Article

Electric Field-Assisted Solid Phase Extraction: Study of Electrochromatographic Parameters with an Anionic Model Compound

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Electric field-assisted solid phase extraction using syringe type cartridges (E-SPE) is a recent approach with a high capacity to obtain differential selectivity and/or recovery in comparison with conventional solid phase extraction (SPE). In this work, E-SPE was employed for the extraction of the anionic dye sunset yellow. During the washing step the electric field was evaluated with the top electrode negatively (E-SPE$_{(-/+)}$) or positively charged (E-SPE$_{(+/-)}$) and the results were compared with conventional SPE to understand how the electrochromatographic parameters can influence the recovery of the dye. Using the E-SPE$_{(-/+)}$ the electric field can increase the elution of the dye from the cartridge by over 57-fold. This notable result can be attributed to the electrophoretic velocity of the dye in the direction of the flow rate of the eluent. These results demonstrated that E-SPE is an interesting approach to elute ionic compounds with reduced volumes of eluent or less amount of organic solvent.

Keywords: electric field-assisted solid phase extraction, sample preparation, electrochromatography

Introduction

The development of analytical separation techniques, such as ultra-high performance liquid chromatography and capillary electrochromatography, both coupled to mass spectrometry, has allowed the determination of trace levels of many classes of substances in complex matrices. However, despite the advances achieved with these modern analytical techniques, sample preparation procedures are often still required and are frequently the critical step in analytical methods.[1-3]

The two most widespread sample preparation techniques are solid phase extraction (SPE) and liquid-liquid extraction, the former being based mainly on sorption mechanisms.[3] Solid phase extraction is widely used due to its advantages, including reduced solvent consumption, high capacity of automation and wide variety of available sorbent materials.[4] Since it was first reported in the literature about forty years ago, SPE has been improved dramatically regarding the development of new sorbent materials and automation systems.[1,2] Innovative sorbent materials, such as molecularly imprinted polymers (MIPs), have enabled high levels of selectivity for the extraction, while other materials, such as restricted access materials (RAMs), have allowed sequential on-line extractions of samples containing high amounts of proteins.[5,6]

Despite the recent advances in automation and sorbent materials for SPE, the mechanisms of this sample preparation technique rely only on liquid chromatographic principles. However, if different compounds demonstrate highly similar affinity to the sorbent, convection due to the overall flow (i.e., pressure) will not be able to separate them, and selectivity will be compromised.

Some reviews have described that the limitations of conventional SPE can be overcome using electric fields as an auxiliary energy source to improve speed, selectivity and extraction efficiency in sample preparation techniques.[7-9]

Recently, Orlando *et al.* described the use of electric-field assisted solid phase extraction (E-SPE) using adapted commercial syringe-type SPE cartridges.[10] The results obtained showed that E-SPE using a C18 sorbent was able to increase or diminish the recovery of the fluoroquinolone marbofloxacin extracted from milk in its cationic form depending on the polarities of the electrodes used.

In E-SPE there are two electrodes, one under and the other above the sorbent, which allow an electric field to be applied across the sorbent (Figure 1).[10]

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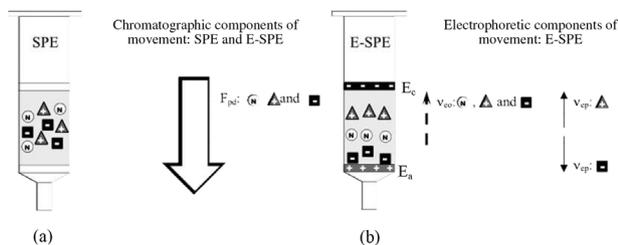
Figure 1. Hypothetical distribution of (N) neutral, (+) cationic and (-) anionic compounds with similar affinities for the sorbent in (a) conventional solid phase extraction (SPE) and in (b) electric field-assisted solid phase extraction (E-SPE). F_{pd}: flow rate; v_{eo}: electroosmotic velocity using a silica-based sorbent with a negative residual charge; v_{ep}: electrophoretic velocity; E_c: cathodic and E_a: anodic electrodes used in E-SPE.

Using the E-SPE approach, the same principles of electrochromatography can be explored during sample preparation. In E-SPE, the electroosmotic velocity (v_{eo}) of neutral and ionic compounds, as well as the electrophoretic velocity (v_{ep}) of cationic and anionic species, in the same or opposite direction of the overall flow rate may also contribute to the overall movement of analytes inside the sorbent bed (Figure 1). Because v_{eo} and v_{ep} are able to act in either the same or opposite direction of the overall solvent flow (i.e., due to pressure), they can be exploited to provide differential selectivity (clean-up) and extraction efficiencies compared to conventional SPE.

Conventional SPE with syringe cartridges is typically carried out in four steps: conditioning, sample loading, washing and final elution. The strategy in E-SPE is to select the correct polarity of the electrodes to improve the elution of the interferents during the sample loading and washing steps and/or to diminish the elution of the analyte of interest. For the final elution step, the strategy is the opposite.

