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Two new electrochemical methods for fast and simultaneous determination of codeine and diclofenac

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ABSTRACT

In this paper, we present two new electrochemical methods for fast and simultaneous determination of codeine (CO) and diclofenac (DCF). The first one is based on batch injection analysis with amperometric detection (BIA-MPA) and the second one is based on capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D). The proposed BIA-MPA method is highly-precise (RSD of 1.1% and 0.9% for DCF and CO, respectively; $n=10$), fast (300 injections h^{-1}) and has low detection limits (1.1 and 1.0 μmol L^{-1} for DCF and CO, respectively). The proposed CE-C^4D method allows the determination of CO and DFC in less than 1 min with high precision (RSD of 0.3% and 0.7% for DCF and CO, respectively; $n=10$) and low detection limits (11 and 21 μmol L^{-1} for DCF and CO, respectively). Both proposed methods were applied to the determination of CO and DCF in pharmaceutical samples with similar results to those achieved by high-performance liquid chromatography (HPLC) at a 95% confidence level.

1. Introduction

Codeine (CO) is one of the most important constituents of opium. It has moderate analgesic and weak cough suppressant effects [1,2]. The analgesic effects are observed because only trace amounts of morphine are formed after the administration of CO. Diclofenac (DCF) is a nonsteroidal anti-inflammatory drug (NSAID) with strong anti-pyretic, analgesic and anti-inflammatory properties [3]. The combination of anti-inflammatory drugs and opioids has recently been used to reduce opioid requirements. The purpose is to achieve an improvement in analgesia without increasing the side effects of the drugs (synergic effect) [4–6].

Few studies in the literature have reported on methods for the simultaneous determination of CO and DCF; among them are capillary electrophoresis with an electromagnetic induction detector [7] and ultraviolet–visible (UV–vis) spectroscopy with reversed matrix representation of the Beer–Lambert law (CPA-matrix method) [8]. To our knowledge, although liquid chromatography has often been used for the separation and detection of multi-analytes, this technique has not been used for simultaneous determination of DCF and CO yet. Only studies in which the determination of both compounds was carried out in sequence (different mobile phases) have been reported [9–12]. This fact can probably be explained based on the differences in the properties of the two drugs. DCF ($pKa=4.2$) is a weak acid and CO ($pKa=8.2$) is a weak base, so that the polarity of each compound is very different if both are dissolved in the same mobile phase [13,14]. In studies conducted in our laboratory using liquid chromatography with a C18 column, it was observed that if one compound is strongly retained, the other is weakly or not retained and vice versa.

Several electroanalytical methods have been reported for the determination of DCF [15–17] or CO [18–20] in single-mode, but to our knowledge, there are no reports on the simultaneous determination of these two compounds using electrochemical methods. Recently, it was demonstrated that flow injection analysis with multiple-pulse amperometric (FIA-MPA) detection can be successfully used for the determination of DCF in pharmaceutical formulations using an unmodified working electrode without contamination/adsorption problems [21]. In addition, it has also been reported that FIA-MPA systems can be used for the simultaneous determinations using a single working electrode. This strategy was used for simultaneous determination of sugars [22], drugs [23–26], antioxidants [27], synthetic colorants [28], as well as the possibility of using the internal standard method in FIA systems [29].

An elegant alternative to FIA methods is batch injection analysis (BIA). In BIA systems, a sample plug is injected through

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a micropipette tip directly onto the working electrode surface (wall-jet configuration), which is immersed in a large-volume of blank solution. This approach renders several desirable characteristics, such requiring only small sample volumes, typically 1–100 μL, high sensitivity, low cost, simplicity and the possibility of developing portable BIA procedures [30,31]. Information and applications involving simultaneous determination using BIA systems with amperometric detection and a single working electrode have also been reported in the literature [32–34].

Another electrochemical technique that is widely used for the separation and simultaneous determination of ionic compounds is capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) [35,36]. This system often has a short analysis time, low cost, high separation efficiency, and low consumption of reagents and samples [37]. In addition, CE is being used as an alternative for fast determination of cations and anions in a single run. This is especially useful for simultaneous quantification of active pharmaceutical ingredients and their counterions (stoichiometric characterization) [38–41].

In this work, we report two new simple and low-cost electrochemical methods (BIA-MPA and CE-C^4D) for fast simultaneous determination of codeine (CO) and diclofenac (DCF).

