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Development of a Background Electrolyte for the Determination of Inorganic Cations in High Ionic Strength Samples by Capillary Electrophoresis with Indirect UV-absorption Detection

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Highlights

- A novel BGE for CE with high electric conductivity tolerance was developed.
- The BGE reduced the electrodispersion when samples with ionic strengths up to 1M were analyzed.
- The method allowed the determination of inorganic cations by indirect UV-absorption detection.
- Na⁺, K⁺, Ca⁺² and Mg⁺² were determined in high ionic strength real samples.

Abstract

In this study, a background electrolyte capable to separate and quantify inorganic cations in high ionic strength samples by UV-absorption indirect detection was designed. In this regard, the four most abundant monovalent and divalent cations in earth crust (K⁺, Na⁺, Ca⁺², Mg⁺²) were selected as model compounds. A group of small carboxylic acids and, several toluidines and pyridines were evaluated as mild strength complexing agents and chromophoric probes, respectively. The optimized background electrolyte was composed of 200 mM 2,4,6-trimethylpyridine as the chromophoric probe, 250 mM lactic acid as the weak complexing agent and pH buffering reagent (adjusted to pH 4.5), and 5 % v/v methanol as organic solvent modifier. Based on a minimum

number of components, it provided outstanding separation performance in less than 4 min in a wide linear dynamic range (10 - 2500 $\mu\text{g}\cdot\text{mL}^{-1}$). Performances were contrasted against a reference method based on conductometric detection. Furthermore, studies of separation efficiency and peak shape were carried out at different analyte concentrations in high electric conductivity solutions. The herein developed method demonstrated exceptional features in terms of limits of detection (~ 10 $\mu\text{g}\cdot\text{mL}^{-1}$), resolution, speed of analysis, sensitivity and peak capacity in high electric conductivity samples. Moreover, the method was successfully applied to high ionic strength samples such as rock digest, sea water, soy sauce and isotonic drinks.

Keywords

Inorganic cations; High ionic strength samples; Capillary electrophoresis; Indirect UV-absorption detection

1. INTRODUCTION

Different approaches have been considered for the separation and quantification of small inorganic and organic ions. However, the preferred techniques in this matter are capillary electrophoresis (CE) and ion chromatography (IC). Although both techniques are widely used, they fundamentally differ in selectivity mechanisms and driving force. IC shows simpler selectivity manipulation whereas CE offers several advantages such as instrumental simplicity, high peak capacity, possibility of miniaturization, and low reagent and solvent consumption [1–5].

Specifically for cation analysis, two main detection techniques have been reported in CE; capacitively-coupled contactless conductivity detection (C4D) [6–11] and single wavelength indirect UV-absorption detection [12–18]. In the latter mode, an absorbing co-ion, called the probe or chromophore probe, is added to the background electrolyte (BGE). Detection is accomplished by displacement of the co-ion based on the electroneutrality principle, leading to a quantifiable decrease in the background absorbance [2,12,19]. In this regard, two aspects must be considered to achieve an acceptable separation without sacrificing sensitivity. The first aspect resides in the probe molar absorptivity: the higher the molar absorptivity of the probe, the better the sensitivity [2]. Whereas the second aspect is the probe concentration, which is related to: a- the maximum detectable concentration of analyte ions, which is upper-limited by the concentration of the probe; b- the baseline absorptivity: the higher chromophore concentration produces a higher and noisier baseline which is translated in worse limits of detection (LOD) and; c- BGE ionic strength: higher chromophore concentration means higher ionic strength.

If the BGE ionic strength or conductivity is similar or lower than the sample, the ionic constituents of the sample will become the charge carriers throughout the separation, generating zone broadening. This issue is not only associated with indirect UV-absorption detection, but is also an inherent characteristic of general electrophoretic separations. Therefore, for attaining good performance in these electrophoretic methods (*i.e. resolution, separation efficiency, sensitivity and*

limit of detection), sample ionic strength should be less or similar to the BGE ionic strength. In this context, most of the published literature tends to minimize the BGE concentration and ionic strength, aiming to improve the limits of detection (LOD) [2,7,20–24]. For instance, several applications have been reported for inorganic ions in low ionic strength samples such as active pharmaceutical ingredients [25], clean water sources [13,22,26] or very diluted high ionic strength samples [27]. Hence, high and moderate ionic strength samples along with inorganic cation determinations by CE have been and still constitute a considerable challenge, leading to the need for more complex BGE formulations [1,2,13], sample pre-treatment strategies (sample cleanup, chromatographic or electromigration enrichment) [27] or alternative detection technologies [6].

In order to overcome this issue, the first strategy was described by Ding *et al* in 1998 [28]. In this work, the authors proposed to use a high concentration of salts in the BGE together with a low concentration of buffer. Despite the success of the methodology when applied to the analysis of UV-absorbing inorganic anions in sea water, the use of background salts prevented its application to the analysis of elemental inorganic cations that were involved in the BGE. Later, the use of conductometric detection with a BGE consisting of a concentrated solution of acetic acid (up to 5 mol·L⁻¹) was applied to the separation of non UV absorbing analytes in challenging samples [6,14,29,30]. However, when inorganic cations were analyzed in high ionic strength matrixes or at very high concentrations, successive dilutions were still needed due to the poor focusing of the bands. Although dilution allows the proper quantification of major cations, it neglects the ones at lower concentrations. Moreover, this BGE presents poor selectivity, hindering the quantification of cations with similar electrophoretic mobilities.

From a physicochemical point of view, the BGE is known to act as a continuous medium that enables the application of an electric potential gradient inside the capillary. Their components are the active agents responsible for conducting electric current, while analytes are usually expected to migrate without exhibiting an active participation [31,32]. However, when a sample contains small inorganic ions as major compounds, it will be characterized by high electric conductivity, *i.e.*

the ions will contribute actively to electric current conducting. Thus, for this kind of samples, the BGE must fulfill two main conditions. On one side, it must have higher conductivity than the sample and, on the other side, the co-ion must match the analyte mobilities. Otherwise, the electrodispersion effect is increased, impairing the separation and eventually limiting the dynamic range of the method.

Having these considerations in mind, the aim of this work consisted in the development of a BGE applicable to the determination of inorganic cations in high ionic strength samples by CE with indirect UV-absorption detection. In this regard, the design and validation of a BGE formulation containing a minimum number of components to reduce electrodispersion, provide high separation selectivity, pH buffering, and a sensitive detection probe has been attained. In addition, electrodispersion studies with standard solutions with different ionic strengths and extremely heterogeneous cation composition were also investigated. Finally, a wide variety of high ionic strength real samples such as rock digests, seawater, soy sauce and isotonic drinks were analyzed.

2. EXPERIMENTAL

2.1. Instrumentation

All experiments were carried out on a Lumex Capel 105M Capillary Electrophoresis system (Lumex Ltd., St. Petersburg, Russia). The detection was performed with a UV spectrophotometric detector which is included in the capillary electrophoresis system or by coupling a contactless conductivity detector ER225 (e-DAQ, Sydney, Australia). An Accumet Research AR25 potentiometer (Fischer Scientific, New Hampshire, USA) connected to a Schott BlueLine 11-pH glass combination electrode (SI Analytics GmbH, Mainz, Germany) was used for pH measurements.

To digest the geological sample, a Nova-8 microwave digestion system (Milestone, Shelton, USA) was employed.

2.2. Chemicals and Materials

Deionized water was provided by a MilliQ® water purification system (Millipore, Bedford, MA, USA). Methanol was HPLC grade (Sintorgan, Buenos Aires, Argentina). 2,4,6-trimethylpyridine (2,4,6-tmP) was obtained from Carlo Erba (Milan, Italy); 2-methylpyridine (2-mP), 3-methylpyridine (3-mP); 4-methylpyridine (4-mP); and o-, m- and p-toluidine (oT, mT and pT) were purchased from Fluka Chemie (Buchs, Switzerland). L (+) lactic acid was provided by J. T. Baker (Phillipsburg, USA), formic acid and acetic acid (glacial) 100 % anhydrous was obtained from Merck (Darmstadt, Germany) and hydrochloric acid was purchased from Cicarelli (San Lorenzo, Argentina). Sodium hydroxide was provided by Mallinckrodt (Dublin, Ireland). Nitric acid, hydrofluoric acid and perchloric acid used for geological samples digestion were trace metal grade and were obtained from Fisher Chemicals (Waltham, USA).

Stock solutions were prepared with sodium chloride, potassium chloride and calcium chloride purchased from Anedra (Buenos Aires, Argentina) and magnesium sulphate heptahydrate was acquired from Merck (Darmstadt, Germany). All chemical reagents used were analytical grade or better.

