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Using multiple short-end injections to develop fast electrophoretic separations—Applications in iodide analysis

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ABSTRACT

The aim of this study was to develop a fast CE separation method by using multiple short-end injections in a capillary coated with quaternary ammonium chitosan (HACC), in order to determine the iodide content of pharmaceutical formulations. The BGE was composed of 20 mM tris(hydroxymethyl)aminomethane and 11 mM hydrochloric acid, at pH 8. The internal standard used was thiocyanate. Separations were performed in a fused silica capillary (32 cm total length, 8.5 cm effective length and 50 μm i.d.) coated with HACC and direct UV detection at 220 nm. EOF was modified by flushing the capillary with polymeric solution, resulting in a semi-permanent coating of controlled and stable EOF. The EOF was anodic at pH 8. Different strategies, using single and multiple injection short-end configurations, were studied to develop a CE method that resulted in a maximum number of iodide samples analyzed per hour: one plug and flush (Sflush) 35 samples/h, one plug without flush (SWflush) 76 samples/h, four plugs and flush (Mflush) 61 samples/h, and four plugs without flush (MWflush) 80 samples/h. Using the multiple injection configuration, it was possible to inject up to four plugs using spacer electrolytes with good separation efficiency and selectivity. The voltage application time needed to separate the eight peaks (iodide and thiocyanate) with MWflush was only 12 s. The method was validated and samples were analyzed using MWflush. Good linearity ($R^2 > 0.999$); a limit of detection 0.4 mg L^{-1}; intermediate precision better than 3.8% (peak area) and recovery in the range of 99–102% were obtained.

1. Introduction

In recent years, capillary electrophoresis (CE) has become one of the preferred separation techniques. This is the result of a number of factors, including improvements in the technology of CE instruments, significant advances in the development of new methodologies for the analysis of various molecules in different types of samples, and the existence of a vast body of literature that supports the understanding and implementation of this technique [1]. An area of great potential for CE is the analysis of pharmaceuticals. CE offers an attractive alternative to Liquid-Chromatography (LC) when conducting quality control for pharmaceuticals. High efficiency, short analysis time, simple instrumentation, and low sample and solvent consumption are the main reasons for this advantage [2]. CE has gained importance in numerous pharmaceutical applications and is increasingly employed for routine analysis.

An interesting characteristic of CE is its potential to quickly separate several analytes. The high degree of efficiency achieved with CE renders it useful in the development of fast separation methods that take only a few seconds. CE plays an essential role in the development of high-throughput analysis systems. The throughput of electrophoretic separations can most obviously be enhanced through the use of multiplexed capillary array instruments, which allow for the simultaneous separation of many samples, and the use of short separation capillaries [3]. The minimum size of the capillary that may be used is limited by the instrumental configuration of the CE, which varies according to the manufacturer [4]. The total analysis time (TAT) for each sample consists of the time necessary for the flush procedure, the sample injection, separation, and vial permutation. Shortening or eliminating one or more operations decreases TAT [5]. Some strategies for decreasing TAT without compromising separation efficiency and selectivity include: using a high electric field; accelerating electroosmotic flow (EOF) by covering the capillary's internal surface with a specific agent or adding an EOF modifier to the background electrolyte (BGE); and employing short-end or multiple-injection procedures. Each strategy, individually or in combination with other strategies, can diminish TAT and consequently increase the analytical frequency of a method [2,4]. In CE, the multiple-injection mode makes it possible to analyze two or more sequentially injected samples within a single run. In pharmaceutical analysis, multiple-injection methodology has been used for local anaesthetic separation (lidocaine) [2,6]; synthetic peptides (e.g. buserelin) [7]; β_2-receptor agonists (e.g. salbutamol) [8]; and hormones (e.g. insulin) [9]. However, before

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employing multiple-injection, a sample must be analyzed using the single-injection mode, to identify possible interferents and indicate whether multiple-injection is possible [10]. The advantage of multiple-injections, when analyzing pharmaceutical formulations, is that in most cases, the principal components are few and known.

Iodide (I^-) compounds are often used in pharmaceutical products, especially to form potassium iodide. I^- may be used as an expectorant agent that facilitates the removal of secretions of the bronchopulmonary mucous membrane and as an inhibitor of the synthesis of thyroid hormones in patients with hyperthyroidism. What is more, in some countries, potassium iodide tablets are sold in drug stores for thyroid protection in the event of a nuclear emergency [11,12]. Some of the methods employed to determine the concentration of I^- in samples are: ion chromatography [13–15]; capillary electrophoresis [16]; reverse-flow injection spectrophotometric [17]; and flow injection, using iodine–starch reactions [11]. However, these methods have the disadvantage of involving chemical reactions and/or analysis that takes several minutes, which often compromises analytical frequency.

