Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 130–136

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Determination of potassium, sodium, calcium and magnesium in total parenteral nutrition formulations by capillary electrophoresis with contactless conductivity detection

Susanne Nussbaumer[a,b], Sandrine Fleury-Souverain[a,*], Lucie Bouchoud[a,b], Serge Rudaz[b], Pascal Bonnabry[a,b], Jean-Luc Veuthey[b]

[a] Hôpitaux Universitaire de Genève–Pharmacie, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland
[b] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, 1211 Geneva, Switzerland

ARTICLE INFO

Article history:
Received 29 September 2009
Received in revised form 25 January 2010
Accepted 26 January 2010
Available online 4 February 2010

Keywords:
Capacitively coupled contactless conductivity detection
Capillary electrophoresis
Inorganic cations
Total parenteral nutrition
Validation

ABSTRACT

A simple method based on capillary electrophoresis with a capacitively coupled contactless conductivity detector (CE-C^4D) was developed for the determination of potassium, sodium, calcium and magnesium in parenteral nutrition formulations. A hydro-organic mixture, consisting of 100 mM Tris-acetate buffer at pH 4.5 and acetonitrile (80:20, v/v), was selected as the background electrolyte. The applied voltage was 30 kV, and sample injection was performed in hydrodynamic mode. All analyses were carried out in a fused silica capillary with an internal diameter of 50 μm and a total length of 64.5 cm. Under these conditions, complete separation between all cations was achieved in less than 4 min. The CE-C^4D method was validated, and trueness values between 98.6% and 101.8% were obtained with repeatability and intermediate precision values of 0.4–1.3% and 0.8–1.8%, respectively. Therefore, this method was found to be appropriate for controlling potassium, sodium, calcium and magnesium in parenteral nutrition formulations and successfully applied in daily quality control at the Geneva University Hospitals.

1. Introduction

Total parenteral nutrition (TPN) is the practice of feeding a person intravenously using nutritional formulas containing essential nutrients such as electrolytes, glucose, amino acids, trace elements and vitamins (see Table 1). These nutritional solutions are prepared daily at the pharmacy of the Geneva University Hospitals (HUG) for paediatric patients. Errors in the concentrations of electrolytes present increased risks for patients, especially for neonates. Therefore, TPN preparations are submitted to quality control before patient administration. Currently, sodium, potassium and calcium are checked at the HUG pharmacy using flame photometry or absorption spectrometry methods in control solutions without amino acids or vitamins. The constituents of real TPN samples (with increased concentrations of glucose, amino acids and vitamins) can interfere with the analysis of ions and contaminate the analytical system. Therefore, other analytical techniques are required.

Capillary electrophoresis (CE) coupled with indirect UV detection was developed for the analysis of inorganic cations [1–7], particularly sodium, potassium, calcium and magnesium, in TPN preparations [8,9]. These methods have been compared with flame atomic spectrometry and ion chromatography [1,9] and were found to be an acceptable alternative. However, UV-absorbing buffer additives and more complex buffer systems were needed to facilitate indirect absorbance detection [10], and weaker quantitative performance was achieved [1,9]. During the past few years, contactless conductivity detection has been recognized as an attractive alternative to optical detection techniques in CE because of its low cost, lack of maintenance requirements, easy handling and simple method development. Among the developed capacitively coupled contactless conductivity detectors (C^4D), we only consider in this paper the instrument used by Zemann [11,12]. The latter presents two metal tube electrodes, placed around the capillary. An oscillation frequency between 75 and 300 kHz is applied to one of the electrodes, and a signal is produced when an analyte zone with a different conductivity passes through the retention gap [2].

Numerous papers have described the analysis of inorganic cations (*e.g.*, sodium, potassium, calcium, magnesium) by CE-C^4D [2–4,10–23]. A buffer based on 2-(N-morpholino)ethanesulfonic

Abbreviations: BGE, background electrolyte; CS, calibration standard; C^4D, capacitively coupled contactless conductivity detector; EOF, electroosmotic flow; HIBA, α-hydroxyisobutyric acid; His, Histidine; HUG, Geneva University Hospitals; IS, internal standard; MES, 2-(N-morpholino)ethanesulfonic acid; SFSTP, Société Française des Sciences et Techniques Pharmaceutiques; TPN, total parenteral nutrition; Tris, tris(hydroxymethyl)-aminoethane; VS, validation standard.

* Corresponding author at: Hôpitaux Universitaire de Genève – Pharmacie, Rue Gabrielle-Perret-Gentil 4, 1211 Genève 14, Switzerland. Tel.: +41 22 382 39 78; fax: +41 22 382 39 65.
E-mail address: sandrine.fleury.souverain@hcuge.ch (S. Fleury-Souverain).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.01.042

Table 1
Composition of TPN at HUG.