2. Experimental

2.1. Reagents and samples

All solutions were prepared freshly with deionized water (18 MΩ-cm) from a Milli Q water purification system (Millipore, Bedford, MA, USA). All reagents were of analytical grade and used without further purification. Sodium diclofenac and codeine phosphate were purchased from Galena (Campinas, SP, Brazil). Lithium hydroxide, lactic acid, sulfuric acid, 2-amino-2-hydroxymethyl-propane-1,3-diol (TRIS), 3-{[2-hydroxy-1,1bis(hydroxymethyl) ethyl]amino}-1-propanesulfonic acid (TAPS), triethanolamine (TEA) and methanol were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Sodium hydroxide and oxalic acid (OXA) were purchased from Synth (Diadema, SP, Brazil). Acetonitrile (HPLC grade), phosphoric acid, and acetic acid were purchased from Merck (Darmstadt, Germany). Sulfuric acid (0.1 mol L^{-1}) was used as the supporting electrolyte in the BIA-MPA experiments. Two buffer solutions were used as background electrolytes (BGE) in CE-C^4D experiments: 10 mmol L^{-1} TRIS/TAPS (pH 8.2) and 10 mmol L^{-1} TEA/1.8 mmol L^{-1} OXA (pH 8.4).

The pharmaceutical samples (tablets) containing sodium diclofenac and codeine phosphate were acquired at local drugstores. Ten tablets were accurately weighed and powdered in a mortar. An adequate amount of the powder was dissolved in water, after stirring and sonication for 10 min in an ultrasonic bath. The sample and standard solutions were further diluted in a suitable electrolyte for subsequent injection in the BIA-MPA system or in water if injected in the CE-C^4D system. The sample and standard solutions were filtered through a membrane filter (pore size of 0.45 μm) before injection in the CE-C^4D system.

2.2. Instrumentation and apparatus

BIA-MPA measurements were performed using μ-Autolab Type III potenciostat/galvanostat (Metrohm Autolab, Utrecht, The Netherlands) connected to a microcomputer and controlled by Autolab Software GPES version 4.9.007. The reference and auxiliary electrodes were a miniaturized Ag/AgCl (saturated KCl) [42] and a platinum wire, respectively. A thin film (around 1.2 μm) of boron-doped diamond (BDD) with a doping level of 8000 ppm on a polycrystalline silicon wafer (Adamant Technologies SA, La Chauxde-Fonds, Switzerland) was used as the working electrode. Before the first use, the BDD electrode was anodically pretreated by applying 0.01 A for 1000 s in a 0.04 mol L^{-1} Britton–Robinson buffer solution (pH=2.0) and then cathodically pretreated by applying −0.01 A for 1000 s in a 0.1 mol L^{-1} H_2SO_4 solution. This electrochemical pretreatment is similar to that used in previously published works [43,44]. After the first pretreatment, the BBD electrode was treated only cathodically once at the beginning of the workday. If the electrode was not used for a few days, both pretreatments (anodic and cathodic) were again performed. The solutions (standards and samples) were injected in the BIA-MPA system with a motorized electronic pipette (Eppendorf® Multipette stream). The homemade BIA cell was previously described [45]. All experiments were carried out with the solution in the BIA cell under stirring by using a micro DC-motor [46].

All electropherograms were performed using homemade CE equipment with two compact and high-resolution capacitively coupled contactless conductivity detectors (CE-C^4D) [47–49]. The detectors were positioned along the capillary at 10 cm from each end. The fused-silica capillary used in all experiments was 40 cm long (effective lengths of 10 and 30 cm) and 50 μm i.d. × 375 μm o.d. (Agilent, Folsom, CA, USA). Prior to use, the capillary was flushed with deionized water for 10 min, 0.1 mol L^{-1} NaOH for 15 min, with deionized water again for 10 min and finally with background electrolyte for 10 min. The samples were injected hydrodynamically for 0.6 s at 25 kPa. All experiments were carried out at −25 kV.

2.3. HPLC analysis

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). For CO determination, the mobile phase was composed of acetonitrile, acetate buffer (pH=6.9) and methanol (10:5:85, v/v) and the UV detector was set at 254 nm [50]. For DCF determination, the mobile phase was composed of acetonitrile and water (60:40, v/v), with the pH value adjusted to 2.1 with acetic acid and the UV detector was set at 280 nm [51]. In both analyses, the flow rate was 1.5 mL min^{-1}.