The first work published on E-SPE showed the great potential of electrophoretic principles associated with SPE and the advantages that can be achieved during sample preparation of real complex matrices.[10] Nevertheless, more studies are necessary to elucidate the phenomena involved.

The present work describes, for the first time, the evaluation of E-SPE for an anionic model compound (sunset yellow). The aim was verify if the electric field can be used to increase or reduce the elution of the model compound during the washing step. For this purpose, five E-SPE parameters were evaluated and the results of the recoveries obtained were compared to those of conventional SPE.

Experimental

Chemicals and reagents

Sunset yellow (E 110) was obtained from Aldrich (Wisconsin, WI, USA). The chemical structure of the anionic dye used as a model compound is shown in Figure 2.
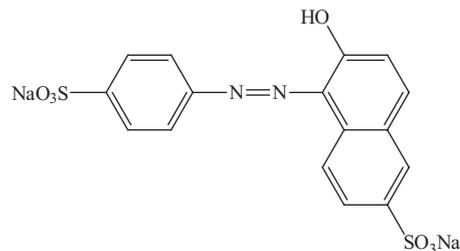
Figure 2. Chemical structure of the anionic dye.

Disodium hydrogen phosphate dodecahydrate, citric acid monohydrate, sodium chloride, sodium dodecyl sulfate purchased from Merck (Darmstadt, Germany) and ammonium phosphate dibasic (Qeel, São Paulo, SP, Brazil) were all analytical grade. Methanol (high performance liquid chromatography (HPLC) grade) was supplied by Tedia (Fairfield, OH, USA). Throughout the studies, high purity water was obtained from a Milli-Q system from Millipore (Bedford, MA, USA).

Buffer solutions

The citric acid-phosphate buffer (CPB) solutions used in this work were all similar to those described by McIlvaine.[11] The buffers were prepared by mixing appropriate quantities of citric acid and disodium hydrogen phosphate solutions to obtain solutions at the desired final pH. Except for the pH studied described in Table 1, all CPB solutions were prepared at pH 4.0.

The concentrations of citric acid and disodium hydrogen phosphate solutions used were as follows:

(*i*) 5 mmol L^{-1} citric acid and 10 mmol L^{-1} disodium hydrogen phosphate, referred to as CPB 5/10;

(*ii*) 10 mmol L^{-1} citric acid and 20 mmol L^{-1} disodium hydrogen phosphate, referred to as CPB 10/20;

(*iii*) 20 mmol L^{-1} citric acid and 40 mmol L^{-1} disodium hydrogen phosphate, referred to as CPB 20/40;

(*iv*) 100 mmol L^{-1} citric acid and 200 mmol L^{-1} disodium hydrogen phosphate, referred to as CPB 100/200.

Standard solutions

The E 110 stock solution (1000 µg mL^{-1}) was prepared by dissolving the standard in methanol:water (1:1, v/v), and the volume was then brought to 25 mL in a volumetric flask.

The working solution of E 110 (80 µg mL^{-1}) was prepared daily by dilution of the stock solution with CPB 100/200. For a better demonstration of the effect of the electric field on each parameter, specific adjustments

Table 1. Common and specific conditions used during the SPE and E-SPE parameters studied

Common conditions			
Samples		Electric field	
1.0 mL E 110 (80 µg mL^{-1}) prepared in CPB 100/200 buffer at the same pH of the washing solution and containing 0.9% (m/v) NaCl		Applied for the E-SPE studies during the washing step with the electric power supply operating at constant electric current	
Specific conditions			
Parameter studied	Washing solution	Electric current / mA	Sorbent (silica C18)
Influence of the concentration of salts in the washing solution[a]	CPB (10.0 mL): 20/40; 10/20; 5/10 pH 4.0 MeOH: 7% (v/v)	10	500 mg
Influence of the content of organic modifier in the washing solution[b]	CPB (10.0 mL): 20/40 pH 4.0 MeOH: 6; 8; 12; 16; 18% (v/v)	7	500 mg
Influence of the pH of the washing solution[a,c]	CPB (10.0 mL): 10/20 pH 3.0; 4.0; 6.0; 8.0 MeOH: 7% (v/v)	20	500 mg
Influence of the volume of the washing solution[a]	CPB (5.0; 10.0; 15.0 mL): 10/20 pH 4.0 MeOH: 7% (v/v)	12	500 mg
Influence of the sorbent amount[a]	CPB (5 mL): 10/20 pH 4.0 MeOH: 10% (v/v)	12	300; 500; 800 mg

[a]Samples containing 10% (v/v) of methanol; [b]samples containing 20% (v/v) of methanol; [c]conductivity of all solutions adjusted to 3.268 mS cm^{-1} by adding appropriate volumes of 25% (m/v) NaCl solution.

to the employed conditions were necessary, so that the pH of the CPB 100/200 used to prepare the working solution, as well as the methanol content added, varied from study to study (Table 1). However, NaCl was added to all of the working solutions to a final concentration of 0.9% (m/v).