Solution	Composition	Manufacturer
NaCl 11.7%	Sodium: 2 mmol mL^{-1}	Bichsel (Interlaken, CH)
Calcium gluco-bionate 10%	Calcium: 0.16 mmol mL^{-1}, lactobionate: 0.16 mmol mL^{-1}, glucobionate: 0.16 mmol mL^{-1}	Bichsel (Interlaken, CH)
Phocytan®	Phosphate: 0.33 mmol mL^{-1}, glucose: 0.33 mmol mL^{-1}, sodium: 0.66 mmol mL^{-1}	Aguettant (Lyon, F)
KCl 7.5%	Potassium: 1 mmol mL^{-1}	Sintetica-Bioren (Couvet, CH)
$MgSO_4$ 5%	Magnesium: 0.2 mmol mL^{-1}	Pharmacy HUG
Sodium acetate 16.4%	Acetate: 2 mmol mL^{-1} Sodium: 2 mmol mL^{-1}	Pharmacy HUG
Vitamins	Vitamin A: 0.4 mg mL^{-1}, D3 1.1 μg mL^{-1}, E 2.04 mg mL^{-1}, C 25 mg mL^{-1}, B1 0.7 mg mL^{-1}, B2 0.83 mg mL^{-1}, B6 0.91 mg mL^{-1}, B12 1.2 μg mL^{-1}, B9 82.5 μg mL^{-1}, B5 3.45 mg mL^{-1}, B8 13.8 μg mL^{-1}, PP 9.2 mg mL^{-1}	Baxter (Volketswil, CH)
Cernevit®		
Trace elements	Fe^{2+}: 0.1 mg mL^{-1}, Zn^{2+}: 0.16 mg mL^{-1}, Mn^{2+}: 27.2 μg mL^{-1}, Cu^{2+}: 38 μg mL^{-1}, Cr^{3+}: 0.5 μg mL^{-1}, Mo(VI): 0.5 μg mL^{-1}, Se(IV): 1 μg mL^{-1}, F^-: 28.5 μg mL^{-1}, I^-: 6.5 μg mL^{-1}, Na^+: 1.9 μmol mL^{-1}, K^+: 0.05 μmol mL^{-1}, Cl^-: 17 μmol mL^{-1}	BBraun (Sempach, CH)
Tracutil® diluted		Pharmacy HUG
Heparin	50 UI mL^{-1}	Pharmacy HUG
Amino acids	Alanine: 6.3 g L^{-1}, arginine: 4.1 g L^{-1}, asparagine acid: 4.1 g L^{-1}, cysteine: 1 g L^{-1}, glutamic acid: 7.1 g L^{-1}, glycine: 2.1 g L^{-1}, histidine: 2.1 g L^{-1}, isoleucine: 3.1 g, leucin: 7.0 g L^{-1}, lysine: 5.6 g L^{-1}, methionine: 1.3 g L^{-1}, phenylalanine: 2.7 g L^{-1}, proline: 5.6 g L^{-1}, serine: 3,8 g L^{-1}, taurine 0,3 g L^{-1}, threonine: 3.6 g L^{-1}, tryptophan: 1.4 g, tyrosine: 0.5 g L^{-1}, valine: 3.6 g L^{-1}	Fresenius Kabi (Stans, CH)
Vaminolact®		
Glucosteril	Glucose 70%	Fresenius (Stans, CH)
Injection water	Water ppi	Bichsel (Interlaken, CH)

acid and histidine (MES/His) has been widely used for the determination of alkali and alkaline earth metals and ammonium ions [2–4,11–20]. Other background electrolytes (BGE) composed of citric, lactic or acetic acids and His or maleic acid/arginine have also been successfully used for the separation of these cations [15,20].

Weak complexing agents have been added to the BGE to modify the separation of inorganic cations, such as α-hydroxyisobutyric acid (HIBA) [4,7,17]. An organic solvent was added (10% methanol) to modify the selectivity and to obtain a complete separation of sodium, calcium and magnesium in blood samples [20]. However, to our knowledge, a validated CE-C^4D for TPN has not yet been reported.

In this study, a simple CE-C^4D method was developed and validated to determine sodium, potassium, calcium and magnesium in TPN and was applied to the quantitation of these cations in daily quality control.

2. Experimental

2.1. Chemicals

Sodium chloride, potassium chloride, calcium chloride, magnesium chloride, lithium chloride and tris(hydroxymethyl)-aminoethane (Tris) were purchased from Fluka (Buchs, Switzerland). Water and NaCl (0.9%) used for pharmaceutical preparations were obtained from Bichsel Laboratories (Interlaken, Switzerland). Acetic acid (glacial, 100%), methanol and acetonitrile were obtained from Merck (Darmstadt, Germany).

Parenteral nutrition solutions were prepared at the HUG pharmacy using the automated compounding system BAXA MM12 (Baxa corporation, Englewood, CO, USA) with the following solutions: Calcium glucobionate (10%) and NaCl (11.7%) obtained from Bichsel Laboratories (Interlaken, Switzerland), KCl (7.5%) from Sintetica-Bioren SA (Couvet, Switzerland), Phocytan from Aguettant (Lyon, France), Aminosteril Hépa (8%), Glucosteril (70%), and Vaminolact from Fresenius Kabi (Stans, Switzerland; Bad Homburg, Germany). Tracutil was diluted in a 1:2 ratio (BBraun, Sempach, Switzerland) and Cernevit was obtained from Baxter (Volketswil, Switzerland). Sodium acetate (16.4%), heparin (50 UI/mL) and magnesium sulfate (5%) were produced by the HUG pharmacy.