3. Results and discussion

3.1. BIA-MPA

Previous investigations using different supporting electrolytes (sulfuric acid, perchloric acid, and acetate and phosphate buffers) and BDD as a working electrode have demonstrated that sulfuric acid media provided the best performance (sensitivity and stability) for electrochemical oxidation of DCF [21]. This result is also in agreement with a previous study that reported the electrochemical behavior of DCF on glassy carbon electrode [53]. Thus, the electrochemical oxidation of CO was investigated in sulfuric acid by cyclic voltammetry (voltammograms not shown). The electrochemical oxidation of CO shows an irreversible behavior, with an oxidation peak around +1.2 V. The electrochemical mechanism of CO oxidation was previously described [1,18,52] and it was assumed that bis-codeine was the only oxidation product.

The electrochemical behavior DCF and CO was also studied using the BIA-MPA system. Ten fast potential pulses, each for 70 ms (0.80, 0.90, 1.00, 1.10, 1.20, 1.30, 1.40, 1.50, 1.60, and 1.70 V) were applied continuously to the working electrode (BDD). The current at each potential pulse (simultaneous acquisition of 10 amperograms) [25] was monitored continuously during three injections in the BIA system of solutions containing 50 μmol L^{-1} of DCF and 50 μmol L^{-1} of CO. The respective current peak at each

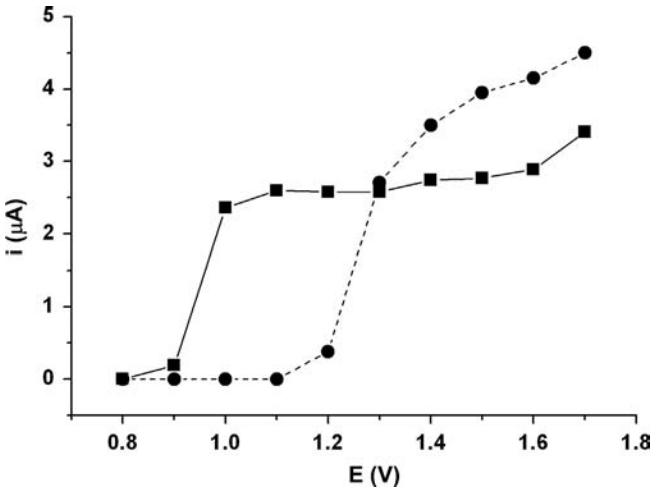

Fig. 1. Hydrodynamic voltammograms obtained by plotting peak current values as function of the corresponding applied potential pulses. (•) Injection of 50 μmol L^{-1} of CO; (▪) Injection of 50 μmol L^{-1} of DCF. Potential pulse times: 70 ms each; supporting electrolyte: H_2SO_4 0.1 mol L^{-1}; dispensing rate: 6.0 mL min^{-1}; injected volume: 100 μL.

potential pulse was measured and used to construct a hydrodynamic voltammogram for the electrochemical oxidation of DCF and CO (Fig. 1).

Based on the hydrodynamic voltammograms, the oxidation current of DCF started to increase at approximately +0.9 V and reached a maximum near +1.0 V, while the oxidation current of CO started at +1.2 V. According to these hydrodynamic voltammograms, the application of potentials lower than +1.1 V would promote the detection of DCF without CO interference, and so this value was selected as the first potential pulse. If potential values higher than +1.2 V were employed, the electrochemical oxidation of both DCF and CO would be verified. Then, a second potential pulse (+1.4 V) was selected at which both compounds were electrochemically oxidized. The oxidation current from CO was obtained by subtraction of the currents detected at the two potential pulses, similarly to previous studies [25,27,32]. A third potential pulse (0.0 V/200 ms) was applied to avoid contamination/passivation of the working electrode surface.

Fig. 2 shows amperograms obtained at +1.1 V (50 ms) and at +1.4 V (50 ms) for duplicate injections of solutions containing 40 μmol L^{-1} of DCF or 40 μmol L^{-1} of CO, or a mixture of 40 μmol L^{-1} of DCF+40 μmol L^{-1} of CO.