E-SPE cartridges and flow extraction system

The E-SPE cartridges were assembled as previously described by Orlando *et al.*[10] and are summarily described in Figure 3.
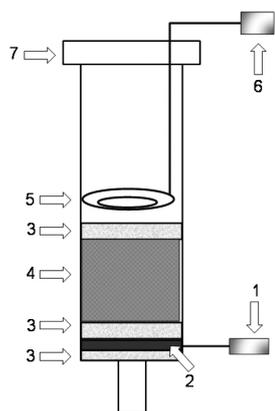
Figure 3. Assembly configuration used for the E-SPE cartridge. 1: electrical connection of the bottom electrode; 2: bottom electrode; 3: PTFE frits; 4: sorbent; 5: top electrodes; 6: electrical connection of the top electrode; 7: empty polypropylene cartridge.

The polypropylene SPE cartridges (6 mL capacity) and the polytetrafluoroethylene (PTFE) frits were obtained from Varian (Palo Alto, CA, USA). The stainless steel grids (120 mesh) used as the bottom electrodes were purchased from SteelMesh (Tatuapé, SP, Brazil). The electrical connection and electrical contact of the bottom electrode consisted of a needle (2.0 mm o.d. × 100 mm length × 1.6 mm i.d.; Höppner, São Paulo, SP, Brazil) coupled to a coil spring (20 mm length × 1.9 mm o.d.; Somol, Guarulhos, SP, Brazil). The top electrode was a wire (0.4 mm o.d.) in a spiral shape (11.5 mm o.d.) (Somol, Guarulhos, SP, Brazil).

Except for the study of the sorbent amount described in Table 1, all experiments employed 500 mg of endcapped Sepra-C18-E (50 µm particle size and 65 Å pore size) C18 silica-based material from Phenomenex (Torrance, CA, USA) as the sorbent.

The electric current, electric potential and flow rate were controlled and monitored during the extraction by the flow extraction system described previously.[10]

Extraction of anionic dyes using SPE and E-SPE

The solid phase extractions of E 110 with and without electric fields were performed in four steps using the following conditions: (*i*) Cartridge conditioning: 2.0 mL of methanol, followed by 2.0 mL of water and 2.0 mL

of CPB 100/200 at a flow rate of 1.0 mL min^{-1}. The CPB 100/200 buffers used for conditioning were prepared at the same pH (4.0, 6.0 or 8.0) as the corresponding standard working solution (Table 1) but without the addition of methanol and NaCl; (*ii*) Sample loading: 1.0 mL of the working solution of E 110, as described in Table 1; (*iii*) Washing: at an average flow rate of 1.0 mL min^{-1}, as described in Table 1; (*iv*) Elution: 5.0 mL of 0.1% (m/v) sodium dodecyl sulfate in methanol at an average flow rate of 0.5 mL min^{-1}.

The washing step was evaluated under three different conditions for each study: (*i*) without an electric field (conventional SPE); (*ii*) with the top electrode negatively charged (as the cathode) and the bottom electrode positively charged (as the anode), termed E-SPE$_{(-/+)}$; and (*iii*) with the top electrode positively charged and the bottom electrode negatively charged, termed E-SPE$_{(+/-)}$. In both E-SPE$_{(-/+)}$ and E-SPE$_{(+/-)}$, the electric field was applied only during the washing step with the electric power supply operating in the constant electric current mode.

The final eluates obtained from the elution step were collected in a 20 mL volumetric flask. The volume was completed with ammonium phosphate dibasic (10 mmol L^{-1}) and filtered through a 0.22 μm membrane polyvinylidene fluoride (PVDF) filter (Millipore, Barueri, SP, Brazil) before quantitation by HPLC-diode array detector (DAD). All extractions were carried out in duplicate (n = 2) using newly prepared cartridges. The recovery results were calculated as:[12]

$$\frac{\begin{array}{c}\text{(Number of moles of analyte originally}\\\text{present in the sample solution)}\end{array}}{\begin{array}{c}\text{(Number of moles of analyte}\\\text{found in the final solution)}\end{array}} \times 100 \qquad (1)$$

The results were presented as the average of the recovery values of the duplicate analysis.

Quantitation of E 110 using high performance liquid chromatography

Quantitation of E 110 was carried out using HPLC in order to separate it from two other anionic dyes that are not discussed in this work.

The HPLC used was a binary gradient chromatographic system from Waters (Milford, MA, USA), model 1525, coupled to a Waters photodiode array detector (PDA) model 2996 and a Rheodyne injector model 7725 (sample loop of 5 μL) (Elkay, Galway, Ireland). Data acquisition was performed by the Millenium 4.0 software from Waters (Milford, MA, USA).