The aim of this study was to develop a fast separation method for CE by using multiple sequential short-end injections in a capillary coated with quaternary ammonium chitosan (HACC) to determine the concentration of I^- in pharmaceutical formulations. The selection of BGE components and the multiple-injection optimizations were performed using Peakmaster software.

2. Experimental

2.1. Chemicals and solutions

Chitosan (CTS), with a deacetylation degree of 90% and a molecular weight of 122.74 kDa, was acquired from Purifarma (São Paulo, Brazil), and glycidyl trimethyl ammonium chloride was purchased from Fluka Chemica (content ≥ 90%, w/w). These were used to synthesize quaternized chitosan (HACC), as described by Qin [18]. A stock solution of 1.0% (w/v) HACC was prepared in deionized water, filtered through a membrane of 45 μm, and reserved for modification of the capillary's surface. 2.5 mM of cetyltrimethylammonium bromide (CTAB) was used to modify the capillary and compared to the capillary coated with HACC. Phosphoric acid, sodium dihydrogen phosphate, tris(hydroxymethyl)aminomethane (Tris), 2-hydroxy-isobutyric (HIBA), acetic acid, morpholine ethanesulfonic acid (MES), sodium hydrogen carbonate, and sodium carbonate, all of analytical-grade purity, were used to prepare buffer solutions and measure EOF. The buffer solutions, and their respective pH values, that were used to measure EOF were: phosphate (pH 2.4 and 2.8); Tris/HIBA (pH 3.3–4.2; and pH 7.6–8.9); Tris/acetic acid (pH 4.7 and 5.3); Tris/MES (pH 5.9–7.4); and carbonate (pH 9.5), all with an ionic strength of around 20 mM and a buffering capacity above 6.0 mM. Stock solutions of hydrochloric acid (44 mM) and Tris (80 mM), reagents purchased from Labsynth (Diadema, Brazil), were used to prepare the running electrolyte. Standard stock solutions (10 mM) of potassium iodide (KI) and internal standard (IS) potassium thiocyanate (KSCN), all purchased from Labsynth (Diadema, Brazil), were dissolved in deionized water. Calibrated solutions of I^- were prepared by diluting the stock solution. Water deionized in a Milli-Q system (Millipore, Bedford, MA, USA), with a resistivity of 18.2 MΩ cm, was used to prepare all solutions.

2.2. Preparation of the sample and the capillary electrophoresis system

Three samples of pharmaco, each containing potassium I^- 100 mg/5 mL and produced by different companies, were purchased in a local market. Prior to CE analysis, the samples were diluted at a 1:100 ratio with deionized water in volumetric flasks, and then diluted 1:10 with water and a SCN^- solution (IS, final concentration 58 mg L^{-1}). To optimize separation, all experiments were conducted on a CE system (HP^{3D} CE, Agilent Technologies, Palo Alto, USA) equipped with a diode array detector set at 220 nm, a temperature control device (temperature maintained at 25 °C), and data treatment software (HP ChemStation). The BGE used to determine I^- concentration was composed of 20 mM Tris and 11 mM hydrochloric acid, at pH 8.0. The standards and samples were introduced at the short end of the capillary and injected using hydrodynamic pressure (50 mbar = 4997.0 Pa), according to the following steps: −50 mbar/3 s (sample or standard); −50 mbar/7 s (spacer electrolyte); −50 mbar/3 s (sample or standard); −50 mbar/8 s (spacer electrolyte); −50 mbar/3 s (sample or standard); −50 mbar/8 s (spacer electrolyte); −50 mbar/3 s (sample or standard). The separation voltage applied was 30 kV, with negative polarity on the injection side. For all experiments, a fused-silica capillary (Microtube, Araraquara, SP, Brazil) of 32 cm (8.5 cm effective length × 50 μm i.d. × 375 μm O.D.) was used. The capillary received a static coating of HACC, in accordance with the following procedure: flush 5 min with HACC 0.2%, maintain static contact for 5 min, and, finally, flush the capillary for 5 min with BGE (without HACC). For purposes of comparison, the same procedure was employed with CTAB. The EOF of the coated capillary was measured according to the procedure described by Williams and Vigh [19], using acetone as a neutral marker.