2.2. BGE preparation

Different BGEs (phosphate pH 2 and 7, borate pH 9, MES/His pH 6.1, citrate pH 3.1 and pH 4.8, lactate and acetate/Tris pH 4.5) were prepared for the method development. The final BGE was composed of a hydro-organic buffer corresponding to a mixture of an aqueous BGE (100 mM Tris-acetate buffer at pH 4.5) and acetonitrile (80:20, v/v). The aqueous BGE was prepared by an adequate dilution of the concentrated acid solution, and a solution of Tris at 1 M was added to adjust the solution to pH 4.5. The solution was then diluted to the final volume with distilled water. The BGE was degassed in an ultrasonic bath for 10 min before use.

2.3. Instrumentation and capillaries

CE experiments were carried out with an $HP^{3D}CE$ system (Agilent Technologies, Waldbronn, Germany) equipped with an autosampler and a power supply able to deliver up to 30 kV. $HP^{3D}CE$ was coupled to a TraceDec detector (Innovative Sensor Technologies GmbH, Strasshof, Austria). The conductivity sensor consisted of two electrodes separated by a detection gap of 1 mm, positioned along the capillary by sliding it into the desired position (14.5 cm from the cathode). A CE ChemStation (Agilent) was used for CE control and data handling, and a C^4D Tracemon (Innovative Sensor Technologies, Austria) was used for conductivity detector control and data acquisition. Analyses were performed in uncoated fused silica capillaries from BGB Analytik AG (Böckten, Switzerland) with an internal diameter of 50 μm, an external diameter of 375 μm and a total length of 64.5 cm (effective length of 50 cm). All experiments were performed in the normal mode (cathode at the outlet end of the capillary). The capillary was thermostated at 25 °C in a high velocity air stream, and a voltage of 30 kV was applied. The generated current was between 5 and 50 μA depending on the buffer solution. Samples were kept at ambient temperature in the autosampler and injected in the hydrodynamic mode to fill approximately 1% of the effective capillary length (40 mbar for 10 s). The final configuration of the C^4D was set at an output frequency of 150 kHz, an output voltage of 40 Vpp, 50% gain and an offset of ~30. The detector acquisition corresponded to the CE mode of 19.8 Hz. Before first use, capillaries were sequentially rinsed with methanol, 0.1 M NaOH, water, methanol, 0.1 M HCl, water and BGE for 5 min. A voltage of 30 kV was then applied for 60 min with the BGE. The

Table 2
Composition of the validation matrix.

Concentration	Nutriment	Composition
42 g/L	Amino acid	160.8 mL of Vaminolact
218.8 g/L	Glucose	78.2 mL of Glucosteril
500 U/L	Heparin	2.5 mL of Heparin
20 ml/L	Oligoelement	5 mL of diluted Tracutil

TraceDec was set to run for 1 h before the first analysis in order to obtain a constant signal. Prior to each sample injection, the capillary was rinsed by pressure (940 mbar) for 1 min with fresh BGE. When not in use, the capillary was rinsed with water and methanol. As the electrophoresis process altered the running buffer pH by electrolysis and subsequently changed the migration times, the separation buffer was refreshed every six runs.

2.4. Method validation

A validation was performed to estimate the quantitative parameters of the method for the analysis of potassium, sodium, calcium and magnesium in parenteral nutritional formulations. The validation was based on ICH recommendations following the guidelines of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) [24] and carried out over three series. Each series involved the injection of a freshly prepared BGE, two calibration standards (CS) at 4 mM for K^+, Na^+ and 2 mM for Ca^{2+}, Mg^{2+}, four validation standards (VS) at 1, 2 and 4 mM for K^+, Na^+ and 0.5, 1 and 2 mM for Ca^{2+}, Mg^{2+}, complete washing of the capillary with water and methanol, and instrument shut-off. Lithium chloride was used as the internal standard (IS). The calculations were performed using normalised area (area/migration time) ratios of the cations on the IS.

2.5. Sample preparation

CS and VS were independently prepared. The IS stock solution was prepared by dissolving lithium chloride in water at a concentration of 50 mM.

2.5.1. Calibration standard

A standard stock solution was prepared by dissolving KCl, NaCl, $CaCl_2{\cdot}2H_2O$ and $MgCl_2{\cdot}6H_2O$ in water to obtain a concentration of 100 mM for K^+ and Na^+, and 50 mM for Ca^{2+} and Mg^{2+}, which were stored at 4 °C until use. Sample solutions were stable for more than 1 week at 4 °C, and no degradation was observed for the tested analytes during analysis. One concentration level sample was prepared by diluting the appropriate volume of standard stock solution in distilled water at a final concentration of 4 mM of K^+ and Na^+ and 2 mM of Ca^{2+} and Mg^{2+}. Lithium chloride was added as an internal standard to obtain a final concentration of 1.25 mM.

2.5.2. Validation standard

A TPN solution with 40 mM of K^+ and Na^+ and 20 mM Ca^{2+} and Mg^{2+} was prepared by diluting NaCl (11.7%), calcium glucobionate (10%), KCl (7.5%) and magnesium sulfate (5%) in a condensed TPN matrix consisting of glucose (Glucosteril, 70%), amino acids (Vaminolact), heparin and trace elements (Tracutil) as shown in Table 2. For VS, three concentration level samples were prepared at 25%, 50% and 100% of the highest value (4 mM K^+ and Na^+, 2 mM Ca^{2+} and Mg^{2+}) by diluting the appropriate volume of the TPN solution in water.