Fused-silica capillaries (50 μm I.D. x 365 μm O.D.) provided by Polymicro Technologies (Phoenix, USA) were cut at a total length (L_t) of 50.0 cm. The window for on-column UV-absorption detection was located 10 cm from the end of the capillary (effective length, L_d : 40 cm). The headstage for conductivity detection was placed at 20 cm from the end of the capillary (L_d : 30 cm). New capillaries were activated for the first use by flushing at 1000 mbar with methanol (5 min), 1 M NaOH (15 min), 0.1 M NaOH (15 min), water (30 min), 0.1 M HCl (10 min), water (5 min), and BGE (20 min). Between runs, capillaries were preconditioned by flushing methanol (1 min), 0.1 M NaOH (1 min), water (1 min) and BGE (2 min).

2.3. Workflow

The optimized aqueous BGE was prepared by mixing the corresponding volume of 2,4,6-tmP to have a final concentration of 200 mM, and the necessary lactic acid to adjust the pH to 4.5

(~250 mM). Methanol was added to a final concentration of 5 % v/v. The BGE was filtered through a 0.22 μm nylon membrane just before use.

Individual stock solutions of each cation were prepared at a final concentration of 20000 $\mu\text{g}\cdot\text{mL}^{-1}$ in deionized water. Standard solutions containing the four studied cations and covering the concentration range 5 - 5000 $\mu\text{g}\cdot\text{mL}^{-1}$ were prepared by mixing adequate volumes of each stock solution and diluting with the same solvent to create ten-point calibration curves. Total ionic strength and individual cation concentrations in each standard solution are detailed in Table S1 from the Supplementary Information.

Samples and standard solutions were hydrodynamically injected by applying a pressure of 30 mbar for 10 seconds. Separations were carried out at a constant separation voltage of 25 kV (electric current 190 μA) and indirect UV-absorption detection was set at 230 nm wavelength. Cation migration order was determined by individually injecting solutions containing each of the analytes. An external standard calibration curve was used for quantification. In order to take into account differences in apparent velocities and possible electroosmotic flow fluctuations between runs, corrected areas (area / migration time) were used to build the calibration curves and perform the analysis of the samples. All solutions were analyzed at least in triplicate.

2.4. Sample pre-treatment

A commercially available soy sauce and two isotonic drinks from different manufacturers were purchased from a local store. Seawater samples from Mar Argentino were collected at San Bernardo beach, Argentina (-36.686367, -56.675709) in glass containers. Soy sauce was diluted 1:10 and 1:20 (v/v) and seawater was diluted 1:10 (v/v), while isotonic drinks did not require dilution. All samples were filtered through a 0.22 μm nylon membrane prior to injection and analyzed without any further treatment.

Geological samples (granite) were collected from Cuesta de Miranda, La Rioja, Argentina (-29.344440, -67.769041), treated by microwave acid digestion. Briefly, 0.1000 g of cleaned, dried

and powdered whole rock was weighed in a PTFE digestion vessel. 1 mL of HClO₄, 5 mL HNO₃, and 4 mL of HF were added. The PTFE vessel was capped, sealed and heated in the microwave system during 30 minutes at 180 °C (800 W). The remaining solution was evaporated to dryness at 85 °C on a hot plate. After cooling down to room temperature, the residue was then taken up in 1 mL of HNO₃ and made up to 100 mL with deionized water. This final solution was filtered through a 0.22 µm nylon membrane before injection.

2.5. Software

Electropherograms transformed to show positive peaks, were recorded and analyzed by the data acquisition and control software Elforun v3.2.2 (Lumex Ltd., St. Petersburg, Russia) for UV-absorption detection and PowerChrome v2.8.2 (e-DAQ, Sydney, Australia) for conductivity detection. Mathematical simulations and BGE optimizations were performed with Peakmaster® 5.3 Complex, developed by the Group of Electromigration Separation Processes from Charles University, Prague, Czech Republic [33,34]. In the latter, electropherograms are shown as obtained by the software.

3. RESULTS AND DISCUSSION

3.1. BGE composition and evaluation

The rationale for the BGE design is presented as a schematic summary in Figure S1. Firstly, potassium (K⁺), sodium (Na⁺), calcium (Ca⁺²) and magnesium (Mg⁺²) were selected as model inorganic cations. Cations having similar hydrodynamic radius, molecular mass and net charge are expected to co-migrate. Therefore, it is necessary to impart separation selectivity by adding an adequate anionic complexing agent. The complex stability constants must be small, and complexation process should occur in different extents to increase relative differences in effective mobility. The initial assessment involved pT 100 mM as the chromophoric probe, pH = 4.5, to maximize the degree of protonation of the chromophoric probe, and several monoprotic complexing

agents such as formic, acetic and lactic acid. In this context, monodentate complexing agents (acetic and formic acids) gave no separation. Lactic acid, a bidentate complexing agent, provided adequate selectivity, being selected for following optimizations. These results are in agreement with results found in literature [21,35–37]. Moreover, its low pK_a value (Table 1) enables its use with a variety of potential chromophoric probes, and aids in ensuring most of the inorganic cations to remain in solution.

Regarding chromophoric probe optimization *o*-, *m*- and *p*-toluidine and, 2-mP, 3-mP, 4-mP, and 2,4,6-tmP were tested (Figure S1). These compounds are small, soluble in water, and contain an UV absorbing group and a basic nitrogen-based moiety, which can be protonated. Thus, by selecting the correct working pH, the acidic pK_a will promote full protonation of the chromophore, ensuring the exclusive exchange with non-absorbing cations in the migrating bands and maximizing the sensitivity of indirect detection protocol. The acidic constants and other physicochemical properties of the tested compounds are listed in Table 1. For such evaluation, several conditions should be ideally fulfilled: 1) A significant part of the complexing agent must be ionized (*i.e.* $pH > pK_{a(c.a.)}$); 2) The BGE should have a good buffering capacity; 3) The chromophoric probe must be almost fully ionized (*i.e.* *approximately* $pH < (pK_{a(c.p.)} - 2)$) and; 4) The chromophoric probe should be the only cation to be displaced quantitatively by analytes. Hence, hydrogen ion concentration should be minimized. Ideally, by at least three orders of magnitude lower than the charged form of the chromophoric probe.

Therefore, there is a narrow pH range that can fulfill these conditions depending on the combination of ligand and chromophoric probe. Considering lactic acid as the complexing and buffering agent; the pH conditions are limited to the range 3.9-4.9 with special interest in the superior limit.

In order to test these initial assumptions, all the possible combinations of chromophore with the complexing agent were firstly simulated in Peakmaster[®]. Table S2 contains all of the combinations simulated, indicating the chromophore and its concentration, the total lactic acid

added and dissociated, final BGE pH and ionic strength. The simulated electropherograms of oT, mT and, pT at different BGE concentrations are shown in Figures S2-S4 respectively. In the cases of oT and mT, the observed selectivity was very poor (Resolution < 1.5) for the lower concentrations of chromophore, especially for the pair sodium/calcium. On the other hand, in the case of pT resolutions are acceptable for 100 mM and 200 mM chromophore concentrations. As a general trend a clear improvement can be observed as the concentration of chromophoric probe increases. Interestingly, the pH is practically constant and only the ionic strength of the BGE is changing. Therefore, this simulation software suggests that separations based on toluidines could be acceptable only at concentrations higher than 200 mM. In agreement with the trend exhibited by toluidines, methylpyridines also showed a consistent trend in increasing resolution with the increase of the chromophore concentration (Figures S5-S8 for 2-mP, 3-mP, 4-mP and 2,4,6-tmP, respectively).

Based on these simulations, the different chromophores were experimentally verified to validate the optimized conditions. For toluidine based BGEs, solutions with concentrations higher than 50 mM for mT and oT and, 100 mM for pT could not be experimentally prepared due to solubility limitations. Since these concentrations are only acceptable when analyzing samples with low ionic strength and low concentration of analytes ($\leq 100 \mu\text{g}\cdot\text{mL}^{-1}$), toluidines were discarded as chromophores. Furthermore, from a practical point of view, rapid oxidation of the BGEs was observed. In contrast, methylpyridines were solubilized at chromophore concentrations up to 270 mM and were found to be more stable in terms of polymerization and oxidation resistance. BGEs evaluation (Figure S9) lead to electropherograms with unstable baselines that ultimately hinder the sensitivity of the assay, with exception of 2,4,6-tmP which proved to be the best option. It is worth to mention that at the working pH (pH = 4.5), the ionization degree for 2,4,6-tmP was 99.4 %. This aspect ensures full ionization of the chromophoric probe, which is one of the most important conditions to fulfill for the BGE. In agreement with Peakmaster's prediction, 200 mM of 2,4,6-tmP will be the selected chromophoric probe to work with.