The separation of E 110 was performed on an endcapped Purospher™ Star column (55 mm × 4.0 mm i.d., 3.0 μm) (Merck, Darmstadt, Germany). The mobile phases were ammonium phosphate (10 mmol L^{-1}, pH 7.8) (A) and methanol (B) under gradient elution. The linear gradient started and was kept constant for 3 min at 10% B. After that B was raised from 10 to 40% over 8 min and maintained constant for 2 min. Then, the percentage of B was linearly decreased to 10% over 2 min and kept constant for 4 min for equilibration. The flow rate and temperature were 1.0 mL min^{-1} and 35 °C, respectively. Quantitation was performed at the maximum absorption wavelength of E 110 (486 nm) using external calibration. The calibration curve in the range of 0.4-9.6 μg mL^{-1} was prepared by diluting the stock solution in 10 mmol L^{-1} ammonium phosphate pH 7.8 and methanol (90:10, v/v).

Results and Discussion

Food, biological and environmental samples, among others, usually have high ion contents, which result in highly electric conductivity matrices. Therefore, to simulate these matrices in terms of conductivity, all of the experiments in this work were carried out by adding 0.9% (m/v) NaCl to the working solutions of the dye E 110. However, the high conductivity of the samples containing NaCl increases the electric current and the temperature inside the cartridge when E-SPE is carried out using a constant electric potential. For this reason, all experiments were performed under constant electric current.

To evaluate the E-SPE of the anionic dye E 110 and the effects of the chromatographic and electrophoretic processes on the extraction procedure, the following parameters were studied: the concentration, percentage of organic modifier, the pH and the volume of the washing solution; and finally, the amount of sorbent material. It is important to consider here that the studies were mainly conducted to evaluate the effect of the electric field on the solid phase extraction process, and that the model compound can be understood as an analyte of interest that should be retained in the cartridge or as an interferent that should be eliminated during the washing step.

In contrast to the first demonstration of E-SPE by Orlando *et al.*,[10] in which an antimicrobial in its cationic form was used, in this work the anionic dye E 110 was used as a model compound. The choice of a dye was particularly interesting because the differences between SPE and E-SPE can be observed visually.

The anionic dye E 110 has been extensively analyzed by capillary electrophoresis, capillary electrochromatography and high performance liquid chromatography.[13-19] Because

this dye has two sulfonate groups, it has been observed that the electrophoretic velocity (v_{ep}) of the dye is directed toward the anode under a wide range of pH conditions.[13] It was also observed that the affinity of the dye to the silica depends on the ionic strength of the eluent.[20] The same standard behavior was observed in the studies described hereafter.

Influence of the buffer concentration in the washing solution

The first study aimed to evaluate the influence of the amount of citric acid and disodium hydrogen phosphate added to the washing solutions used during the extraction of the anionic dye E 110 (Figure 4a). Three different molar ratios of citric acid and phosphate were employed: 5/10, 10/20 and 20/40, all at pH 4.0.
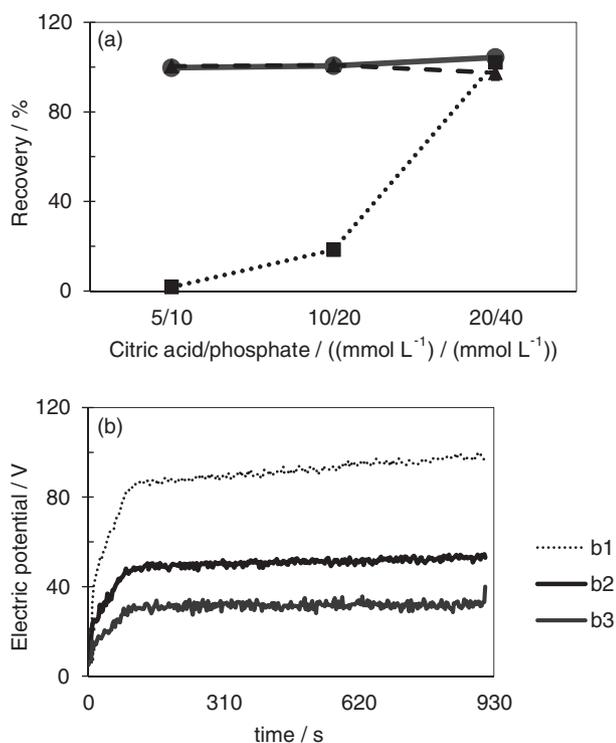
Figure 4. (a) Effect of citric acid and phosphate concentrations used to prepare the washing solution on the recoveries of E 110 (mean, maximum and minimum values, n = 2). Triangle-dashed line: E-SPE$_{(+/-)}$; square-dotted line: E-SPE$_{(-/+)}$; circle-solid line: SPE. (b) Typical electric potential values monitored during the study: b1 = 5/10; b2 = 10/20 and b3 = 20/40 [citric acid/phosphate; (mmol L^{-1}) / (mmol L^{-1})].