2.6. Application to TPN solutions

The four cations were determined in TPN solutions prepared at the pharmacy of HUG. Therefore, the formulations were diluted in distilled water to obtain a final concentration between 1 and 4 mM for K^+ and Na^+, and 0.5 and 2 mM for Ca^{2+} and Mg^{2+}. The quantitative analysis was repeated twice ($N = 2$) for each formulation.

3. Results and discussion

Paediatric TPN are produced daily in the HUG pharmacy and submitted to a quality control before patient administration. A CE-C^4D method was developed and validated for determining potassium, sodium, calcium and magnesium in these preparations.

3.1. Method development

3.1.1. Buffer selection

The selection of the BGE was based on conductivity detection of the four cations and selectivity toward other compounds of the TPN, such as amino acids, glucose or vitamins. In C^4D, the response arises from the difference in conductivity between analytes and BGE co-ions. For obtaining the highest signal-to-noise ratio, a large difference between the conductance of the analytes and the electrolyte is needed. Moreover, CE requires BGEs with a higher ionic strength compared to the sample zone to take advantage of the stacking effect. The compromise consists of using an amphoteric or low conductance buffer at high ionic strength [12]. Among the different BGEs tested, a good separation of the four cations was achieved as expected with the commonly used MES/His BGE [2–4,11–20], but also with the acetate/Tris buffer system (pH 4.5). With both BGEs, the resolution between sodium, calcium and magnesium had to be improved to determine magnesium and calcium in presence of sodium at high concentration, as it is generally the case in TPN. The acetate/Tris BGE was chosen for further development because it gave satisfactory results for the analysis of suxamethonium by CE-C^4D [25] and it possesses a low conductivity and can be used at a concentration of 100 mM without generating a high current (~20 μA). Lithium chloride was chosen as IS because it is not a constituent of TPN and it presents a much lower mobility than the four cations tested.

3.1.2. Influence of acetate concentration in the BGE

The first analyses were performed with an acetate/Tris buffer at 20 mM to reduce the background conductivity. Nevertheless, BGEs with different concentrations were tested (10, 20, 30, 50, 75 and 100 mM) to improve the resolution between sodium, calcium and magnesium. As shown in the literature, interactions of the analytes with BGE components could enhance selectivity in CE [4,5,7,8,17,21,26]. In these studies, a weak complexing agent (for example HIBA) was added to the BGE to modify the separation of the cations. The mobility of Mg^{2+} and Ca^{2+} was found to decrease due to a stronger interaction with HIBA [8]. In this work, acetate is a weak complexing agent that can interact with the studied cations [5]. Indeed, calcium and magnesium have higher complexation constants with acetate than potassium or sodium [27]. Increasing the acetate concentration therefore changed the migration order to potassium, sodium, calcium and magnesium (see Fig. 1). Furthermore, as expected, the electroosmotic flow (EOF) decreased with increasing acetate concentration, resulting in a net increase of the migration times of all cations.

The pH of the BGE modifies the EOF and the proportion of acetate, which can influence the separation of the cations. Therefore, acetate/Tris solutions with different pH were tested in the buffer region. In this work, the migration order of the cations changed with the pH value (pH 4.1: calcium–sodium–magnesium;

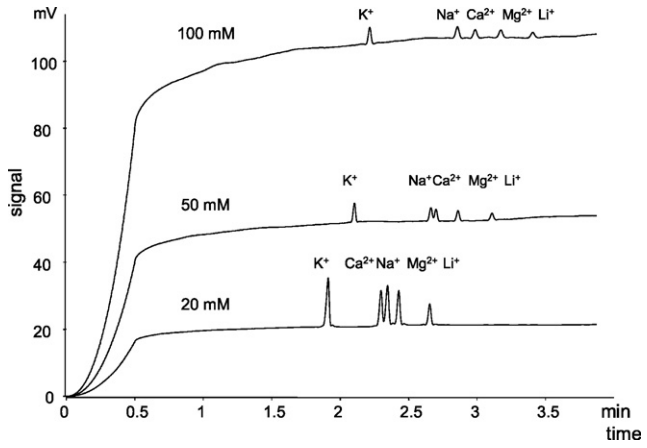

Fig. 1. Influence of BGE concentration: electropherograms of a sample containing sodium (1 mM), potassium (1 mM), calcium (0.5 mM), magnesium (0.5 mM), and lithium (1.25 mM) in an aqueous solution. BGE: 20, 50 and 100 mM Tris-acetate at pH 4.9. All other experimental conditions are described in Section 2.3.

pH 4.9: sodium–calcium–magnesium). This change of selectivity can also be explained by interactions of the cations with acetate. The best separation was obtained with a buffer pH of 4.9, but the signal-to-noise ratio was lower due to the higher conductance of the BGE (shown in Fig. 2).

Finally, a 100 mM Tris acetate buffer at pH 4.5 was selected since the signal-to-noise ratio of the cations was significantly enhanced compared to a BGE at pH 4.9 and the current generated was still inferior to 30 μA. Under these conditions, the complete separation of the four cations was achieved and the mobilities of the compounds were in the following decreasing order: potassium, sodium, calcium, magnesium and lithium.