As can be seen, only DCF was oxidized at +1.1 V, while at +1.4 V both DCF and CO were oxidized. It can be also observed that the oxidation current detected for DCF at +1.1 V was similar in the presence or absence of CO, indicating the absence of any chemical interaction between the compounds or CO detection at 1.1 V. In addition, although reasonable results were obtained without applying the third potential pulse (0.0 V for 200 ms), improved stability was observed after long-term experiments if the third potential pulse was applied.

It is worth mentioning that DCF oxidation currents were not the same at the two potential pulses (+1.1 V and +1.4 V). The oxidation current of DCF at +1.4 V was higher than the current detected at +1.1 V. Therefore, a simple subtraction between the currents detected at the two potential pulses does not directly yield the absolute value of CO oxidation current at +1.4 V. Since the selective determination of CO depends on subtraction of the DCF current due to its oxidation at +1.4 V, a *correction factor* (*CF*) can be calculated based on the ratio of the current responses to DCF oxidation registered at +1.1 and +1.4 V. This *CF* was obtained by injecting a solution containing only DCF in the BIA-MPA system and the following equation:

$$CF = \frac{i_{\text{DCF at } +1.4\text{ V}}}{i_{\text{DCF at } +1.1\text{ V}}} \quad (1)$$

Then, if solutions or samples containing both compounds are injected in the BIA-MPA system, the current originating from CO oxidation detected at +1.4 V can be calculated using the *CF* value and the following equation:

$$I_{CO} = i_{+1.4\text{ V}} - (CF \times i_{+1.1\text{ V}}) \quad (2)$$

It is important to emphasize that the *CF* value was obtained by the injection of different concentrations of DCF in the linear concentration range (5.5–60 μmol L^{-1}) and the value was constant (1.10 ± 0.04; RSD=3.6%, $n=7$) using the following conditions: dispenser rate of 4.5 mL min^{-1} and injection volume of 150 μL.

The influence of stirring of the solution into the BIA cell (electrolyte) was also evaluated. The response was considerably influenced by solutions with and without stirring, similar to that observed in a previous work [46]. Under stirring, the peak currents from both compounds decrease rapidly to baseline and excellent analytical frequency could be obtained (~300 injections h^{-1}). However, if the solution was maintained without stirring, the peak currents slowly decreased to baseline and analytical frequency considerably diminished (25 injections h^{-1}). This occurs because, under stirring, both compounds are quickly removed from the electrode surface. Furthermore it is likely that the cleaning or activation procedure is more effective if the solution is under stirring. The stability of the BDD electrode coupled to the BIA-MPA system was examined by successive injections ($n=10$) of solutions containing DCF and CO (60 and 43 μmol L^{-1}, respectively). The relative standard deviation was 1.1% for DCF (detected at 1.1 V) and 0.9% for CO (detected at 1.4 V and using the *CF*).

The calibration curves for both compounds were obtained by taking into consideration the concentration range for which the *correction factor* (*CF*) was constant and the molar concentration proportion found in commercial pharmaceutical samples (approximately 1.4-fold more of DCF than CO). Fig. 3A shows the amperograms obtained at +1.1 V and +1.4 V for triplicate injections of two solutions containing only DCF (a and b) which were used for calculation of the *CF*, five solutions containing increasing concentrations of both DCF (c–g: 10.0–50.0 μmol L^{-1}) and CO (c–g: 7.1–35.7 μmol L^{-1}), and two properly diluted samples (1 and 2). The current response from CO oxidation was calculated using the *CF* and Eq. (2). The respective calibration curves are also presented (Fig. 3B and C).

It can be observed in Fig. 3 that the standard solutions injected in ascending or descending order presented similar responses that confirm that the phenomenon of electrode contamination or memory effect is prevented even using a solid and unmodified working electrode (BDD). The analytic characteristics of the BIA-MPA method are shown in Table 1.

3.2. CE-C^4D

The strategy adopted in the present study for simultaneous separation of anions (DCF) and cations (CO) using a regular CE system was the use of high magnitude normal electroosmotic flow (EOF). Under this condition (pH > 7), both anions (counter-EOF mode) and cations (co-EOF mode) are swept downward in the same direction toward the detector [40].This approach is especially recommended for the separation of anions with low electrophoretic mobility, such as DCF. Initially, the BGE composed of TRIS/TAPS (10 mmol L^{-1} each; pH 8.2) previously used for simultaneous separation of DCF and its common counter-ions was tested [54] (Fig. 4a).