3. Results and discussion

3.1. Optimizing parameters to develop a rapid method for iodide determination

3.1.1. Choosing BGE components and internal standard

The I^- anion is a species that absorbs in the ultraviolet region (UV) and is also a fast anion ($\mu = -79.6 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$) that is fully ionized between pH of 1 and 14. Thus co- and counter-ion components of the electrolyte are independent of pH, whereas other species with characteristics similar to those of I^- (high mobility and UV absorption) are not present in the sample. The co-ion selected as the running electrolyte is chloride, since it has a mobility ($\mu = -79.1 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$) very close to that of I^-, thus avoiding the effects of asymmetric peaking for the analyte quantified by electromigration dispersion (EMD). For I^- the EMD is equal to 0.06 with chloride as co-ion—data obtained using Peakmaster® software [20–22]. Furthermore, this species does not absorb UV, allowing it to be used in the direct detection of I^-. The counter-ion electrolyte chosen was the Tris, the separation pH was about 8, since this molecule has a pK_a of around 8, thus the buffering capacity of the electrolyte was maximized. The IS selected was SCN^-, a species that, like I^-, absorbs in UV and also has mobility ($\mu = -68.5 \times 10^{-9}\ m^2\ V^{-1}\ s^{-1}$) close to that of I^-.

3.1.2. Optimizing the ionic strength of the BGE

The BGE ionic strength was selected by observing how the current changed in relation to the amount of voltage applied. For this study, solutions of BGE Tris/HCl, at pH ~ 8, with ionic strength ranging from 11 to 43 mM and voltages ranging from −5 to −30 kV, were employed. Of all the solutions tested, the lower ionic strength showed the best correlation coefficient (0.998) for the entire range of applied voltage, which indicates a separation free of Joule heating. Thus, the ionic strength chosen for the running electrolyte was 11 mM. With an interest in obtaining the fastest separation possible, the voltage selected was −30 kV.

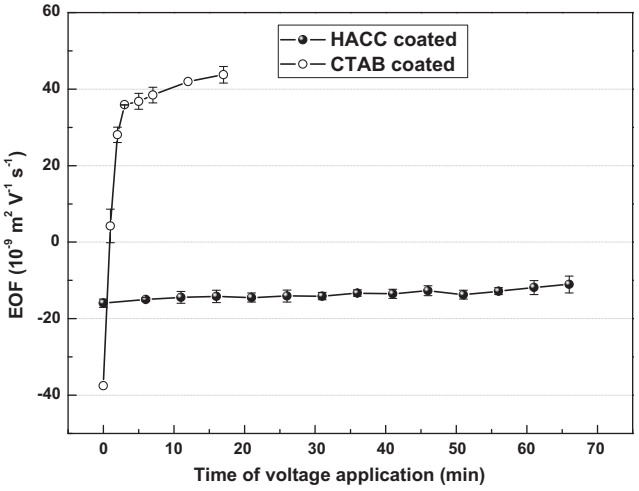

Fig. 1. Influence of duration of voltage application (−30 kV) on EOF stability, using the modifiers HACC and CTAB. Experimental conditions: electrolyte Tris 20 mM/HCl 11 mM, pH 8.0, ionic strength 11 mM; capillary 32 cm (L_{tot}), 50 μm i.d. and 23.5 cm (L_{det}); EOF measurements using acetone as flow marker, migration time 30 s and voltage −15 kV. EOF values expressed as the mean and standard deviation: HACC, $n = 3$ (one cycle in one capillary and two cycles in another capillary) and CTAB, $n = 2$ (two cycles in one capillary).