Finally, the addition of solvent to fine tune peak shape and selectivity by modifying the relative permittivity and ion solvation capabilities of the BGE was explored [38,39]. In this regard, acetonitrile, and methanol at compositions between 5 % and 30 % (v/v) by volume were evaluated. The addition of methanol 5 % (v/v) improved significantly the electropherogram without producing significant shifts in neither the pK_a of lactic acid and 2,4,6-tmP nor the complex stability constants [40,41]. Therefore, the BGE formulation found as the more suitable resulted in 200 mM 2,4,6-tmP and lactic acid up to $pH = 4.5$ (250 mM) with 5 % v/v methanol.

The electrodispersion produced by the BGE was experimentally evaluated in terms of peak shape and width, resolution, and maximum allowed analyte concentration for different ionic strengths. The results are presented in Figure 1: panel I-A-C shows the separation efficiency for Na^+ at different concentrations for different ionic strengths of the BGE. The acceptance criterion was set at peak widths up to 0.2 min and a clearly defined maximum and symmetry. It can be observed that peak shape is lost above $500 \mu g \cdot mL^{-1}$ when 50 mM BGE is used (Figure 1-I-A), while for 100 mM (Figure 1-I-B) and 200 mM (Figure 1-I-C) the limit concentrations are $500 \mu g \cdot mL^{-1}$ and $2500 \mu g \cdot mL^{-1}$. Although the limiting concentration is the same for 100 mM (Figure 1-I-B) and 50 mM (Figure 1-I-A), there are crucial differences in the peak shape. While at 50 mM peak shape is not acceptable above $1000 \mu g \cdot mL^{-1}$, at 100 mM there is an acceptable peak shape in the whole range of concentrations. In panel II of Figure 1, the results for the pair Ca^{+2} / Mg^{+2} are represented in the same conditions as Na^+ for different BGE ionic strengths and analyte concentrations. The limit concentrations for Ca^{+2} and Mg^{+2} are 100, 500 and $2500 \mu g \cdot mL^{-1}$ for 50 mM, 100 mM and 200 mM of BGE, respectively (Figure 1-II-A-C). Peak shape and width are suitable when using 200 mM 2,4,6-tmP. Moreover, in this condition, the resolution between both compounds is also adequate (Resolution ≥ 1.5). Noteworthy, the limiting concentrations are higher for monovalent cations than divalent cations. This can be explained based on the higher ionic strength of the divalent cation bands in contrast to the monovalent ones [32]. When the concentration of the analytes increases, the electrodispersion produced by this BGE is compromised in a larger extent if cations are divalent

than if they are monovalent. It is important to highlight the change in the electrodispersion patterns for Ca^{+2} and Mg^{+2} from fronting (Figure 1-II-A, B) to tailing (Figure 1-II-C), [2] accordingly to the change in transport numbers of chromophore and analytes in the separation zones at different relative concentrations. The same conclusions were drawn for the remaining analyte K^+ whose results are presented in the supplementary information (Figure S10).

In addition, plots of the number of theoretical plates (N), expressed as the logarithm of N as a function of analyte concentration are shown in Figure 2. Increasing the ionic strength of the BGE improves the separation efficiency and enables wider concentration ranges for Na^+ and K^+ (Figure 2-a)-b)). In the case of Ca^{+2} , Figure 2-c), the less concentrated BGE (50 mM) shows a particularly sharp decrease of the number of theoretical plates as the analyte concentration increases (approximately 1 order of magnitude between 10 and 100 $\mu\text{g}\cdot\text{mL}^{-1}$). Moreover, for BGEs with concentrations of 100 mM and 200 mM of 2,4,6-tmP there is a similar decrease in separation efficiency for increasing concentrations of the analyte. Finally, for Mg^{+2} , contrarily to Ca^{+2} , the most diluted BGE extends the limiting concentration to 1000 $\mu\text{g}\cdot\text{mL}^{-1}$ and shows the best separation efficiency for the 100 mM BGE (Figure 2-d)). However, the trend for the rest of the compounds does not correspond with Mg^{+2} . For all the tested compounds, the number of theoretical plates converges when the analyte concentrations tend to infinite dilution, fulfilling the classical electrophoretic theory.

3.2. BGE performance

The aim of the proposed BGE formulation is to separate and quantify K^+ , Na^+ , Ca^{+2} and Mg^{+2} in medium to high ionic strength samples. In order to prove its suitability, calibration curves were prepared and analyzed in the range within 5 – 5000 $\mu\text{g}\cdot\text{mL}^{-1}$. However, based on the statistical evaluation of the obtained data, linearity was found to be established in the range between 10 and 2500 $\mu\text{g}\cdot\text{mL}^{-1}$ for the four studied cations. Regression analysis plots can be seen in Figure S11 along with method performance parameters, which can be found in Table S3. Precision was evaluated in

terms of repeatability by performing 5 replicate measurements from a standard solution containing all the analytes at a $100 \mu\text{g}\cdot\text{mL}^{-1}$ concentration. The LOQ was determined to be the lowest calibration point that had a signal to noise ratio higher than 10 ($S/N > 10$) and a relative standard deviation, calculated from the corrected areas of replicate measurements, below 0.20 ($RSD < 0.20$). LOD was estimated from the LOQ in each case as $3\cdot(\text{LOQ}/10)$.

Lastly, the separation was evaluated in the presence of one of the analytes at an excessively high concentration respect to the others (Figure 3). In Figure 3-A, a standard mixture of K^+ ($5000 \mu\text{g}\cdot\text{mL}^{-1}$) and Na^+ , Mg^{+2} and Ca^{+2} ($25 \mu\text{g}\cdot\text{mL}^{-1}$) was analyzed. Although the concentrations of the minor components are close to the LOQ, the shape of the peaks and the estimated concentrations remained within acceptable values when compared with the original spiked concentrations. On the other hand, in Figure 3-B, the major compound is Ca^{+2} ($5000 \mu\text{g}\cdot\text{mL}^{-1}$) and K^+ , Na^+ and Mg^{+2} are at low levels ($25 \mu\text{g}\cdot\text{mL}^{-1}$). In this case, Na^+ and K^+ kept satisfactory peak shapes and relative areas. However, in the case of Mg^{+2} , the separation efficiency is slightly decreased, hindering the detection at this concentration level (very close to the LOQ). These experiments definitively proved the versatility of the BGE for a wide variety of potential applications where heterogeneous concentration distributions can be encountered.

Aiming to provide a fair comparison between the proposed BGE for indirect UV-absorption detection and a conventional method for high ionic strength samples [6], both analyzed the same standard mixture containing all the analytes at concentration of $250 \mu\text{g}\cdot\text{mL}^{-1}$ (Figure 4). As reported, for the conventional method, only 5 M acetic acid was used as BGE and conductometric detection was used (Figure 4-A). Despite the same electric field strength being applied in both assays, the conventional approach there was no separation between the analytes Ca^{+2} , Mg^{+2} and Na^{+2} , while using the proposed BGE, the separation of all analytes was performed without any issues (Figure 4-B). Therefore, although the acetic acid BGE presents good baseline stability, the improved BGE approach proposed in this manuscript proved to be superior in selectivity and sensitivity for this type of applications.

3.3. Analysis of real samples

Different samples covering a wide range of ionic strengths were selected to test the versatility of the proposed BGE for the quantitative determination of cations. Moreover, these samples were selected to be representative of different application areas and they present different relative concentrations of their constituents. In this regard, seawater and soy sauce can be considered samples having very high ionic strength, while isotonic drinks and the digested geological sample can be classified as high ionic strength samples. Electropherograms obtained for each sample analyzed using the optimized BGE and separation conditions are depicted in Figure 5. It can be observed that successful separations are achieved in all cases, this means resolutions above 1.5, peak width below 0.2 min and acceptable peak shapes. Although a dilution step was required for soy sauce, this was not driven to avoid electrodispersion losses but to fit cation concentration within the calibration range and to reduce the high viscosity of the sample. The latter leading to low repeatability of the injection and migration times. In the case of seawater, a 1:10 dilution was needed only for Na^+ quantitation. Nevertheless, no analyte peak became not detectable due to the dilution, even when some compounds were more concentrated than others. For instance, the soy sauce sample presented a 10 to 500 times higher concentration of Na^+ respect to the other analytes. Similarly, in the seawater sample, Na^+ is about 10 to 50 times higher than the rest of the analyzed compounds. The quantitative results for all the samples are summarized in Table 2. Besides, in Figure 5 it can be observed the presence of a band, or baseline instability, with no apparent physical explanation. This issue was observed for real sample analysis and further work must be done to overcome this issue for specific applications. Nevertheless, it is worth to mention, that this migrating band had not affected the quantitation performance of the method.