3.1.3. Addition of an organic solvent

Ion-pair formation can be favoured by non-aqueous solvents due to their lower permittivity constant [28]. Organic solvents change the solvation radii of ions, which contributes to a modification of their mobilities [29–31]. They also alter the viscosity of BGE and directly affect the mobility of the analytes. Therefore, resolution can be enhanced by the addition of organic solvents. Separation of cations in purely non-aqueous buffers was achieved by Salimi-Moosavi and Cassidy and the effect of acetonitrile in methanol was demonstrated to be useful [31]. The addition of organic solvents

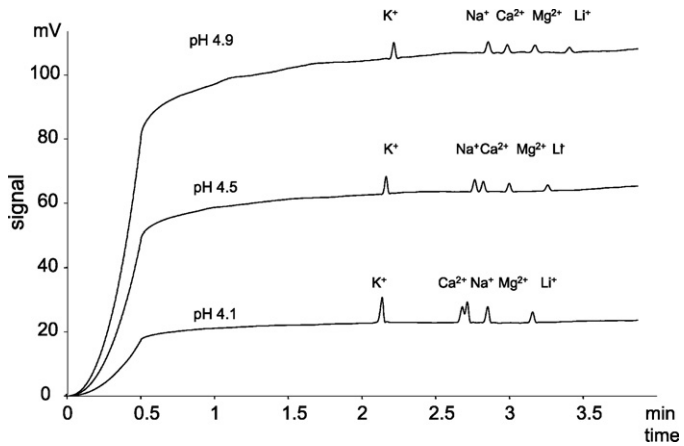

Fig. 2. Influence of BGE pH on the conductivity detection: electropherograms of a sample containing sodium (1 mM), potassium (1 mM), calcium (0.5 mM), magnesium (0.5 mM), and lithium (1.25 mM) in an aqueous solution. BGE: 100 mM Tris-acetate at pH 4.1, 4.5 and 4.9. All other experimental conditions are described in Section 2.3.

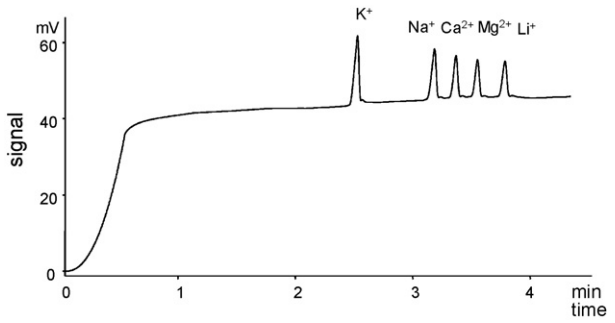

Fig. 3. Electropherogram obtained for the CE-C^{4}D analysis of a sample containing sodium (1 mM), potassium (1 mM), calcium (0.5 mM), magnesium (0.5 mM) and lithium (1.25 mM) in an aqueous solution. BGE: 100 mM Tris-acetate at pH 4.5, acetonitrile (80:20, v/v). All other experimental conditions are described in Section 2.3.

to the electrophoretic medium can modify the selectivity through changes in the solvent pH and analyte pK_a. The increase of the pK_a values of aromatic acids with increasing concentration of acetonitrile was studied by Sarmini and Kenndler [32]. This is most easily understood in terms of a solvent-induced change of the analyte charge [33].

The addition of 10–30% methanol did not change the separation in the presented work, while it has been shown useful for changing the selectivity in other studies [29]. However, the addition of acetonitrile enhanced the separation of sodium, calcium and magnesium. Different concentrations of acetonitrile were added to the BGE (data not shown). The addition of 20% acetonitrile to the acetate/Tris BGE improved the separation significantly, without modifying the migration order.

Therefore, 100 mM acetate/Tris BGE, pH 4.5, 20% acetonitrile (v/v) was selected for the separation of the four cations (Rs > 1.5) (see Fig. 3).

3.1.4. C^{4}D parameters

Different oscillation voltages and oscillation frequencies of the C^{4}D were tested (data not shown). An oscillation voltage of 40 Vpp and a frequency of 150 kHz gave the best results with the selected BGE. The response of potassium, sodium, calcium and magnesium and lithium as a function of the excitation frequency was studied by Pavel and Hauser, where a maximal output voltage was observed at a frequency of 250 or 400 kHz with a BGE of His and acetic acid at pH 2.75 [14]. The difference of BGE did not allow a direct comparison of the optimal set-up parameters, but, in both cases, the detector response was enhanced with increasing output frequency.

3.2. Method validation

TPN is produced daily on prescription and the concentration of the different constituents varies in each case. The validation of the method could not include all dilutions and compositions possible, but was based on a worst case situation according to 4 years of TPN prescription at the HUG (internal unpublished data). In general, sodium is the most abundant cation in the TPN, while magnesium is the less concentrated. Calcium and magnesium are present at much lower concentrations than sodium or potassium and, therefore, the CS of Ca^{2+} and Mg^{2+} were chosen to be half of the concentration of Na^+ and K^+. First, the response function in the concentration range of 0.2–4 mM for Na^+ and K^+ and 0.1–2 mM for Ca^{2+} and Mg^{2+} was evaluated with ordinary linear regression using five concentration levels (5%, 10%, 25%, 50% and 100%). A linear response function ($r^2 > 0.999$) was achieved for all cations in the tested concentration range. Therefore, a 1-level calibration at 4 mM Na^+ and K^+ and 2 mM Ca^{2+} and Mg^{2+} (100%) was chosen for the validation in order

to shorten the analysis sequence time. The LOD of the method was estimated at 0.02 mM for all cations, while lowest quantification level obtained after dilution of the TPN was at 1 mM for Na^+ and K^+ and 0.5 mM for Ca^{2+} and Mg^{2+}.