It can be observed that the E-SPE$_{(-/+)}$ applied during the washing step changed the recovery of the dye E 110 from approximately less than 1.7 to 99.6% (57-fold improvement in elution) by varying the buffer concentration from 5/10 to 20/40 [citric acid/phosphate; (mmol L^{-1}) / (mmol L^{-1})].

In E-SPE, two main processes contribute to the recovery of the analytes: the chromatographic process (distribution

of the analyte between the stationary and mobile phases) and the electrophoretic process (v_{ep} and v_{eo}).

When the concentration of salt in the solution is increased, the salting out effect becomes relevant, and, as a consequence, the dye is more retained in the sorbent. The improvement of the chromatographic effect (sorption) due to the salting out and the reduction of the electrophoretic phenomena (v_{ep}) as a result of the reduction of the electric field are in agreement with the results observed for the anionic dye E 110 when the buffer concentration was increased.

Using the polarity of the top electrode as anode (E-SPE$_{(+/-)}$) and comparing to SPE, no significant differences were observed in the recoveries (99.6 and 100.3% for SPE and E-SPE$_{(+/-)}$, respectively). This situation was also observed for almost all of the extraction parameters evaluated. However, the use of E-SPE$_{(+/-)}$ revealed that the heating that occurs during the application of the electric field (ca. 7 °C) is not strong enough to reduce the recovery of E 110. This is a significant result and can be extended to E-SPE$_{(-/+)}$ because both methods generate the same electric power. These results reinforce that the change in recovery of 99.6% (SPE) to 1.7% (E-SPE$_{(-/+)}$) obtained with CPB 5/10 cannot be attributed only to the nonspecific heating effect.

The electric potential was monitored during the extraction and was found to be stable for all concentrations of buffer used (Figure 4b). As expected, when the salt concentration was reduced, the applied electric potential and, consequently, the electric field were also improved because the experiment was carried out using a constant electric current mode.

Influence of the content of organic modifier in the washing solution

Another important parameter to study in E-SPE processes is the content of organic modifier used in the washing solution. The effect of the amount of methanol (%) added to the buffer of the washing solution on the recovery of E 110 is presented in Figure 5a.

It was verified that the recoveries of E 110 in all of the situations (SPE and E-SPE) were only influenced when methanol was added at a proportion higher than 12% (v/v) to the buffer solution. With 16% (v/v) methanol, the recovery of E 110 using E-SPE$_{(-/+)}$ was approximately 1/3 less than that of the SPE process.

The amount of the organic modifier in solution can change both the chromatographic and electrophoretic processes. Organic solvents in aqueous solution can modify the hydrodynamic radius of the analytes and, consequently, their electrophoretic mobility.[20] The conductivity and the
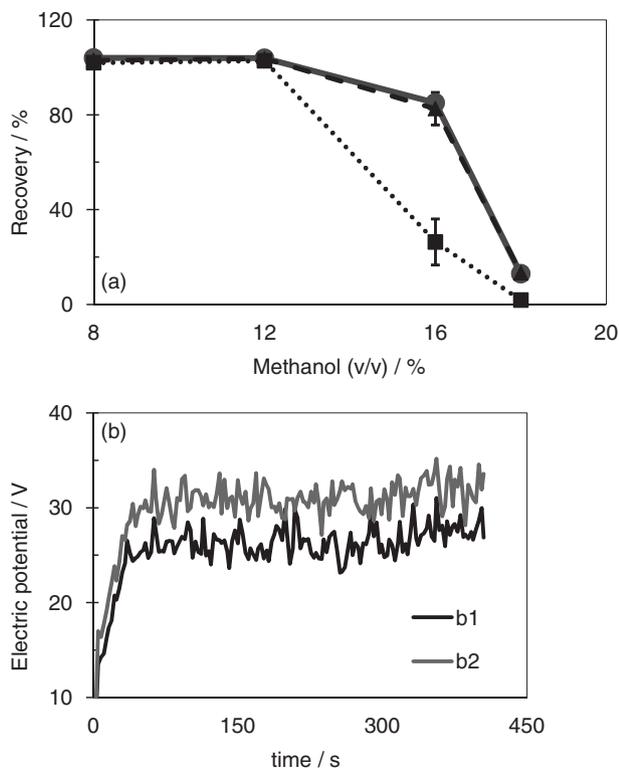
Figure 5. (a) Effect of the content of methanol in the washing solution on the recoveries of E 110 (mean, maximum and minimum values, n = 2). Triangle-dashed line: E-SPE$_{(+/-)}$; square-dotted line: E-SPE$_{(-/+)}$; circle-solid line: SPE. (b) Typical electric potential values monitored during the study with b1 = 8% and b2 = 18% of methanol.

electric double layer are also affected by the presence of organic modifiers and the hydrophobic interactions of the analyte with the C18 groups are reduced. If a compound is retained too strongly, the v_{ep} will not develop to an appreciable extent because the compound will predominantly be retained on the sorbent.