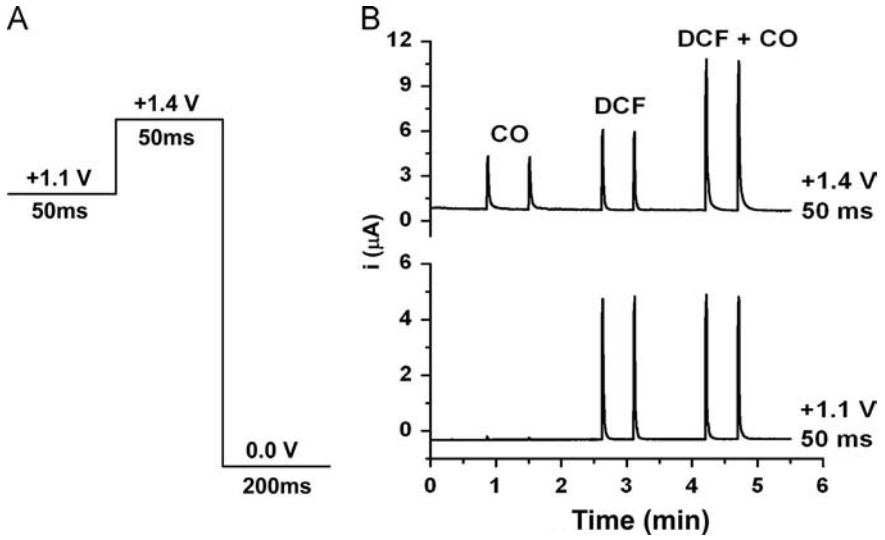

Fig. 2. (A) Potential pulse scheme; (B) Amperometric responses ($n=2$) obtained after injections in the BIA-MPA system of solutions containing only DCF (40 μmol L^{-1}), only CO (40 μmol L^{-1}) or DCF+CO (40+40 μmol L^{-1}). Applied potential pulses: +1.1 V/50 ms; +1.4 V/50 ms; 0.0 V/200 ms (cleaning potential pulse; signal not shown); dispensing rate: 6.0 mL min^{-1}; injected volume: 100 μL.