For the VS, reconstituted dosage forms were obtained by a blank matrix built of glucose, amino acids, heparin and trace elements in the highest possible concentration, to mimic the highest concentrated samples, spiked with sodium, potassium, calcium and magnesium at usual TPN concentrations. The blank matrix composition is shown in Table 2.

The developed method was validated according to ICH guidelines following the SFSTP recommendations [24]. Quantitative performance was estimated in three separate series ($j = 3$) with the V1 protocol. This protocol involves one level ($k = 1$) at the upper end of the investigated range with two repetitions ($n = 2$) for CS and three concentration levels ($k = 3$) with four repetitions ($n = 4$) for the VS.

The concentrations of VS (25%, 50% and 100% of the target value) were computed from the analytical response to obtain trueness, repeatability and intermediate precision. Trueness was expressed in percent as the ratio between the theoretical and average measured values at each concentration level. Repeatability and intermediate precision were expressed as the coefficient of variation (CV %) of the ratio of the intra-day standard deviation (s_r) and between-day standard deviation (s_R), respectively, on the theoretical concentrations as described in [34]. The s_r and s_R values were obtained using ANOVA analysis. As reported in Table 3, the trueness and precision values were in accordance with regular recommendations for the analysis of pharmaceutical formulations over the tested concentration range. The CV (repeatability and intermediate precision) was lower than 2%, with trueness between 98.6 and 101.8% for all cations. To visualise the overall method variability, the accuracy profile of each cation was built combining trueness and intermediate precision as the confidence interval [35]. As presented in Fig. 4, the total error did not exceed the acceptance limits (±5%) for all concentration levels. Consequently, the developed CE-C^4D method is validated for determining the four cations over the tested concentration range.

Table 3
Validation results: trueness, repeatability and intermediate precision of the developed CE-C^4D method for the determination of the four cations in a pharmaceutical formulation.

	Trueness	Repeatability (CV)	Intermediate precision (CV)
Theoretical concentration of potassium [mM]			
1	100.6%	1.0%	1.3%
2	101.8%	1.2%	1.4%
4	101.6%	1.1%	1.1%
Theoretical concentration of sodium [mM]			
1	100.9%	1.2%	1.5%
2	100.9%	1.1%	1.5%
4	99.7%	0.9%	1.2%
Theoretical concentration of calcium [mM]			
0.5	100.5%	1.1%	1.1%
1	100.4%	1.3%	1.8%
2	99.0%	0.4%	1.1%
Theoretical concentration of magnesium [mM]			
0.5	99.1%	1.0%	1.2%
1	99.2%	0.8%	1.1%
2	98.6%	0.8%	0.8%

3.3. Application in the quality control laboratory of the HUG pharmacy

In order to demonstrate the applicability of the CE-C^4D method to real samples with different concentrations, quantitation of the four cations was achieved on several formulations prepared at the pharmacy of HUG. The concentrations of sodium, potassium, calcium and magnesium were calculated with reference to a central point, which was replicated twice. All concentrations of the four cations were found to be in the tolerated concentration of ±15% of the target value (internal fixed limits) by CE-C^4D. The results for