The results presented in Figure 5a can be attributed mainly to an increase in the solubility of the dye in the washing solution (e.g., lower affinity of the analyte to the sorbent), as this behavior was clearly observed without an electric field (SPE). When the E 110 presented a weaker interaction with the stationary phase, the electric field applied was able to affect the movement of the molecule. Therefore, the distribution constants of the compounds must be situated within a specific range to allow the electrophoretic effects to contribute significantly to the overall extraction process.

When 16 and 18% methanol in the washing solution were used, no differences in the recoveries were observed between E-SPE$_{(+/-)}$ and conventional SPE. An improvement in the recovery is expected in this situation due to the electrophoretic attraction of the anionic dye to the positively charged top electrode. In this case, the electrophoretic velocity most likely was annulled by the flow rate employed

and/or by the v_{eo} in the opposite direction (to the luer of the cartridge).

In this study, a slight electric potential increase (current of 7 mA) was observed when the organic modifier added to the buffer was changed from 8 to 18% (v/v) (Figure 5b), which could be due to the reduction of the conductivity of the washing solution by the organic solvent.

Influence of the pH of the washing solution

The recoveries of E 110 using E-SPE$_{(-/+)}$ were also influenced by the pH of the washing solution (Figure 6a). It is important to note that this study was carried out under a controlled conductivity maintained at 3.268 mS cm^{-1} by the addition of NaCl solution (Table 1). This approach was important to maintain a constant electric field for all washing solutions at different pH values (Figure 6b).
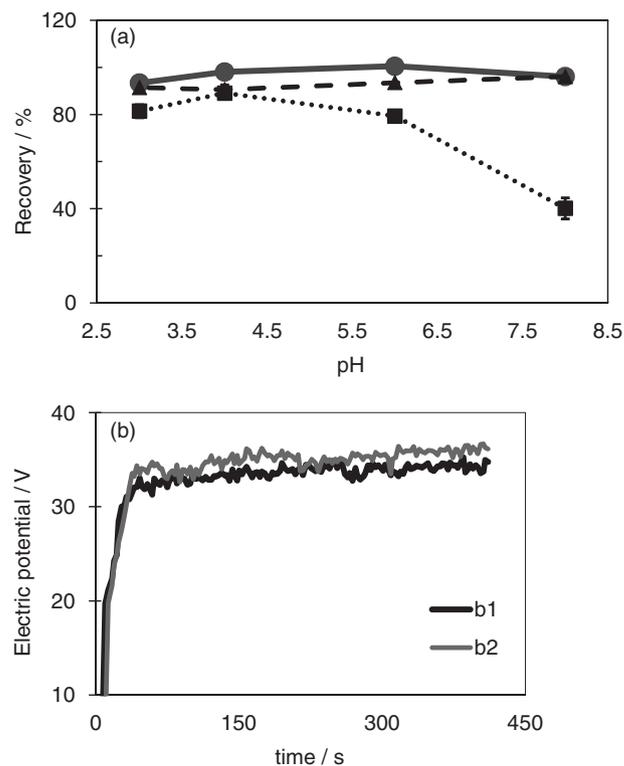
Figure 6. (a) Effect of the pH of the washing solution on the recoveries of E 110 (mean, maximum and minimum values, n = 2). Triangle-dashed line: E-SPE$_{(+/-)}$; square-dotted line: E-SPE$_{(-/+)}$; circle-solid line: SPE. (b) Typical electric potential values monitored during the study with b1 = pH 3.0 and b2 = pH 8.

It was verified that the extraction of the dye under the experimental conditions employed was more affected at pH 8 using E-SPE$_{(-/+)}$.

The pH of the solution controls the ionization degree of the dye, which can change the v_{ep} (electrophoretic process) and also the affinity of the dye to the sorbent

(chromatographic process). When compounds possess ionizable groups, such as the phenol group of E 110, hydrophobic interactions with the C18 silica sorbents can be significantly reduced. The anionic model compound used in the extraction studies presents different functional groups, including phenyl, sulfonate and azo groups. The degree of ionization of the phenyl group changes according to the pH of the solution. The effective v_{ep} of azo dyes usually increases with increasing pH due to the increase in the number of negative charges (phenyl group) *per* molecule.[13] The lower affinity of the dye to the sorbent associated with higher v_{ep} in the direction of the flow rate may be responsible for the recoveries obtained with E-SPE$_{(-/+)}$.