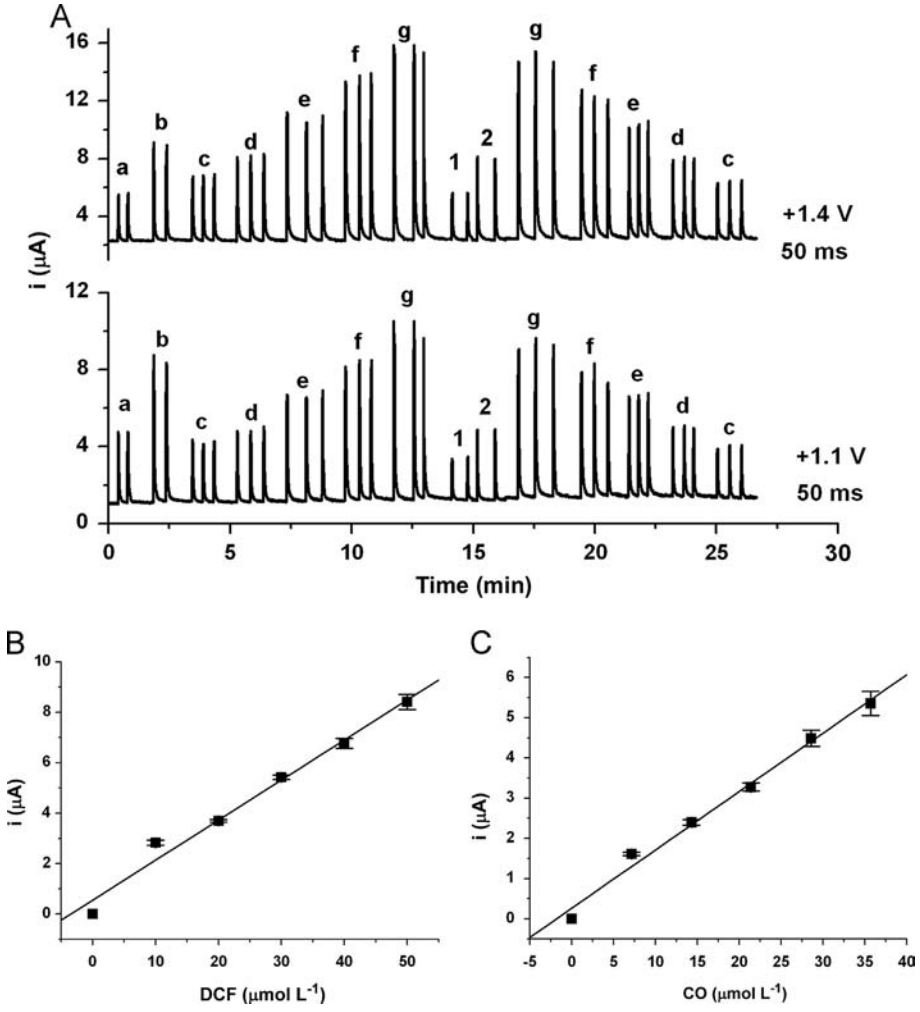

Fig. 3. (A) BIA-MPA amperograms obtained after injections of 2 solutions containing only DCF (a and b); 5 solutions containing simultaneously increasing concentration of DCF (c–g: 10.0–50.0 μmol L^{-1}) and CO (c–g: 7.1–35.7 μmol L^{-1}); and 2 appropriately diluted samples (1 and 2). (B) Calibration curve of DCF ($r=0.991$); (C) calibration curve of CO ($r=0.995$). Dispensing rate: 4.5 mL min^{-1}; injected volume: 150 μL. For other conditions see Fig. 2.

The 10 mmol L^{-1} TRIS/TAPS buffer did not reached the deliver results since its conductivity was similar to CO and the sensitivity (conductometric detector) of the method was compromised (Fig. 4a). To identify a better BGE for simultaneous determination of CO and DCF, TEA which shows higher conductivity and an adequate *p*Ka value (8.4) was evaluated as a component of the

Table 1
Analytical characteristics of the proposed methods.

Characteristics	BIA-MPA		CE-C^4D	
	CO	DCF	CO	DCF
LR (μmol L^{-1})	7–36	10–50	250–1250	250–1250
r	0.995	0.991	0.996	0.997
LOD (μmol L^{-1})	1.0	1.1	21	11
AN (h^{-1})	300	300	90	90
Intra-day RSD ($n=10$)	0.9%	1.1%	0.7%	0.3%
Inter-day RSD ($n=3$)	8.7%	9.2%	1.5%	3.8%
RT ($n=3$)	112 ± 3%	98 ± 4%	97 ± 2%	101 ± 3%

LR: linear range; *r*: correlation coefficient; LOD: limit of detection; AN: analytical frequency; RSD: relative standard deviation; RT: recovery test; Confidence interval = 95%.

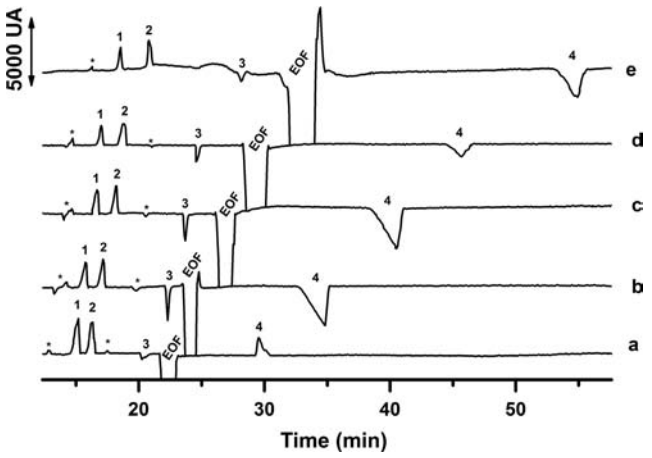

Fig. 4. Electropherograms obtained from standard solution containing sodium, DCF and CO (350 μmol L^{-1} each) and Li^+ as an IS (300 μmol L^{-1}) using the BGE composite with (a) 10 mmol L^{-1} TRIS/TAPS (pH 8.2) or 10 mmol L^{-1} TEA and pH adjusted to 8.4 with: (b) oxalic acid; (c) sulfuric acid, (d) lactic acid, and (e) phosphoric acid. Peaks: (*) system peaks; (1) Na^+; (2) Li^+ (IS); (3) CO; (4) DCF^-. CE conditions: normal EOF; separation voltage: 25 kV, effective capillary length: 10 cm, hydrodynamic injection: 25 kPa for 0.6 s.