It should be noted that cation analysis in some of these samples has been previously reported [6,14]. In those studies, a BGE composed of 5 M acetic acid was used in combination with C4D detection. In the case of a seawater sample, the application of this method required a dilution

between 50 and 1000 times for quantification of the inorganic cations [6]. In another report, although it was not the main focus of the work, for the detection of sodium in soy sauce, the sample had to be diluted at least 100 times to obtain an appropriate peak shape [14]. Certainly, these high dilution requirements to quantify major and minor compounds hinder the determination of other species that may be present in lower amounts. In contrast, the BGE system herein implemented has demonstrated to extend the linear range of some inorganic cations up to $2500 \mu\text{g}\cdot\text{mL}^{-1}$ in samples with ionic strength higher than 0.6 M. This emphasizes the purpose of the work herein reported, showing improvements from 1 to 3 orders of magnitude in the band focusing performance without substantially sacrificing the sensitivity of the method.

4. CONCLUSIONS

In this work a high capacity and high performance BGE for the analysis of cations in samples with high ionic strength by CE-UV with indirect detection was developed. The optimum BGE was composed by 200 mM 2,4,6-trimethylpyridine, 250 mM lactic acid to adjust $\text{pH} = 4.5$, and 5% v/v methanol. The formulation not only reduces dramatically the electrodispersion effect of highly concentrated analytes but also establishes efficiently the charge transport in the capillary at very high sample conductivity. The developed method using this BGE provided complete resolution and proper quantification of the model analytes in less than 4 minutes. Furthermore, the BGE solution has been capable to suppress the electrodispersion effect having maximum peak resolution in ionic strength samples up to 1M, where the model analytes can be quantified at any concentration (or combination of extreme relative concentrations) within the range of $10\text{-}2500 \mu\text{g}\cdot\text{mL}^{-1}$. LODs and LOQs attained in all cases were around 3 and $10 \mu\text{g}\cdot\text{mL}^{-1}$, respectively. Even though these values can be lowered by using less concentrated BGEs, the scope of this work was to detect and quantify highly concentrated compounds in high ionic-strength samples.

Aiming to demonstrate the BGE performance in real matrixes, samples having high ionic strengths such as sea water, rock digest, soy sauce, and isotonic drinks were analyzed. The results

have proven the remarkable versatility of the BGE formulation. It must be highlighted that the developed method is not limited to these applications but can easily be extended to the direct analysis of body fluids such as serum or urine. Therefore, this work is not limited to the conceptual development of the BGE, but it has also extended the applicability of CE to many diverse fields such as food analysis, bioanalysis and geochemistry, among others.

Credit Author Statement

CL has been involved in the experimental work, data curation, and preparation of the original draft.

JA has been involved in the experimental work, and manuscript review & editing.

MT has participated in the design of experiments and methodology, supervision, experimental work, data curation, conceptualization and preparation of the original draft.

LGG has worked in the funding acquisition, design of experiments and methodology, supervision, data curation, conceptualization, manuscript review & editing.

Declaration of interests

No.

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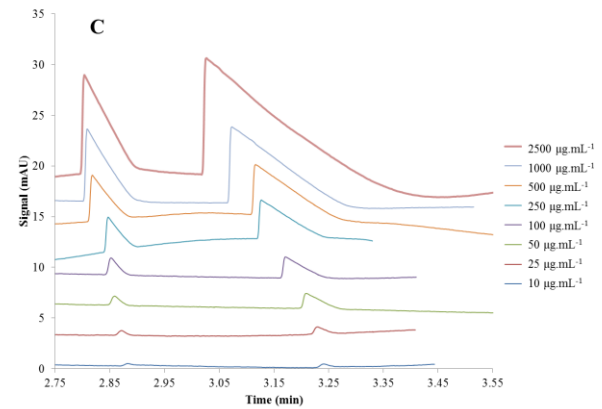
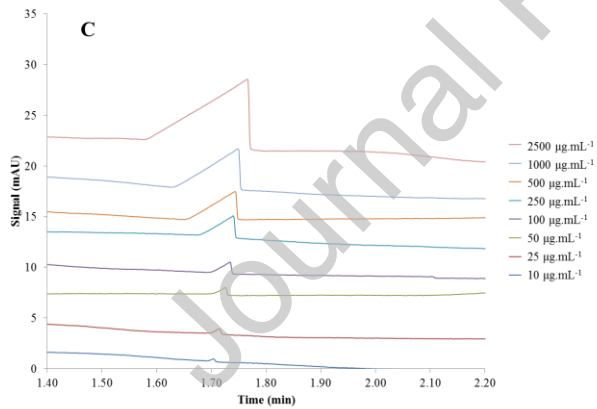
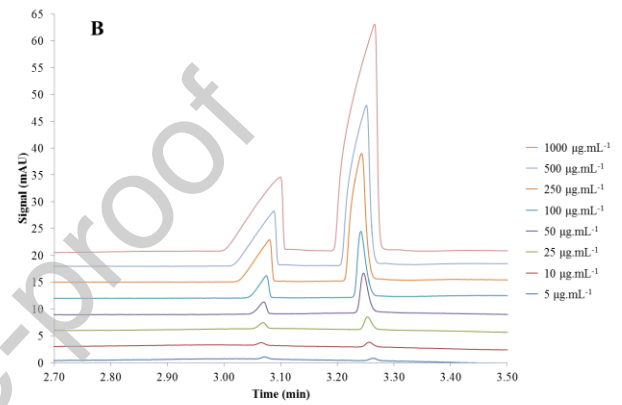
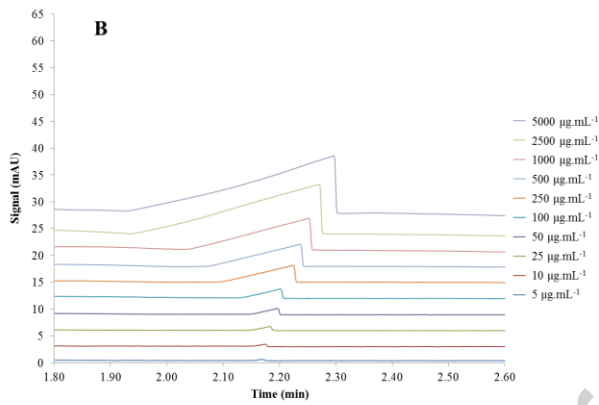
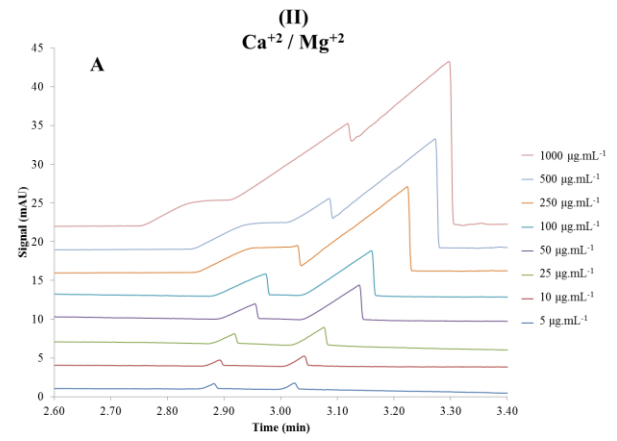
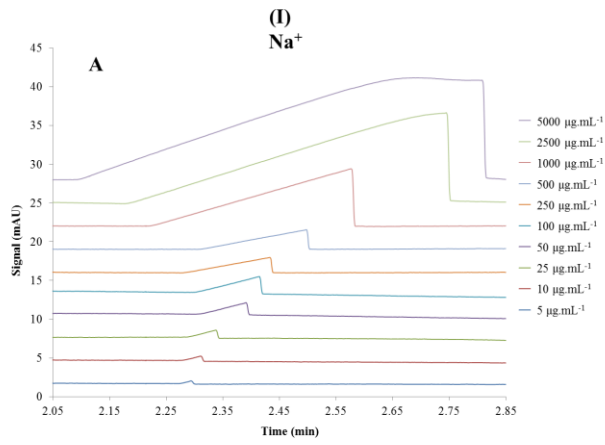
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Figure Captions

Figure 1. Superimposed electropherograms for the evaluation of peak shape as a function of BGE and standard solutions ionic strength. 2,4,6-trimethylpyridine: A- 50 mM, B- 100 mM, C- 200 mM. Standard solutions concentration detailed in legends. Panel I corresponds to Na^+ and Panel II to the pair $\text{Ca}^{+2} / \text{Mg}^{+2}$. Analysis conditions as described in section 2.3. Workflow.