Another electrophoretic component of movement in the SPE process is the electroosmotic velocity (v_{eo}). Regardless of the polarity of the electrodes, the v_{eo} is always in the opposite direction to the electrophoretic mobility of the anionic dye if sufficient free silanols on the sorbent are present to form an appreciable electric double layer at the surface of the particle.[21,22] It has been reported that the electric double layer formed on the surfaces of silica particles is favored at higher pH values due to an increase in the number of dissociated silanol groups, which favors the v_{eo}.[21,22] If the v_{eo} could overcome the v_{ep}, a reduction in the recovery of E 110 with E-SPE$_{(+/-)}$ would be observed. However, the recoveries obtained with E-SPE$_{(+/-)}$ were all similar to SPE, and both were nearly 100%, which suggests that the v_{eo} does not contribute significantly to the process as a whole.

Influence of the volume of the washing solution

In conventional SPE, the recovery of the analytes is also dependent on the volume of washing solution employed during the clean-up step.[3] If an excessive volume of washing solution is used, losses of the analytes are observed. On the other hand, when an insufficient volume is applied, unsatisfactory clean-up occurs.[3] The effect of the volume of the washing solution on the recovery of E 110 during the SPE and E-SPE procedures is shown in Figure 7a.

Increasing the volume of the washing solution displaces the molecules from the sorbent into the eluent, which moves in the direction of the exit of the cartridge, as described by chromatographic distribution.[3] However, the results of SPE demonstrate that greater volumes of the washing solution were not able to change the recoveries of the dye E 110 without an electric field.

On the other hand, when an electric field was employed with a cathodic top electrode (E-SPE$_{(-/+)}$), the recovery of the dye was fairly dependent on the volume of the
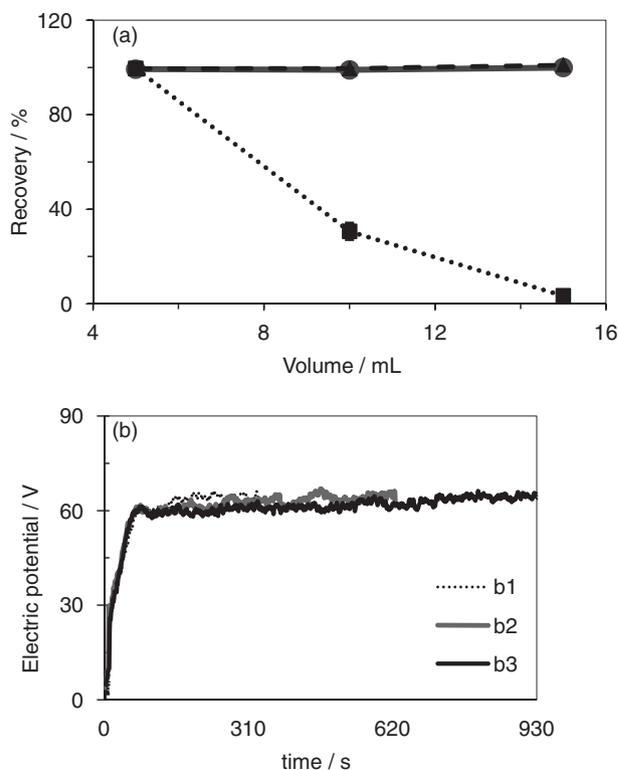
Figure 7. (a) Effect of the volume of the washing solution on the recoveries of E 110 (mean, maximum and minimum values, n = 2). Triangle-dashed line: E-SPE$_{(+/-)}$; square-dotted line: E-SPE$_{(-/+)}$; circle-solid line: SPE. (b) Typical electric potential values monitored during the study with b1 = 5 mL; b2 = 10 mL and b3 = 15 mL of washing solution.

washing solution. It was observed that the contribution of the electrophoretic process became more intense as a higher volume of eluent was used. This most likely can be explained by the longer time required to percolate a higher volume of washing solution, which prolongs the action of the electric field.

During these studies, the electric potential was found to be stable for all three volumes of washing solution evaluated (Figure 7b).

Influence of the sorbent amount

The efficiency of the extraction is dependent on the amount of sorbent. In conventional SPE, a greater amount of sorbent leads to a higher number of interactions for the chromatographic process.[3,23] The effect of the sorbent amount on the recoveries of the E 110 dye is depicted in Figure 8a.