BGE. Oxalic, sulfuric, lactic, and phosphoric acids were evaluated for adjusting the pH of the BGE to 8.4. The results are presented in Fig. 4. As can be observed, the BGE composed of 10 mmol L^{-1} of TEA and 1.8 mmol L^{-1} of OXA (pH = 8.4) provided a better signal-to-noise ratio, analysis time, and sensitivity. This BGE was selected for the following experiments.

The effects of variables such as separation potential and injection time were also studied. An applied potential of 25 kV and injection time of 0.6 s (25 kPa) yielded the best compromise in terms of efficiency, resolution, signal-to-noise ratio, and analysis time. Fig. 5 presents electropherograms corresponding to the injection of standard solutions containing sodium, DCF and CO at different concentrations (from 250 to 1250 μmol L^{-1}). Lithium was added in all solutions as an internal standard (IS).

A linear relationship was observed between peak areas and concentrations of sodium, CO, and DCF. The CE-C^4D system has an additional advantage over the BIA-MPA system. In the same experiment, the CE-C^4D system also allows the determination of sodium (in addition to CO and DCF) and the stoichiometric characterization of sodium DCF salt (not shown) is also possible in less than 1 min (a single run). The analytic characteristics of the CE-C^4D method are shown in Table 1.

3.3. Comparison of the two proposed methods (BIA-MPA and CE-C^4D)

Table 1 shows the analytical characteristics of the two new proposed methods.

It can be seen that both proposed methods presented appropriate analytical characteristics for simultaneous determination of CO and DCF. Both methods exhibited similar standard deviations for replicate analysis ($<1.1\%$), percentage of recovery of close to 100%, and correlation coefficients of better than 0.99. However, the BIA-MPA method presented a lower limit of detection and higher analytical frequency than CE-C^4D. On the other hand, only the CE-C^4D method allows a fast stoichiometric characterization of sodium DCF salt, because it enables the simultaneous determination of sodium and DCF. In addition, the CE-C^4D method presented a better inter-day precision, probably due to the use of an internal standard.

The effect of some potential interferents (used as excipients in this pharmaceutical formulation), such as starch, colloidal silicon dioxide, dibasic calcium phosphate, magnesium stearate, talc, titanium dioxide, polyethoxylated castor oil, hydroxypropyl methylcellulose, and ferric oxide was also investigated. These compounds were added to standard solutions containing 30 μmol L^{-1} (BIA-MPA) or 500 μmol L^{-1} (CE-C^4D) of CO and DCF. These studies were performed with the concentration ratio of CO:DCF:interferent of 1:1:10. The corresponding responses were compared with those obtained in the absence of each interferent. There were no significant differences ($<3.0\%$) between the responses of the solutions without and with addition of the potential interferents. Consequently, CO and DCF can be accurately determined in the concomitant presence of those compounds using the proposed methods.

The proposed BIA-MPA and CE-C^4D methods were also used to determine CO and DCF in two pharmaceutical samples. For comparison, the pharmaceutical samples were also analyzed by HPLC. However, in this case, CO and DCF were determined in two different chromatographic measurements. Table 2 shows the results of the analysis of these samples with their respective standard deviations ($n=3$).

All results obtained by the proposed methods were in agreement with those obtained by HPLC. At the 95% confidence level, the calculated *t*-test values were smaller than the critical value ($t_{n-1}=2.78$; $n=6$), indicating that there were no significant differences between the results. According to the statistical *F*-test, no significant difference was detected in the standard deviations between the results of the two methods at 95% confidence level (in all cases, $F_{calculated} < F_{critical} = 19.0$). The accuracy of the proposed BIA method was also evaluated by recovery tests using samples spiked with DCF and CO and the values obtained ranged from 97 to 112% (Table 1).