3.1.3. Modifying and characterizing the electroosmotic flow

In order to separate anions as fast as possible using CE, is necessary to reverse EOF to promote the migration of analytes in the same direction as EOF (co-electroosmotic mode). The HACC modifier was employed as an EOF-reverser [23], used as an additive in the BGE for anion separations. In this study, HACC was used to reverse EOF simply by washing the capillary with a polymer solution, i.e., without being added to the BGE. The HACC concentration used in the wash solution to ensure saturation of the surface of the capillary with the polymer and to generate a constant anodic EOF was 0.20%. The EOF stability in the capillary coated with HACC was compared with a capillary coated with CTAB (EOF-reverser commonly used in CE), using the same modification procedure. Fig. 1 shows variations in EOF as a function of the application time of voltage in capillaries coated by being washed with solutions of HACC or CTAB. After about 1 min of applying 30 kV to the capillary modified with CTAB, EOF was reversed, indicating the removal of much of the CTAB from the capillary surface. Meanwhile, in the capillary coated with HACC, the EOF remains almost constant until about 60 min of voltage application, showing that the modification is stable. The coefficient of variation (CV) of the EOF values measured during 31 min of voltage application was about 4.6%, and for 66 min of voltage application was 9.0%. Thus, the semi-permanent coating with HACC proved much more stable than the semi-permanent coating with CTAB. Furthermore, when CTAB is used as an EOF-reverser (as an additive in the BGE) creates a system peak very near that of I^-, since the bromide present in the BGE is the counter-ion of CTAB, which undermines separation. With HACC, this peak does not occur because its counter-ion is chloride. Also the noise in the baseline electropherogram is reduced with HACC. Thus, HACC may potentially be used as a semi-permanent EOF-reverser in I^- analysis.

3.1.4. Optimizing the injection strategy

3.1.4.1. Simulation of the multiple injections. The injections tested were: single injection (a plug of the sample), flushing the capillary with the BGE between runs (Sflush) and not flushing it between runs (SWflush); and multiple injections (over a plug of the sample) flushing the capillary with the BGE between runs (Mflush) and not flushing it between runs (MWflush). All injections were made on the short-end path from the capillary to the detector. For multiple injections, the maximum number of plugs that could be injected was calculated, and the separation was simulated using the Peakmaster. The number of plugs that may be injected (η_S) when multiple injections are being performed [10] can be calculated using the following equations:

$$\eta_S = \frac{t_{mig(SCN)}}{(\Delta t_{mig} + 12\sigma)} \quad (1)$$

$$\sigma = \frac{W_{50\%}}{2.35} \quad (2)$$

where $t_{mig(SCN)}$ is the migration time of the SCN^-, Δt_{mig} is the difference in migration time between SCN^- and I^-, σ is the broadest peak, and $W_{50\%}$ is the peak width at half height. The values of $t_{mig(SCN)}$, Δt_{mig}, $W_{50\%}$, were obtained by using the Peakmaster to simulate the separation of I^- and SCN^- (for simulation conditions, see Fig. 2). The value of $W_{50\%}$ for the SCN^- (which had a broader peak, limiting resolution), obtained using the simulated electropherogram, was 0.24 s, and the migration times of I^- and SCN^- were 9.96 and 10.98 s, respectively. These values were used to calculate an η_S of 4.89. As the value obtained was not an integer, we deemed it wise to make the maximum number of injected plugs four, in order to avoid compromising resolution. The simulation also estimated the time between spacer electrolytes, in order to separate the peaks of the injected plugs. The partial electrophoresis time of separation between pairs of neighboring peaks (t_{PE}—Fig. 2b), $t_{PE} = (\Delta t_{mig} + 12\sigma)$ – which represents sufficient separation between them, with a good resolution – was calculated. Considering that the number of plugs injected was 4 ($n = 4$), we predicted the migration time of the SCN^- of the P3 plug (injected prior to P4), as follows:

$$t_{mig(SCN)(Pn-1)} = t_{mig(SCN)(Pn)} - t_{PE} \quad (3)$$

where $t_{mig(SCN)(Pn-1)}$ is the migration time of the SCN^- of the P3 plug and $t_{mig(SCN)(Pn)}$ is the migration time of the SCN^- of the P4 plug. Thus, the predicted migration time of the SCN^- of the transferred plug (P3 plug) was 8.73 s. With $t_{mig(SCN)(Pn-1)}$ it is possible to calculate the introduction time of the plug to detect the SCN^- exactly on predicted migration time. This was done using the following equations:

$$t_{mig(SCN)(Pn-1)} = \frac{L_{det} - L_{inj}}{\mu_{SCN} E} \quad (4)$$

being that,

$$L_{inj} = \frac{\Delta P t_{int} r^2}{8 L_{tot} \eta} \quad (5)$$

$$\mu_{SCN} = \mu_{osm} + \mu_{eff} \quad (6)$$

substituting Eqs. (5) and (6) in Eq. (4), yielding:

$$t_{mig(SCN)(Pn-1)} = \frac{L_{det} - ((\Delta P t_{int} r^2)/(8 L_{tot} \eta))}{(\mu_{osm} + \mu_{eff}) E} \quad (7)$$

