a, b), 5 solutions containing simultaneously increasing concentrations of PA (c–g: 7.6 to 196 mg L^{-1}) and CA (c–g: 1.0 to 25.7 mg L^{-1}); and, 4 appropriately diluted pharmaceutical samples (h–l). Supporting electrolyte: Acetic acid/acetate buffer (0.1 mol L^{-1}; pH 4.7); Flow rate: 4.5 mL min^{-1}; Sample injection volume: 100 μL.

Table 3. Comparison of results obtained from simultaneous determination of PA and CA in pharmaceutical samples using FIA-MPA versus HPLC ($n=3$). $E_1=100\times$(FIAMPA–label value)/label value; $E_2=100\times$(FIAMPA–HPLC)/HPLC.

Samples		Label value (mg)	HPLC (mg)	FIA–MPA (mg)	E_1 (%)	E_2 (%)
01	PA	500	502 ± 5	493 ± 12	−1.4	−1.8
	CA	65	58 ± 2	54 ± 3	−16.9	−6.9
02	PA	500	501 ± 9	512 ± 5	+2.4	+2.2
	CA	65	58 ± 3	63 ± 3	−3.1	+8.6
03	PA	500	515 ± 12	507 ± 15	+1.4	+0.2
	CA	65	57 ± 2	55 ± 2	−15.4	−3.5
04	PA	500	496 ± 11	500 ± 8	0	+0.8
	CA	65	58 ± 2	56 ± 2	−13.8	−3.4

CA. The *LOD* values determined in the present study for both compounds are slightly higher than previously reported *LOD* values using voltammetry in steady-state batch systems [18,20]. However, importantly, the *LOD* values determined in the present study are adequate for analysis of the pharmaceutical samples available on the Brazilian market. In addition, the analytical frequency of the proposed method is very high (approximately 140 per hour), which is desirable for routine analysis. Table 3 presents results obtained using the proposed FIA-MPA method for the simultaneous determination of PA and CA in 4 pharmaceutical samples. Results were compared to those obtained by an HPLC method, and with the known sample concentrations (from the package labels). Statistical analysis (paired Student's *t*-Test) was used to compare values obtained by HPLC with those found using the proposed FIA-MPA method. At a 95% confidence level, resulting *t* values (1.85 for PA and 2.04 for CA) were smaller than the critical value (3.18; $\alpha=0.05$), demonstrating that there are no significant differences between results obtained by either methods.

Finally, recovery studies were carried out at 2 different levels for both PA and CA (50 and 100 %), to evaluate matrix effects after addition of standard-solution. Based on these experiments, matrix effects did not significantly influence determination of either compound (102 ± 3; $n=4$).

4 Conclusions

In this work, we report for the first time a simple strategy (use of the *correction factor*) for simultaneous analysis using FIA with amperometric detection and a single working electrode. This approach was successfully applied for fast and simultaneous determination of paracetamol and caffeine in pharmaceutical samples. Determination was found to be possible using acetic acid/acetate buffer as the supporting electrolyte, boron-doped diamond as the working electrode, and a home-made electrochemical wall-jet cell coupled to a single-line flow system. The analytical frequency of this FIA-MPA method was estimated

to be 140 injections per hour. In addition, results obtained for PA and CA in FIA were similar to those obtained by HPLC method. Furthermore, it is important to emphasize that the proposed methodology (using a *correction factor*) open new gates for simultaneous analysis of other compounds.

The strategy used for simultaneous determination of paracetamol and caffeine is also possible using two sequential working electrodes and conventional amperometry (bipotentiostatic model). However, in this case, the flow cell configuration would be more complex and the commercially-available bipotentatiostats often do not perform pulsed amperometry at the two electrodes, which can be necessary to overcome contamination of the working electrode surface. The possibility of using a potential pulse for cleaning step can be crucial if analytes with adsorptive characteristics are analyzed.

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