Journal Pre-proof



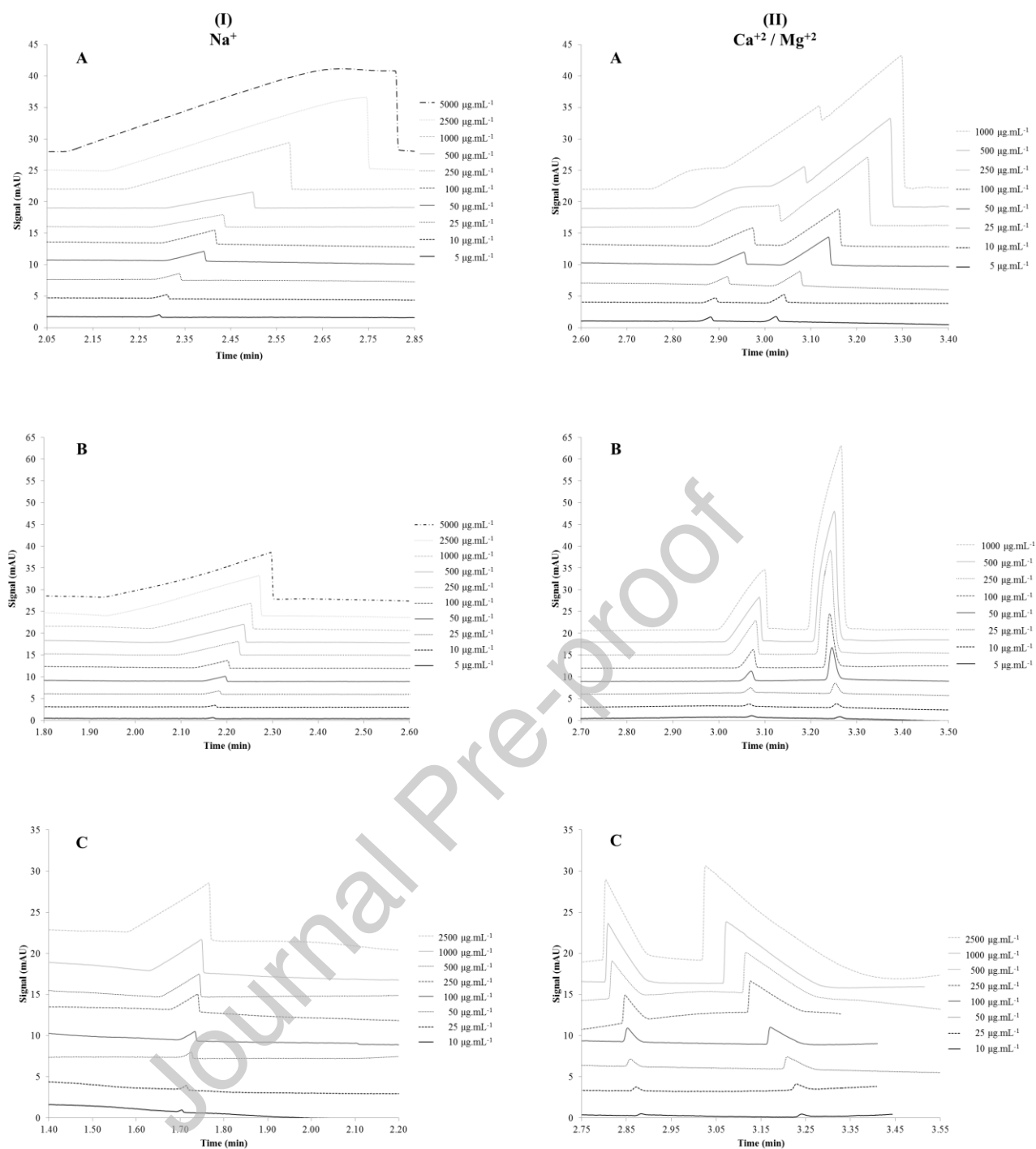
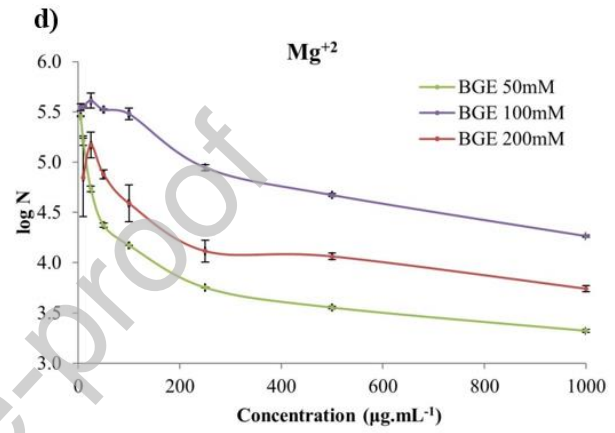
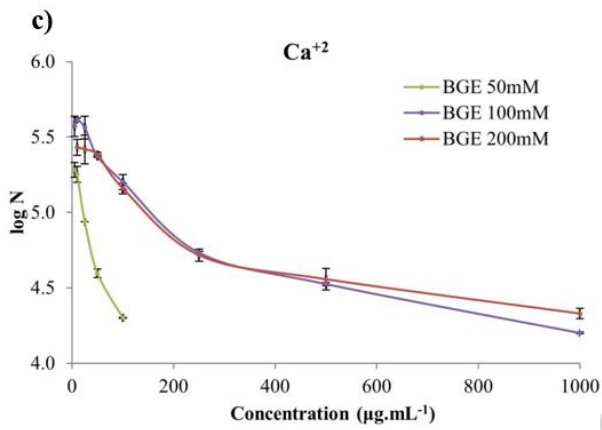
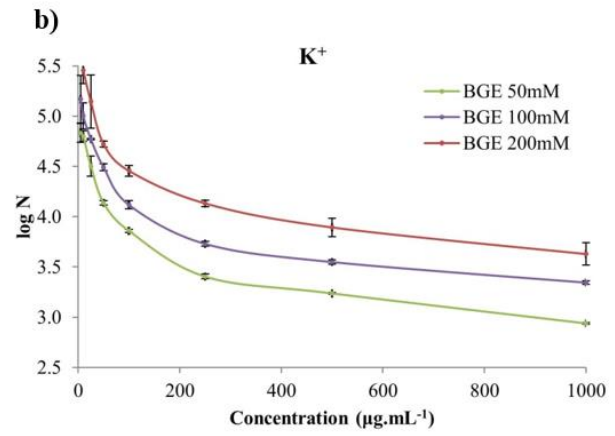
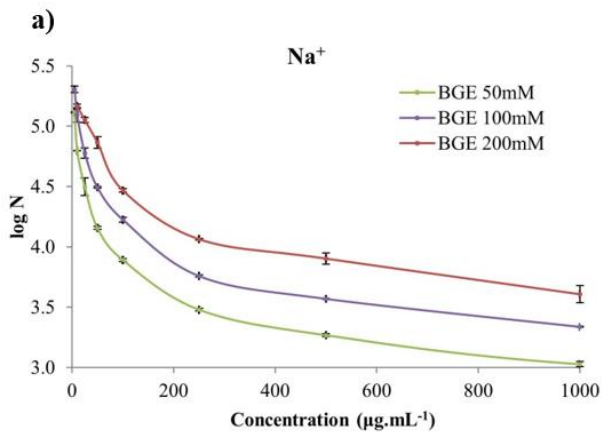


Figure 2. Plots of theoretical plate number (N) (plotted as $\log N$) as a function of analyte concentration at different BGE ionic strength (50, 100 and 200 mM 2,4,6-trimethylpyridine) for a) Na^+ , b) K^+ , c) Ca^{+2} , and d) Mg^{+2} . $N = 5.54 \cdot (t_m/W_{1/2})^2$ where t_m is the migration time and $W_{1/2}$ is the peak width at half-height.