It is well known that, for the same sorbent material, the plate number increases with increasing sorbent amount, and, as a consequence, an increase in recovery is achieved.[3,23] This effect was observed for all extractions evaluated (E-SPE$_{(+/-)}$, E-SPE$_{(-/+)}$ and SPE) by varying the sorbent amount from 300 to 500 mg. Above 500 mg,
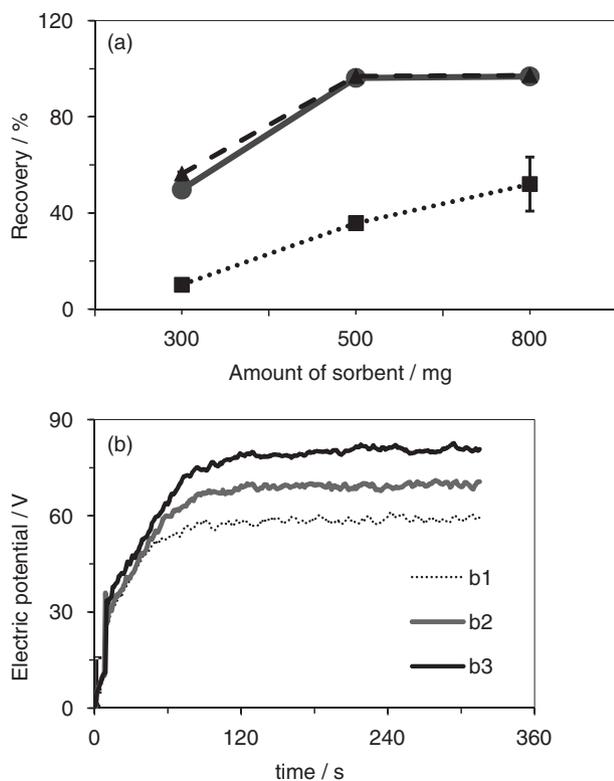
Figure 8. (a) Effect of the amount of sorbent on the recovery of E 110 (mean, maximum and minimum values, n = 2). Triangle-dashed line: E-SPE$_{(+/-)}$; square-dotted line: E-SPE$_{(-/+)}$; circle-solid line: SPE. (b) Typical electric potential values monitored during the study with b1 = 300 mg; b2 = 500 mg and b3 = 800 mg of sorbent.

no changes were observed with conventional SPE and E-SPE$_{(+/-)}$ because the recovery had already reached a maximum of approximately 100%. Using E-SPE$_{(-/+)}$, however, the recoveries increased slightly compared with SPE and E-SPE$_{(+/-)}$. The results obtained with E-SPE$_{(-/+)}$ demonstrate that the v_{ep} in the direction of the flow rate can be effective even when using a higher amount of sorbent.

It should be noted that the use of a constant electric current of 12 mA resulted in a more intense electric potential when higher amounts of sorbent were used (Figure 8b). As a consequence, equal electric fields (V cm^{-1}) were applied.

All of the results with E-SPE$_{(-/+)}$ are in agreement with the concepts of electrochromatography. However, no significant changes in the recovery of the anionic dye were observed when the top electrode was used as the anode (E-SPE$_{(+/-)}$) under any parameters evaluated when compared with conventional SPE. In fact, the recovery results obtained with E-SPE$_{(+/-)}$ were usually very similar to SPE.

These results could indicate that there was an almost perfect annulment of the v_{ep} by the action of the flow rate (convection) in the opposite direction under the experimental conditions employed. However, in a previous work, Orlando *et al.*[10] demonstrated that the electric field can improve the recovery of an analyte even if the v_{ep} is acting in the opposite direction of the flow rate.

Another possibility is that the v_{eo} acting in the opposite direction of the v_{ep} of the anionic dye may cancel the expected improvement in recovery.

Nevertheless, the possible effect of the residual movement of the electric double layer during the extraction process cannot be ruled out. When an electric field is applied, it can compress the electric double layer overlapping inside the particle pores and induce a concentration polarization (CP) at that region of the particle sorbent.[24] The CP reduces the concentration of co-ions and increases the concentration of counter ions on the sorbent surface inside the particle. The compressed electric double layer reduces the concentrations of anions at the silica surface and promotes their displacement in the direction of the diffuse layer (Gouy-Chapman layer). This effect may reduce the interactions of the anionic compounds with the C18 groups of the sorbent.[24]

Conclusions

The E-SPE studies with the anionic sunset yellow dye revealed that an electric field can be used as an important driving force to modulate the recoveries of anionic compounds.

Using the top electrode as the cathode (E-SPE$_{(-/+)}$), significant improvements in the elution of the dye were obtained during the washing step in comparison with conventional SPE. This indicates that E-SPE$_{(-/+)}$ can be used to selectively eliminate anionic interferents during the washing step and/or improve the elution of an anionic analyte during the final elution step by applying electric fields, avoiding the need for high volumes of eluents or organic solvents.

On the other hand, using the top electrode as an anode (E-SPE$_{(+/-)}$), the recoveries were usually very close to those obtained with conventional SPE under almost all conditions evaluated, especially when the recovery obtained with SPE was close to 100%.

These results indicate that in E-SPE, the overall effect of the electric field on the extraction process is a complex combination of the contributions of the electrophoretic and electroosmotic velocities, convection, diffusion and sorption. These driving forces in E-SPE can act in favor or in opposition to each other depending on the analyte charge, the polarity of the electrode and the charge of the surface of the sorbent.

In addition, the concentration polarization in the sorbent material may also reduce the retention of the analytes on

the silica-modified surface. However, additional studies are necessary to prove this hypothesis.

The results presented here have helped characterize the E-SPE process and broaden the field of sample preparation techniques.

Acknowledgments

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