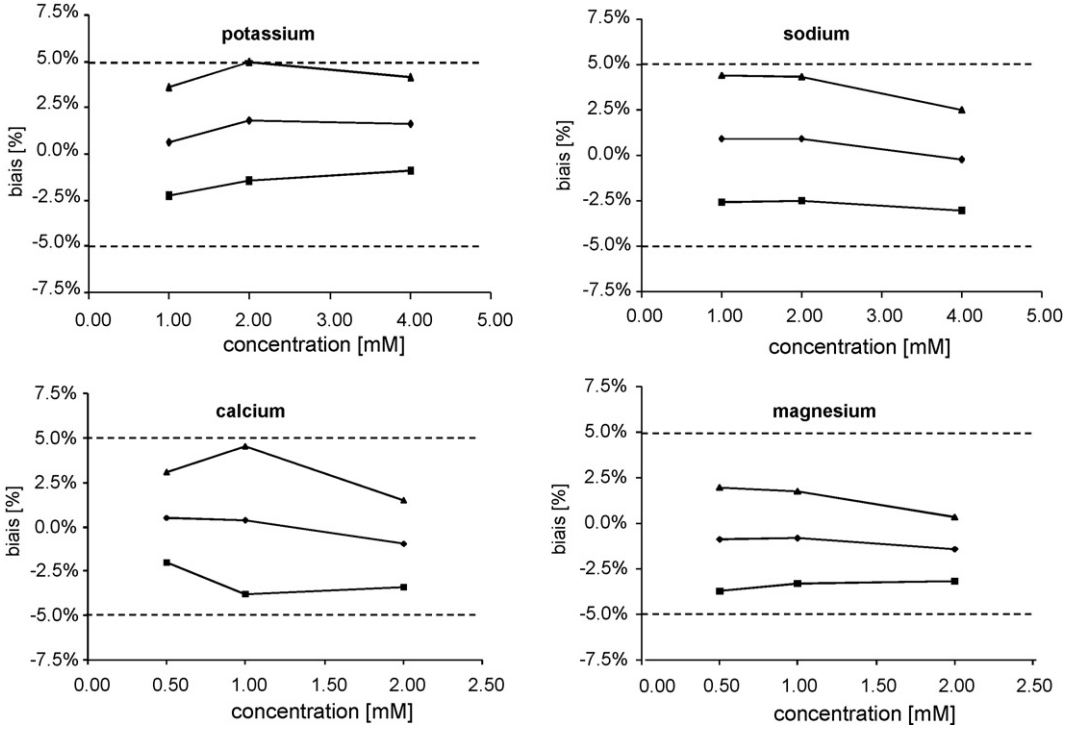

Fig. 4. Accuracy profile of the developed CE-C^4D method for the determination of sodium, potassium, calcium and magnesium in total parenteral nutrition. The dashed lines represent the acceptance limits of ±5%.

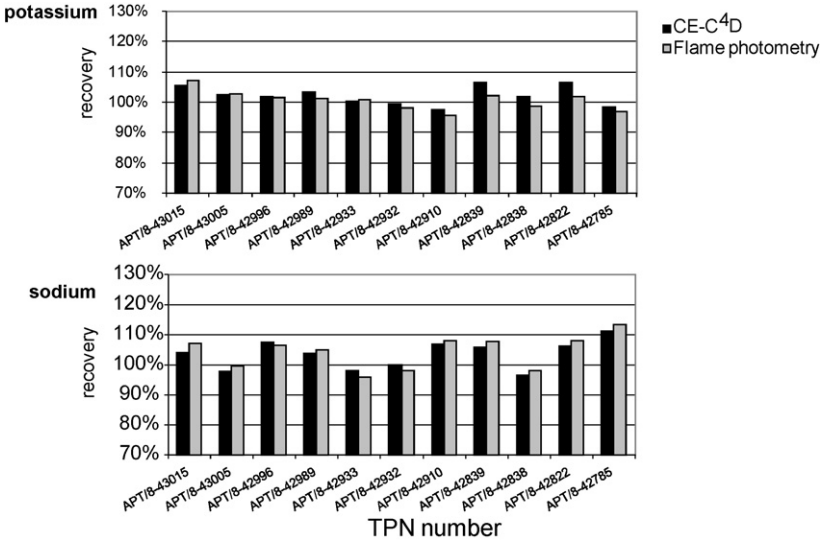

Fig. 5. Comparison of the results obtained by the developed CE-C^4D method and the determination by flame photometry for potassium and sodium.

sodium and potassium were confirmed by flame photometry (IL 243 flame photometer, Instrumentation Laboratory (Italy)) used as a reference method at the pharmacy of HUG. The results are shown in Fig. 5. The two methods were compared with the *t*-test for paired samples and were statistically identical for the determination of sodium and potassium (data not shown).

4. Conclusions

A simple method was developed for the quantitative determination of potassium, sodium, calcium and magnesium in TPN solutions by CE-C^4D. Under these conditions, even if the tested compounds did not possess chromophore groups, the developed method exhibited very good quantitative performance in terms of accuracy and precision with an analysis time of less than 4 min for all cations. The results demonstrated that CE-C^4D analysis is very useful for the determination of cations in parenteral nutrition formulations and the method was successfully applied in daily quality control.