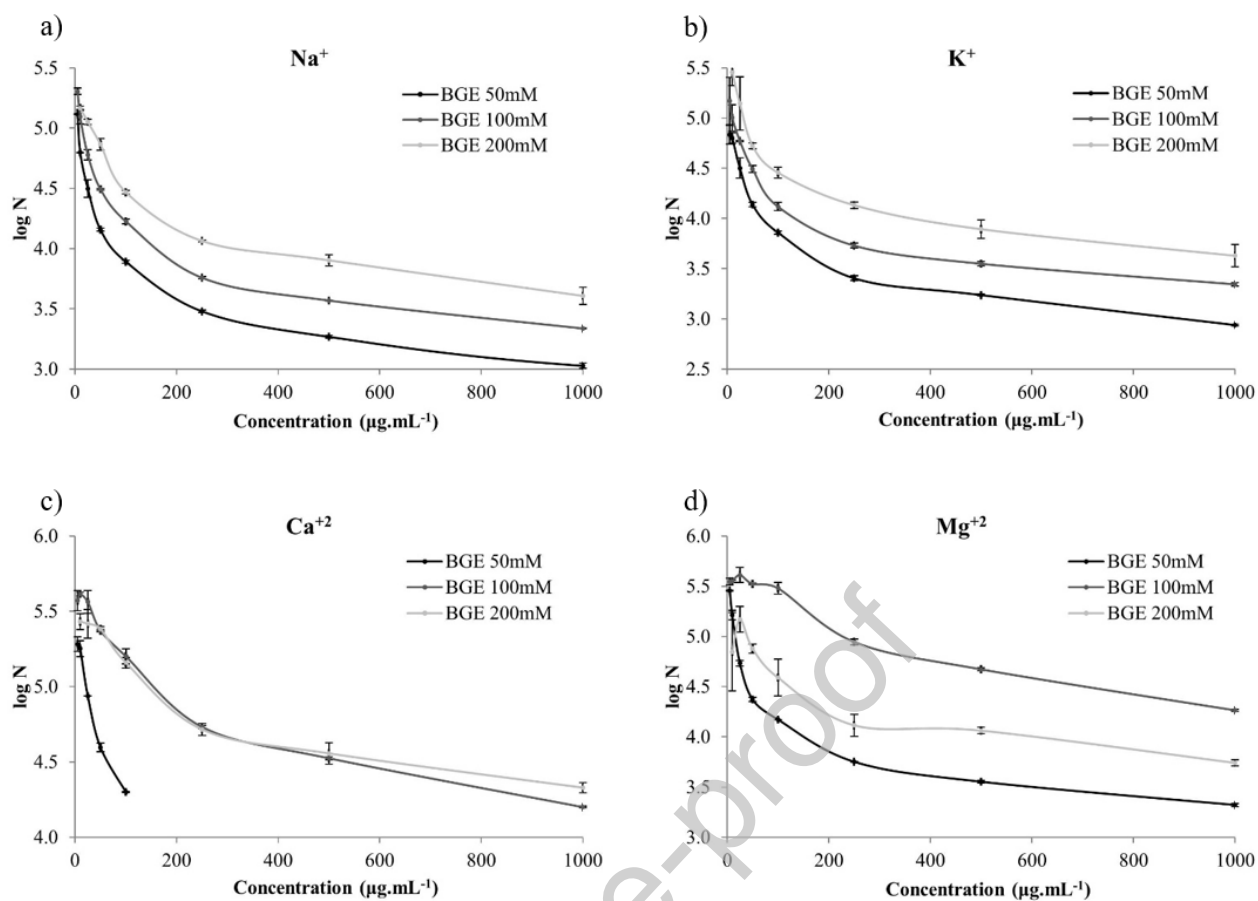
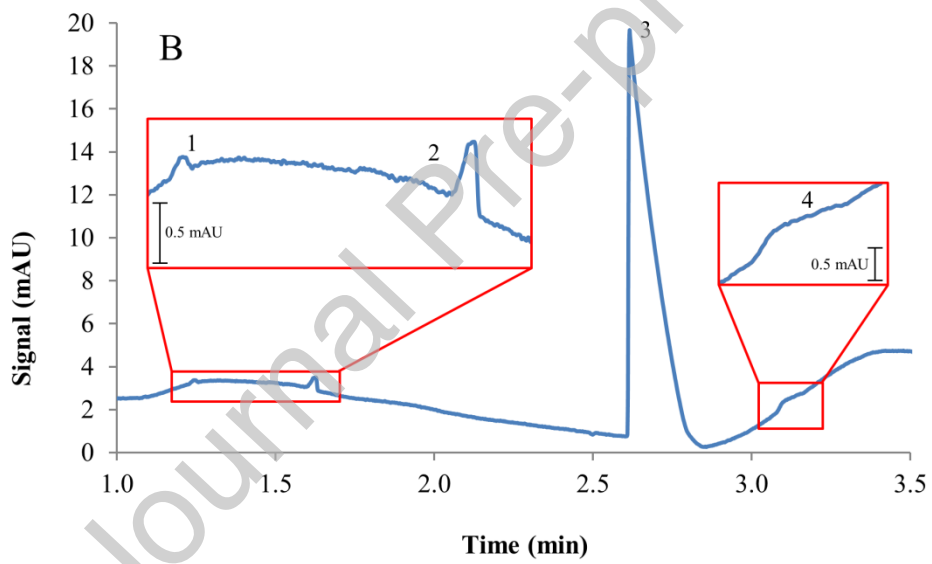
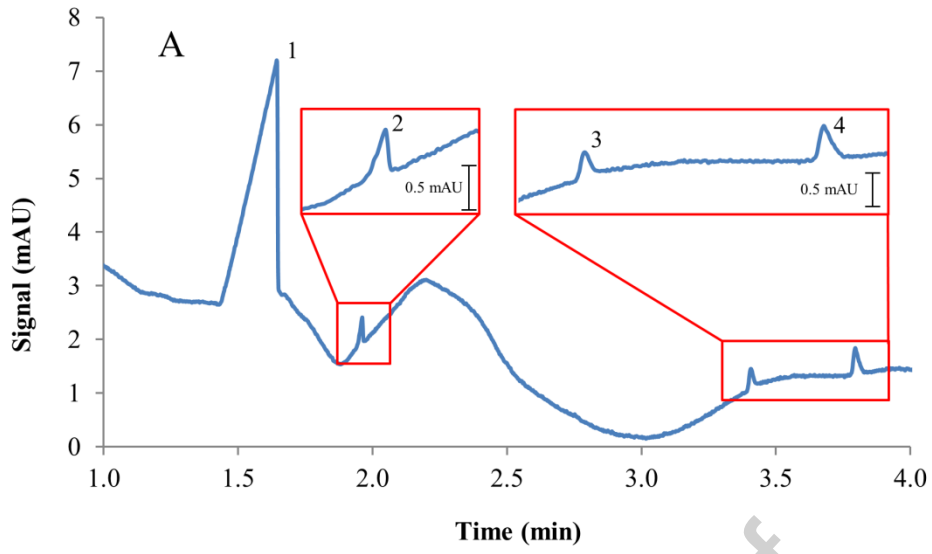


Figure 3. Electropherograms performed using 200 mM 2,4,6-trimethylpyridine BGE, showing the separation of standard mixtures containing one of the cations at an excessively high concentration compared to the others. A- K^+ $5000 \mu\text{g}\cdot\text{mL}^{-1}$; Na^+ , Ca^{+2} and Mg^{+2} $25 \mu\text{g}\cdot\text{mL}^{-1}$; B- Ca^{+2} $5000 \mu\text{g}\cdot\text{mL}^{-1}$; K^+ , Na^+ and Mg^{+2} $25 \mu\text{g}\cdot\text{mL}^{-1}$. Analysis conditions as described in section 2.3. Workflow.