4. Conclusions

Two new and simple electrochemical methods for simultaneous determination of CO and DCF are presented here. The first one is by using batch injection analysis with multiple pulse amperometric detection (BIA-MPA); the second is by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D). Both methods are highly precise (RSD < 1.1%; $n=10$), accurate (confirmed by comparison with HPLC results and recovery tests), very fast (300 h^{-1} by BIA and 90 h^{-1} by CE), and exhibit good linearity ($r>0.99$ in all cases). The limits of detection were 1.0 and 1.1 μmol L^{-1} (BIA) and 21 and 11 μmol L^{-1} (CE) for CO and DCF, respectively. In addition to the desirable characteristics, it is important to emphasize that both proposed systems

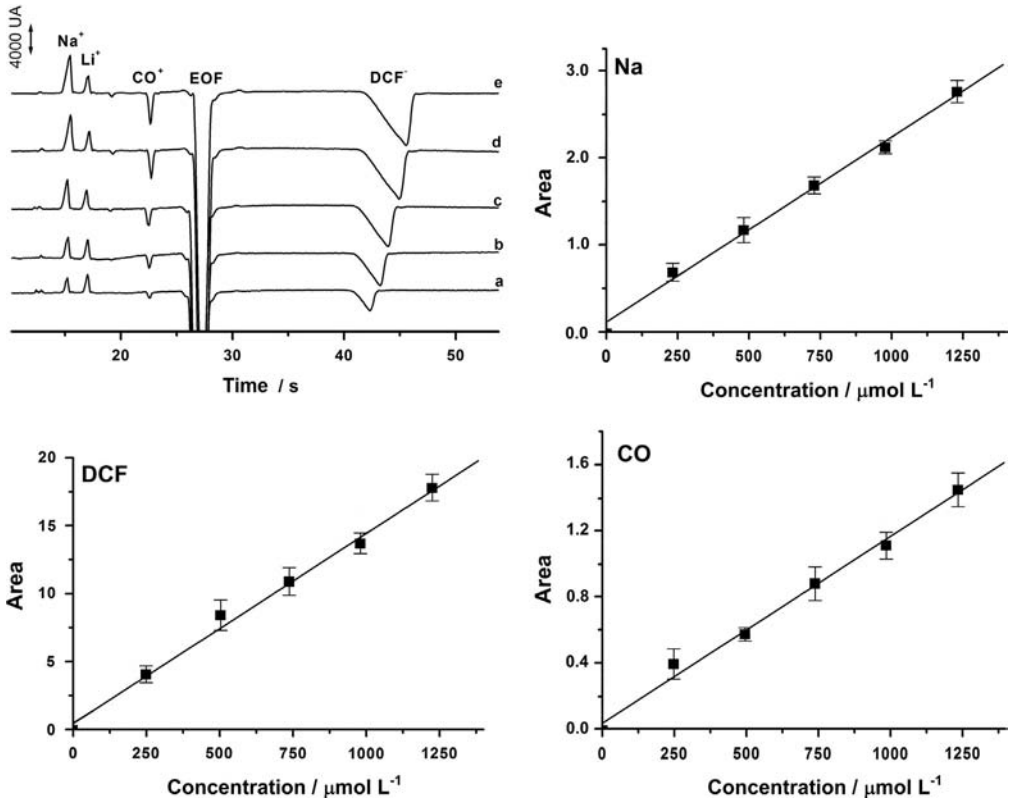

Fig. 5. Electropherograms obtained from injection of standard solutions containing Na, DCF and CO from 250 to 1250 μmol L^{-1} and their respective calibration curves. IS: Li 600 μmol L^{-1}. For other conditions see Fig. 4.

Table 2
Comparison of the results obtained for simultaneous determination of CO and DCF in pharmaceutical formulations by BIA-MPA, CE-C^4D and HPLC ($n=3$).

Sample	Analyte	Label value (mg/tablet)	BIA-MPA (mg/tablet)	CE-C^4D (mg/tablet)	HPLC (mg/tablet)
A	CO	38.0	37.6 ± 1.3	34.5 ± 0.9	34.8 ± 0.6
	DCF	46.4	42.6 ± 0.9	41.8 ± 1.1	42.2 ± 0.8
B	CO	38.0	39.4 ± 1.6	37.1 ± 1.2	37.4 ± 0.7
	DCF	46.4	46.1 ± 1.8	45.9 ± 1.1	46.2 ± 1.0

allow an analysis that is not possible using HPLC (C18 column) with a single chromatogram.

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