rearranged as,

$$t_{int} = \left(\frac{8 L_{tot} \eta}{\Delta P r^2}\right) [L_{det} - (t_{mig(SCN)(Pn-1)} E \{\mu_{osm} + \mu_{eff}\})] \quad (8)$$

where $t_{mig(SCN)(Pn-1)}$ and t_{int} are the migration time of the SCN^- of the transferred plug and the introduction time of this plug, respectively, in seconds; L_{det}, L_{inj} and L_{tot}, are the length of the capillary until the detector, the length of the introduction of the injected plug and the total length of the capillary, respectively, in meters; μ_{SCN}, μ_{osm} and μ_{eff} are the apparent mobility, the electroosmotic flow mobility and the effective mobility of SCN^-, respectively, in $m^2\ V^{-1}\ s^{-1}$; E is the applied electric field in $V\ m^{-1}$; ΔP is the injection pressure, equal to 4996.2 $N\ m^{-2}$ (99.92 $N\ m^{-2}$ = 1 mbar); and η

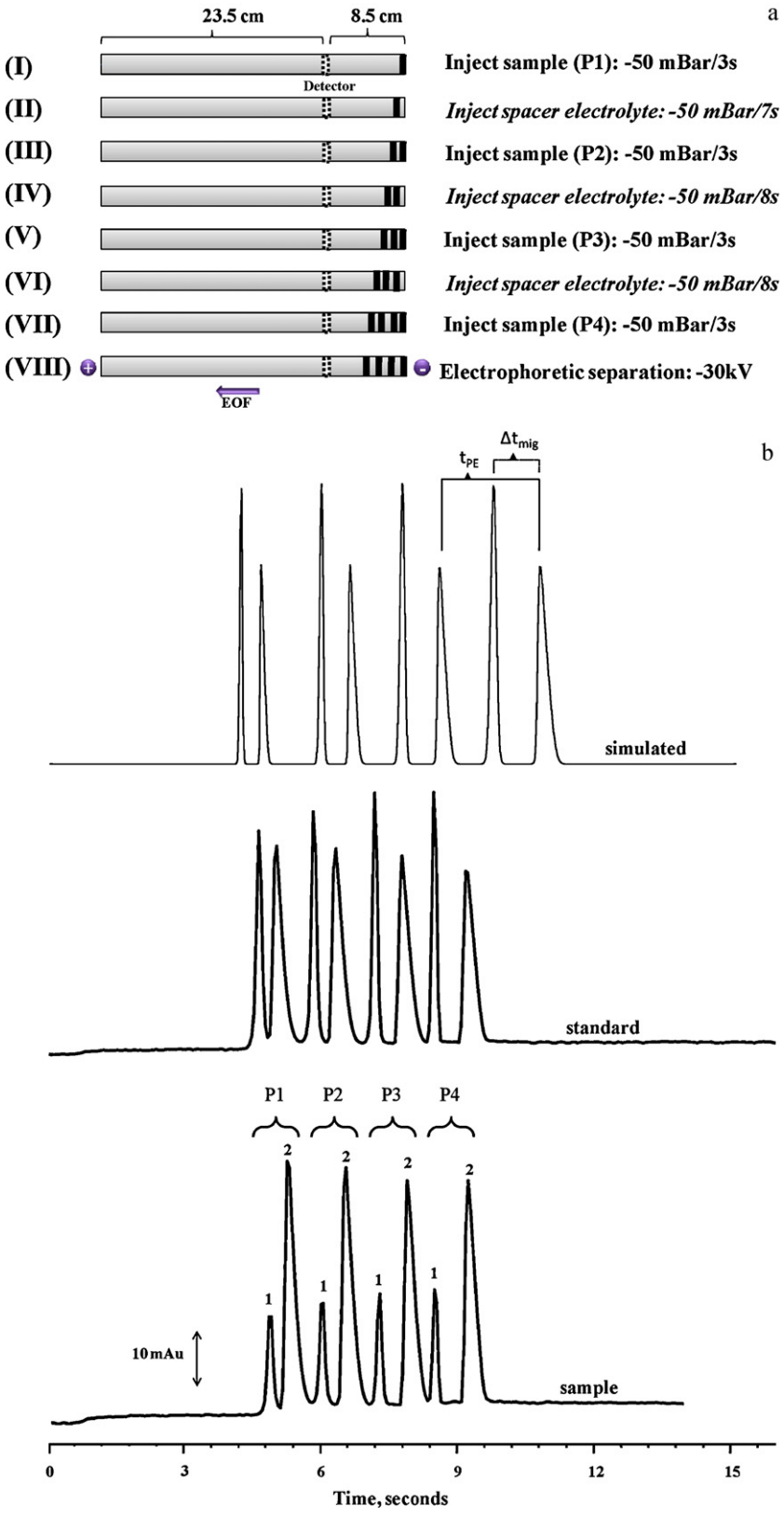

Fig. 2. (a) Scheme representing the multiple-injection mode and (b) electropherograms resulting from the MWflush multiple-injection method: simulation (using Peakmaster); injection of standard (I^- 63 mg L^{-1} and SCN^- 58 mg L^{-1}); injection of sample (I^- about 15 mg L^{-1} and SCN^- 58 mg L^{-1}). Experimental conditions: BGE Tris 20 mM/HCl 11 mM; pH 8.0; voltage −30 kV; L_{det} 8.5 cm, L_{tot} 32 cm × 50 μm i.d.; simulation using identical conditions and μ_{EOF} -16×10^{-9} m^2 V^{-1} s^{-1}. Peaks legend: 1: I^-, 2: SCN^-; P1: first injected plug, P2: second injected plug, P3: third injected plug, and P4: fourth injected plug.

is the solution viscosity, equal to 8.9×10^{-4} N s m^{-2} at 25 °C. Assuming values for each condition in Eq. (8) (for other conditions, see Fig. 2), the calculated value of the t_{int} of the P3 plug was 13.98 s. Knowing that the time of introduction is given by:

$$t_{\text{int}} = \sum t_{inject} + \sum t_{espa}$$
$$t_{\text{int}} = t_{inject\text{-P1}} + t_{inject\text{-P2}} + t_{inject\text{-P3}} + t_{inject\text{-P4}}) \quad (9)$$
$$+ (t_{espa\text{-P1/P2}} + t_{espa\text{-P2/P3}} + t_{espa\text{-P3/P4}}$$

where t_{inject} is the injection time of the plug and t_{espa} is the time of the spacer used, the injection time for each plug 3 s can be estimated by calculating the time of the spacer electrolyte between plugs P3 and P4:

$$t_{espa\text{-P3/P4}} = t_{\text{int}} - (t_{inject\text{-P3}} + t_{inject\text{-P4}}) \quad (10)$$

Using this equation, a spacer of 8 s was obtained. This value was taken to be the time between plugs in the multiple-injection simulations. With the time of introduction of each these plugs, $t_{int\text{-P4}}$ = 3 s, $t_{int\text{-P3}}$ = 14 s, $t_{int\text{-P2}}$ = 25 s and $t_{int\text{-P1}}$ = 36 s, it is possible to calculate the L_{det} for each plug transferred, input these values into the Peakmaster, and simulate the separation of the four plugs injected, $L_{det(Pn)} = L_{det} - L_{inj(Pn)}$. Knowing that L_{inj} is given by Eq. (5), the same conditions are stipulated: $L_{det(P4)}$ = 8.28 cm, $L_{det(P3)}$ = 6.58 cm, $L_{det(P2)}$ = 5.07 cm and $L_{det(P1)}$ = 3.57 cm. The electropherogram resulting from the simulation is shown in Fig. 2.

3.1.4.2. Minimization of total analysis time using different modes of injection. To determine which injection strategy maximized the number of samples analyzed per hour, we observed the total analysis time (TAT) of each mode of injection. The results, summarized in Table 1, reveal that the largest number of samples analyzed per hour – up to 80 – was obtained with MWflush injection. Also, experiments were performed in which the vial's position in the carousel was changed, to in order evaluate the influence of position on TAT. Four vials were placed in several positions in different sectors of the carousel (positions 08, 09, 10, 11, 20, 21, 30, 31, 40 and 41). The TATs of six different combinations of positions, measured as triplicates, had a coefficient of variation that was less than 1.2%, indicating that TTA is independent of the positions occupied by the samples in the equipment. An estimate of the time spent on each step of analysis of the Mflush injection revealed that flushing took up 23% (54 s) of TAT, injection 72% (168 s), and separation just 5% (12 s). This clearly shows that most of TAT is related to the instrumental speed of exchange of the vial during injection and flushing and that decreasing the time spent on electrophoretic separation will not lead to significant gains in TAT, i.e., that the effect of electrophoretic separation time on TAT was totally maximized.