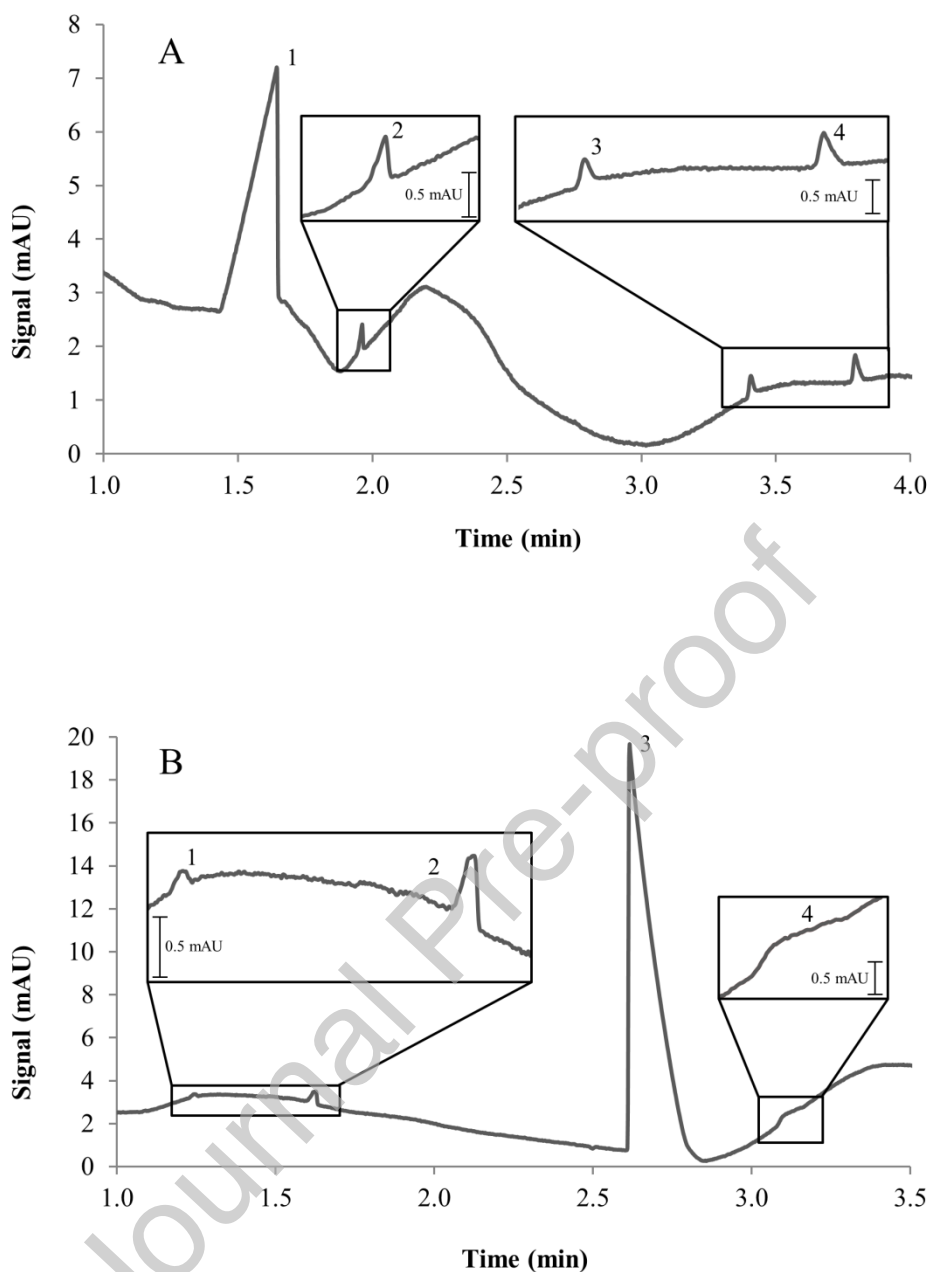
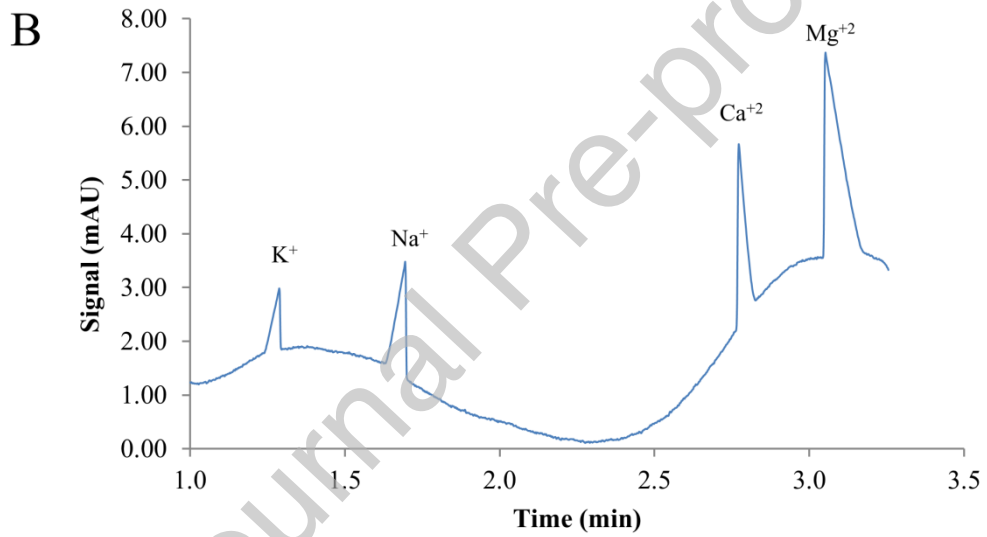
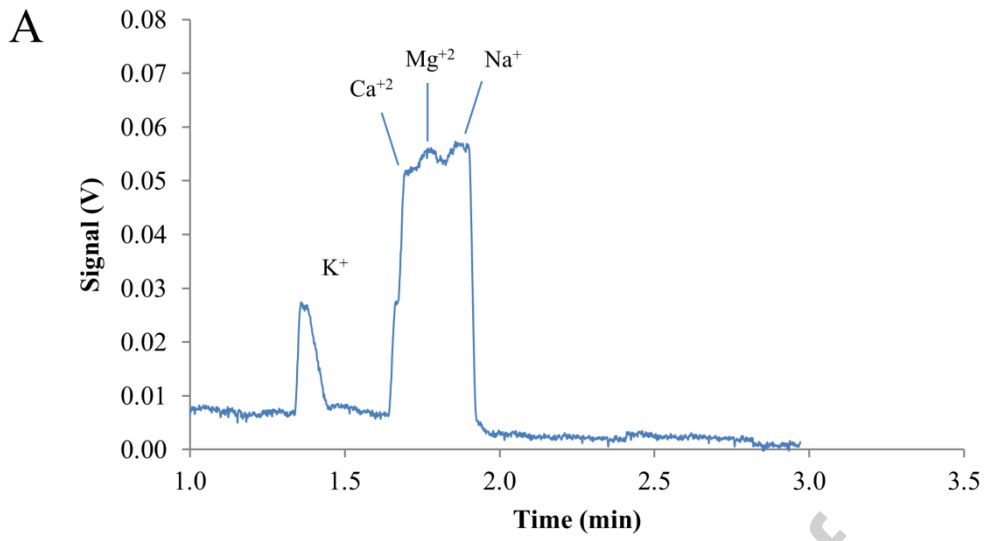


Figure 4. Electropherograms of the analysis of the 250 µg·mL⁻¹ standard solution. Comparison between A- 5 M acetic acid BGE and conductivity detection; and B- 200 mM 2,4,6-trimethylpyridine BGE and indirect UV-absorption detection (230 nm). Other analysis conditions as described in section 2.3. Workflow.