References

[1] M. Pantsar-Kallio, P.K.G. Manninen, Determination of sodium, potassium, calcium and magnesium cations by capillary electrophoresis compared with ion chromatography, Anal. Chim. Acta 314 (1995) 67–75.
[2] J.S. Fritz, Recent developments in the separation of inorganic and small organic ions by capillary electrophoresis, J. Chromatogr. A 884 (2000) 261–275.
[3] P. Kubáň, B. Karlberg, P. Kubáň, V. Kubáň, Application of a contactless conductometric detector for the simultaneous determination of small anions and cations by capillary electrophoresis with dual-opposite end injection, J. Chromatogr. A 964 (2002) 227–241.
[4] A.R. Timerbaev, Capillary electrophoresis of inorganic ions: an update, Electrophoresis 25 (2004) 4008–4031.
[5] T.I. Lin, Y.H. Lee, Y.C. Chen, Capillary electrophoretic analysis of inorganic cations—role of complexing agent and buffer pH, J. Chromatogr. A 654 (1993) 167–176.
[6] C. Johns, M. Macka, P.R. Haddad, Highly sensitive indirect photometric detection of cations by capillary electrophoresis with the cationic dye chrysoidine, J. Chromatogr. A 997 (2003) 87–94.
[7] M. Chiari, Enhancement of selectivity in capillary electrophoretic separations of metals and ligands through complex formation, J. Chromatogr. A 805 (1998) 1–15.
[8] M. Koberda, M. Konkowski, P. Youngberg, W.R. Jones, A. Weston, Capillary electrophoretic determination of alkali and alkaline-earth cations in various multiple electrolyte solutions for parenteral use, J. Chromatogr. A 602 (1992) 235–240.
[9] Q. Yang, M. Jimidar, T.P. Hamoir, J. Smeyers-Verbeke, D.L. Massart, Determination of alkali and alkaline earth metals in real samples by capillary ion analysis, J. Chromatogr. A 673 (1994) 275–285.
[10] E. Baltussen, R.M. Guijt, G. van der Steen, F. Laugere, S. Baltussen, G.W.K. van Dedem, Considerations on contactless conductivity detection in capillary electrophoresis, Electrophoresis 23 (2002) 2888–2893.
[11] A.J. Zemann, Conductivity detection in capillary electrophoresis, Trends Anal. Chem. 20 (2001) 346–354.
[12] A.J. Zemann, Capacitively coupled contactless conductivity detection in capillary electrophoresis, Electrophoresis 24 (2003) 2125–2137.
[13] A.J. Zemann, E. Schnell, D. Volgger, G.K. Bonn, Contactless conductivity detection for capillary electrophoresis, Anal. Chem. 70 (1998) 563–567.
[14] P. Kubáň, P.C. Hauser, Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part I: Frequency behavior and cell geometry, Electrophoresis 25 (2004) 3387–3397.
[15] P. Kubáň, P.C. Hauser, Contactless conductivity detection in capillary electrophoresis: a review, Electroanalysis 16 (2004) 2009–2021.
[16] R.M. Guijt, C.J. Evenhuis, M. Macka, P.R. Haddad, Conductivity detection for conventional and miniaturised capillary electrophoresis systems, Electrophoresis 25 (2004) 4032–4057.
[17] J. Tanyanyiwa, P.C. Hauser, High-voltage contactless conductivity detection of metal ions in capillary electrophoresis, Electrophoresis 23 (2002) 3781–3786.
[18] P.S. Vuorinen, M. Jussila, H. Sirén, S. Palonen, M.-L. Riekkola, Integration of a contactless conductivity detector into a commercial capillary cassette: detection of inorganic cations and catecholamines, J. Chromatogr. A 990 (2003) 45–52.
[19] V. šolínová, V. Kašička, Recent applications of conductivity detection in capillary and chip electrophoresis, J. Sep. Sci. 29 (2006) 1743–1762.
[20] Q.J. Wan, P. Kubáň, J. Tanyanyiwa, A. Rainelli, P.C. Hauser, Determination of major inorganic ions in blood serum and urine by capillary electrophoresis with contactless conductivity detection, Anal. Chim. Acta 525 (2004) 11–16.
[21] P. Janoš, Role of chemical equilibria in the capillary electrophoresis of inorganic substances, J. Chromatogr. A 834 (1999) 3–20.
[22] J.A. Fracassi da Silva, N. Guzman, C.L. do Lago, Contactless conductivity detection for capillary electrophoresis: hardware improvements and optimization of the input-signal amplitude and frequency, J. Chromatogr. A 942 (2002) 249–258.
[23] P. Kubáň, P.C. Hauser, Fundamental aspects of contactless conductivity detection for capillary electrophoresis. Part II: Signal-to-noise ratio and stray capacitance, Electrophoresis 25 (2004) 3398–3405.
[24] P. Hubert, J.J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.A. Compagnon, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Validation des procédures analytiques quantitatives - Harmonisation des démarches, STP Pharma Pratiques 13 (2003) 101–138.
[25] S. Nussbaumer, S. Fleury-Souverain, S. Rudaz, P. Bonnabry, J.-L. Veuthey, Determination of suxamethonium in a pharmaceutical formulation by capillary electrophoresis with contactless conductivity detection (CE-C4D), J. Pharm. Biomed. Anal. 49 (2009) 333–337.
[26] V. Pacáková, P. Coufal, K. štulík, Capillary electrophoresis of inorganic cations, J. Chromatogr. A 834 (1999) 257–275.
[27] S. Kotrlý, L. Šůche, Handbook of Chemical Equilibria in Analytical Chemistry, John Wiley & Sons, New York, 1985.
[28] A. Varenne, S. Descroix, Recent strategies to improve resolution in capillary electrophoresis—a review, Anal. Chim. Acta 628 (2008) 9–23.
[29] A. Jouyban, E. Kenndler, Theoretical and empirical approaches to express the mobility of small ions in capillary electrophoresis, Electrophoresis 27 (2006) 992–1005.

[30] M. Jimidar, Q. Yang, J. Smeyers-Verbeke, D.L. Massart, Method development and optimization for small ion capillary electrophoresis, Trends Anal. Chem. 15 (1996) 91–102.
[31] H. Salimi-Moosavi, R.M. Cassidy, Control of separation selectivity and electroosmotic flow in nonaqueous capillary electrophoretic separations of alkali and alkaline earth metal ions, J. Chromatogr. A 749 (1996) 279–286.
[32] K. Sarmini, E. Kenndler, Capillary zone electrophoresis in mixed aqueous-organic media: effect of organic solvents on actual ionic mobilities and acidity constants of substituted aromatic acids: IV. Acetonitrile, J. Chromatogr. A 833 (1999) 245–259.
[33] K.I. Roy, C.A. Lucy, Capillary electrophoresis in aqueous-organic media: Ionic strength effects and limitations of the Hubbard-Onsager dielectric friction model, J. Chromatogr. A 964 (2002) 213–225.
[34] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, B. Boulanger, P. Hubert, Analysis of recent pharmaceutical regulatory documents on analytical method validation, J. Chromatogr. A 1158 (2007) 111–125.
[35] L. Geiser, S. Rudaz, J.-L. Veuthey, Decreasing analysis time in capillary electrophoresis: validation and comparison of quantitative performances in several approaches, Electrophoresis 26 (2005) 2293–2302.