Fig. 2 shows the scheme of multiple injections and electropherograms, simulated and experimental, using optimal MWflush conditions. The electropherograms predicted and obtained from the standard and the samples are close. The differences in the migration times of peaks P2, P3, and P4 can be attributed to the pas-

Table 1
Total analysis time (TAT) by different modes of injection.

Mode	Number of plug	TAT (min)	Time for each sample (min)	Number of samples analyzed per hour
Sflush[a]	1	1.68	1.68	35
Mflush[a,b]	4	3.89	0.972	61
SWflush	1	0.783	0.783	76
MWflush[b]	4	2.99	0.747	80

[a] Results were expressed as average times (measured in triplicate) and flush time 0.42 min.
[b] Times electrolyte spacer: 7 s; 8 s; 8 s.

Table 2
Figures of merit for iodide determination using the MWflush method optimized. For other conditions see Section 2.

Parameter		Value
Linearity-calibration range (mg L^{-1})	n = 6 (levels of concentration)	6.3–63
Linearity-slope (L mg^{-1})		0.0252
Slope standard deviation		0.0002
Linearity-intercept		0.0142
Intercept standard deviation		0.0029
Linearity-determination coefficient		0.999
LOD (mg L^{-1})[a]		0.4
LOQ (mg L^{-1})[a]		1.2
Instrumental precision, CV (%)—peak area[b]	n = 20	0.36–1.8 (0.60–0.87)
Instrumental precision, CV (%)—migration time[b]	n = 20	0.33–2.8 (0.41–2.1)
Intra-day precision, CV (%)—peak area[b]	n = 8	0.83–3.2 (1.3–2.1)
Intra-day precision, CV (%)—migration time[b]	n = 8	4.0–6.4 (2.3–6.6)
Intermediate precision, CV (%)—peak area[b]	n = 16	1.4–3.8 (3.0–3.3)
Intermediate precision, CV (%)—migration time[b]	n = 16	5.1–6.4 (3.6–6.3)
Number of plates per meter (N m^{-1}; I$^-$)[c]	15,220 (P1); 23,491 (P2); 46,296 (P3); 90,033 (P4)	
Peak symmetry (I$^-$)[c]	0.771 (P1); 1.05 (P2); 0.584 (P3); 0.873 (P4)	
Asymmetry factor (I$^-$)[c]	1.12 (P1); 1.03 (P2); 0.905 (P3); 0.973 (P4)	
Tailing factor (I$^-$)[c]	1.05 (P1); 1.02 (P2); 1.00 (P3); 0.997 (P4)	
Resolution (I$^-$:SCN$^-$)[c]	0.85 (P1); 0.92 (P2); 1.10 (P3); 1.41 (P4)	
Accuracy (% recovery)[d]	Add 12.7 mg L^{-1}	102
Accuracy (% recovery)[d]	Add 25.4 mg L^{-1}	99.6
Accuracy (% recovery)[d]	Add 38.1 mg L^{-1}	99.5
Accuracy (% recovery)[d]	Add 50.8 mg L^{-1}	100

[a] Limits of detection and quantification calculated according to the equations: LOD = (3.3 × s)/S; LOQ = (10 × s)/S, where s is the intercept standard deviation and S is the slope of the analytical curve equation, respectively.
[b] Coefficient of variation calculated with the equation: CV = ($P/\bar{u}$) × 100, where P is the absolute standard deviation and $\bar{u}$ is the arithmetic mean of the area ratios of the analyte to internal standard or arithmetic mean of the migration time. Values referents to a standard and a sample, outside and inside in the brackets, respectively. CV range relative to four peaks injected (P1, P2, P3 and P4).
[c] P1, first plug injected; P2, second plug injected; P3, third plug injected; P4, fourth plug injected. Number of plates and resolution calculated according to the equations, $N = 16(t_n/w_n)^2$ and $Rs = 2(t_n - t_{n-1})/(w_n + w_{n-1})$, respectively, where t is the migration time in minutes and w is the baseline peak width in minutes. Peak symmetry was obtained from ChemStation® software. Asymmetry factor was calculated using the distance from the center line of the peak to the back slope divided by the distance from the center line of the peak to the front slope, with all measurements made at 10% of the maximum peak height. Tailing factor was calculated using the distance from the front slope of the peak to the back slope divided by twice the distance from the center line of the peak to the front slope, with all measurements made at 5% of the maximum peak height.
[d] Experiments performed using diluted sample 1000 times.

sage of the last plug injected by the plugs initially injected (analyte plugs in deionized water), since the ionic strength of these plugs is smaller than that of the BGE, which influences the effective mobility of I^- and SCN^- when passing through regions of different ionic strengths.