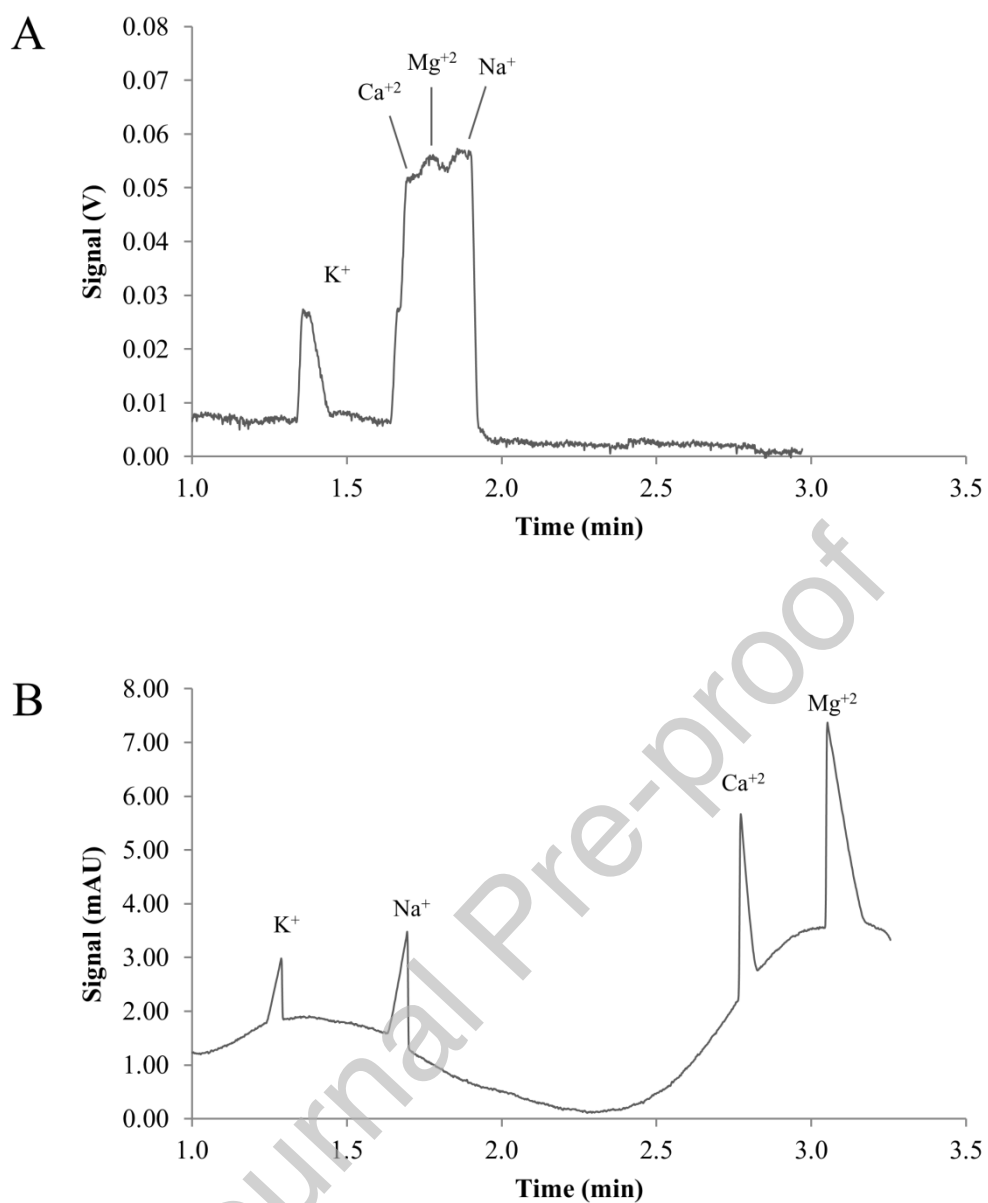
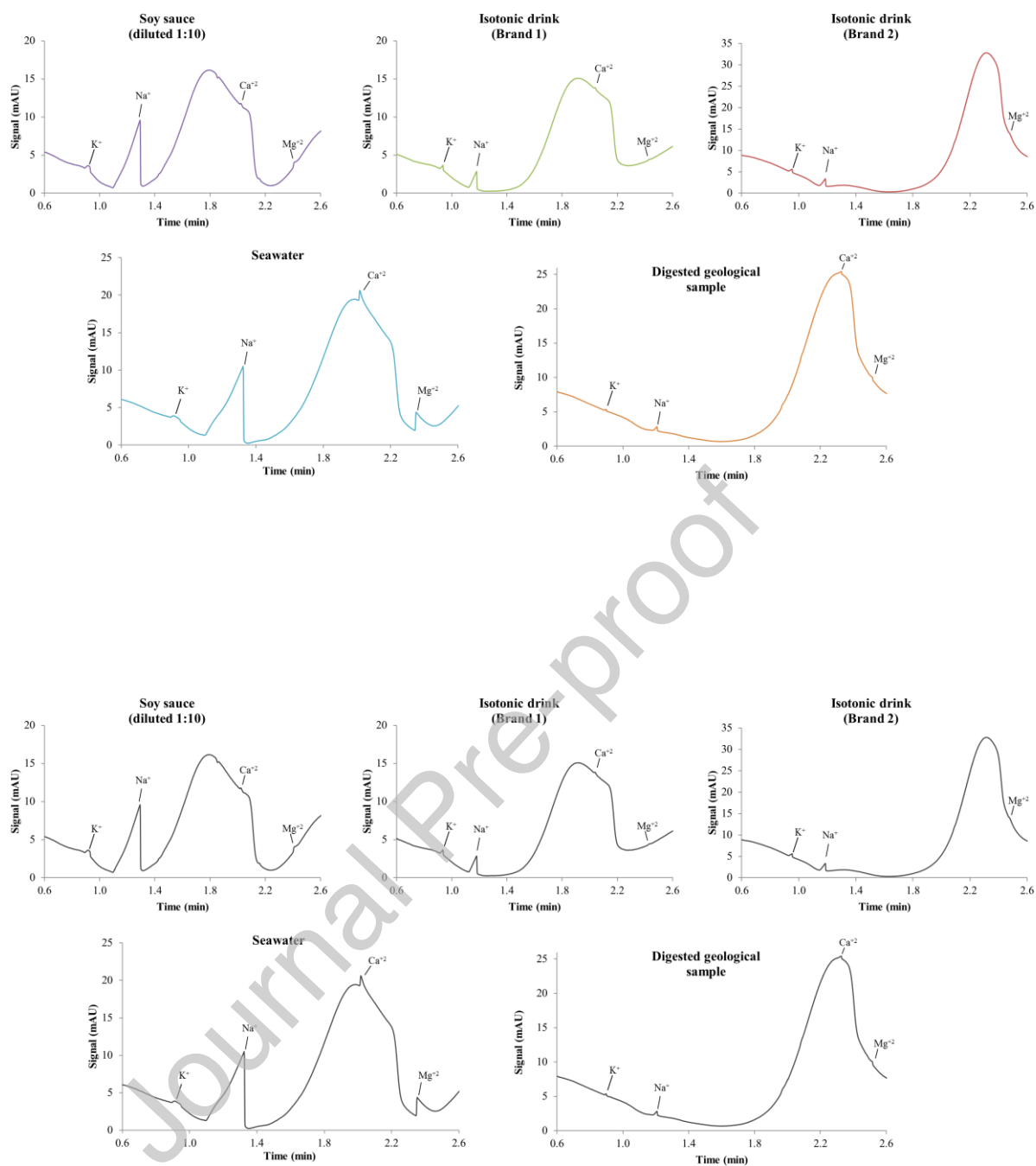


Figure 5. Electropherograms obtained employing the optimized BGE and separation conditions described in section 2.3. Workflow for each real sample quantified: Soy sauce (dilution 1:10), two different brands of isotonic drinks without dilution, seawater and digested geological sample.



Tables

Table 1. Physical properties and pK_a of the chromophores and complexing agents.

Compound (state at r.t.)	m. p. / b.p. (°C)	<i>M</i> (g·mol⁻¹)	Density (g·mL⁻¹)	pKa
o-toluidine (L)	-23.7 / 199	107.17	0.962	4.4
m-toluidine (L)	-30 / 203	107.17	0.984	4.7
p-toluidine (L)	-43 / 204	107.17	0.998	5.1
2-methylpyridine (L)	-70 / 129.5	93.13	0.943	6.2
3-methylpyridine (L)	-19 / 144	93.13	0.957	4.9
4-methylpyridine (L)	2.4 / 145	93.13	0.957	6.1
2,4,6-trimethylpyridine (L)	-43 / 170	121.18	0.917	6.7
formic acid (L)	8.4 / 100.8	46.03	1.220	3.75
lactic acid (L)	18 / 122	90.08	1.357	3.86
acetic acid (L)	16.6 / 118	60.05	1.049	4.75

1 **Table 2.** Quantitative results of the cations found in the analyzed samples: Concentration (c) and
 2 standard deviation of the determination (SD) in $\mu\text{g}\cdot\text{mL}^{-1}$. Analysis conditions as described in section
 3 2.3. Workflow.

	K^+		Na^+		Ca^{+2}		Mg^{+2}	
	c ($\mu\text{g}\cdot\text{mL}^{-1}$)	SD ($\mu\text{g}\cdot\text{mL}^{-1}$)	c ($\mu\text{g}\cdot\text{mL}^{-1}$)	SD ($\mu\text{g}\cdot\text{mL}^{-1}$)	c ($\mu\text{g}\cdot\text{mL}^{-1}$)	SD ($\mu\text{g}\cdot\text{mL}^{-1}$)	c ($\mu\text{g}\cdot\text{mL}^{-1}$)	SD ($\mu\text{g}\cdot\text{mL}^{-1}$)
Soy sauce [§]	3240 [#]	30	41900 [†]	300	140 [#]	20	90 [#]	20
Isotonic drink (Brand 1)	120	20	420	20	<i>N.Q.</i>		<i>N.Q.</i>	
Isotonic drink (Brand 2)	140	20	220	20	<i>N.D.</i>		<i>N.Q.</i>	
Seawater	270	20	8700 [#]	70	360	20	880	20
Digested geological sample	30	20	60	20	20	20	<i>N.Q.</i>	

4 N.D.: not detected. N.Q.: not quantified.

5 [§] Required dilution due to it high sample viscosity.

6 [#] Quantitation performed by a 1:10 dilution of the sample.

7 [†] Quantitation performed by a 1:20 dilution of the sample.

8

9