Knowing that the HACC coating is relatively stable (for about 30 min when voltage is being applied—CV = 4.6%), we can estimate that there is enough time to perform about 150 runs, equivalent to analyzing 600 samples, using the MWflush injection method (each separation requires about 12 s of voltage). Thus, it is possible to perform analysis with the same coating over the course of about 7 h. After this period, the capillary must be re-coated.

3.2. Method figures of merit and determination of iodide in samples

To evaluate the suitability of the MWflush methodology when determining I^- in pharmaceutical formulations, the following validation parameters were evaluated according to ICH guidelines [24]: linearity, limit of detection, limit of quantification, repeatability (instrumental and intra-day), intermediate precision, and selectivity.

The selectivity of the proposed method was performed using the method of standard addition. The slopes of the standard addition curve (0.0239 ± 0.0014) and the external calibration curve (0.0252 ± 0.0002) were compared. The *t*-test showed that the slopes are statistically equal ($t_{calc} = 0.08$ and $t_{critic} = 2.3$, at 95% confidence level). Proximity between the slopes indicates that the method is suitably selective for the analysis of I^- in pharmaceutical formulations.

Instrumental precision was determined using a mixture of I^- and SCN^- (IS) with the MWflush mode, injecting the same sample several times (plugs 1, 2, 3 and 4) for $n = 20$. The CV for both of the corrected areas of the peaks, as well as for the migration time of each plug, was less than 3.0%. For intra-day precisions and intermediate determinations, higher CV were observed during migration times (<7%) than for the corrected peak area (<4%). However, these values may be appropriate, considering that migration time not only depends on the effective mobility of I^-, but on the precision of the pressure system of the equipment that injects the electrolyte spacers to separate the plugs. Intermediate precision was evaluated in accordance with daily variations (between two different days).

The calibration curve showed good linearity (coefficient of determination > 0.99) in a wide range of concentrations ($\sim$ 6.0–60.0 mg L^{-1}). The linear model showed significance with $F > 6700$ and did not show a lack of fit ($F_{calc} = 2.77$ and $F_{critic} = 4.96$, at 95% confidence level). Limits of detection and quantification (LOD and LOQ, respectively, calculated using the parameters of the calibration curve) were appropriate for the determination of I^- in the samples.

The values obtained for analysis of addition/recovery at four levels of concentration showed good agreement with the reference values, indicating that the method is accurate.

All the pharmaceutical formulations analyzed (average results with a confidence limit of 95%, for $n = 3$; samples prepared in triplicates and injected in triplicates) showed a small increase in I^-, in relation to the values reported, ranging from 6.1 to 9.7%. Of the three samples analyzed, one contained sodium benzoate as a preservative. The presence of benzoate anion in the sample could interfere in the separation of I^- when the MWflush method was applied, because benzoate presents an electrophoretic mobility of -33.6×10^{-9} m^2 V^{-1} s^{-1} and, in optimized separation conditions, migrates a few seconds after the separation of I^- and SCN^-. When a sample containing benzoate was injected at positions P1 or P2, the peak of this anion co-migrated with the peaks of subsequent injections (P3 and P4), interfering with separation. To resolve this problem, it was sufficient to inject the samples containing benzoate at positions P3 and P4; when this was done, the peaks of the benzoate migrated from P4 after the last peak of SCN^- (Table 2).

4. Conclusions

The simulations performed with Peakmaster allowed for the selection of appropriate components of the BGE. They also made it possible to accurately predict the experimental electropherogram and optimize spacer electrolyte times when the multiple-injection method was applied. The coating of capillaries with HACC to reverse EOF and to develop a method that allowed for rapid analysis of I^- proved to be simple, easy, and quick (only 15 min)—and also resulted in stable EOF. Also, the MWflush injection mode resulted in high analytical frequency (80 samples/h). Since instrumental speed limited analytical frequency, improving the technology of CE equipment is suggested. The proposed fastest method is very attractive for use in the quality control of pharmaceutical formulations.

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