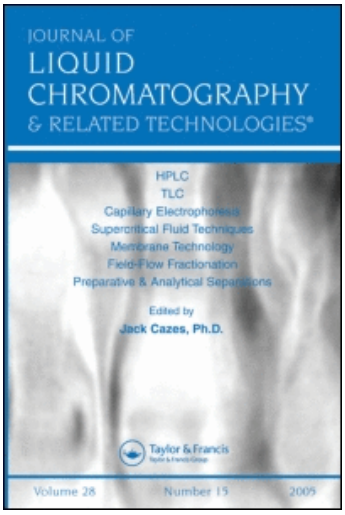

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DETERMINATION OF CATIONS IN WATER AND BIOLOGICAL FLUIDS BY CAPILLARY ZONE ELECTROPHORESIS

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ABSTRACT

The parameters of greatest importance in the process of Capillary Electrophoresis (CE)–buffer pH, voltage, temperature, and buffer concentration–were studied in respect of their influence upon the migration times (t_m) of the following cations: Na^+, K^+, Ca^{+2}, Mg^{+2}, Ni^{+2}, Zn^{+2}, Fe^{+2}, and Mn^{+2}. With the figures obtained from this research it proved possible to understand, in greater depth, the behaviour of these cations in this modern analytic technique.

The data obtained permitted the design of an analytical procedure using CE with the optimal combination of values for each variable. This was thereafter applied to the analysis of these cations in samples of water, milk, blood plasma, and urine.

The conditions seen as the most suitable were the following: the electrolyte was an aqueous solution of imidazole 15 mM, pH = 4.0, voltage V = 15 kV, and temperature T = 30°C. The procedure proved to have good precision, linearity, a coefficient of correlation CC = 0.999, and detection limits for the cations involved of between 0.01 and 5.00 ppm.

INTRODUCTION

Cations are of importance to a range of biological, research, and industrial activities. Ion chromatography has been used to determine cations in many analytical procedures. In recent years, capillary electrophoresis has been attracting considerable attention as an alternative to traditional chromatographic techniques, particularly in the biological sciences.[1-5] Many papers have been published concerning the analysis of anions and cations by means of CE having been shown to be both highly efficient and highly sensitive.

In determining cations by capillary electrophoresis, Capillary Zone Electrophoresis (CZE) is the most extensively used approach.[6-11] The applications of CZE are wide ranging covering amino acids, peptides,[12] and numerous organic[13,14] and inorganic ions.[15-18] In this case indirect UV detection is necessary,[2,8,15] owing to the lack of chromophores in these items. Indirect detection is carried out by the use of a highly absorbent substance such as chromate or imidazole[7] in the electrolyte which will allow us to detect negative absorbances once the cations get to the detector.

The electro-osmotic flow (EOF) varies greatly[2,3,4,9,12,19,20] with the pH, the concentration of electrolyte, the temperature, and the working voltage. This affects the migration times, therefore, a knowledge of the changes in these parameters is extremely important in order to design a successful analytic method in CZE.

The aim of this study is to establish an analytical procedure to check for the Na^+, K^+, Ca^{+2}, Mg^{+2}, Ni^{+2}, Zn^{+2}, Fe^{+2}, and Mn^{+2} cations, using Capillary Zone Electrophoresis(CZE) on samples of water, milk, blood plasma and urine.

To obtain the data needed to achieve this aim, standard solutions for each cation under investigation were analysed at a variety of settings for those parameters having a major influence on CZE, with the migration times being measured for all the cations. The parameters considered were voltage, buffer pH, working temperature, and buffer concentration. In all cases the electrolyte used was an aqueous solution of imidazole at various concentrations and pH levels, thus serving as buffer and also as background, since the cations are determined by use of indirect photometric detection.[2,8,11,15]

The analytical procedure we are proposing was designed using the data obtained from this work. The voltage, temperate, imidazole concentration, and pH values which appeared to be the best for determining the cations under investigation in samples of water, milk, blood plasma, and urine were established.

EXPERIMENTAL

Instruments

The capillary electrophoresis equipment used for analyses was a P/ACE model Beckman System 2000 fitted with a high voltage power supply allowing operation over the range 1 kV to 30 kV. It had a recirculation system for liquid coolant that enabled temperature to be kept steady throughout the process of analysis. Detection was by means of UV. The equipment was run under the control of System Gold software, which also processed the results obtained. This software also permits inversion of peaks on the electropherogram when they are oriented downwards because of indirect photometric detection. For measuring cations, the cathode must be located near the detection window. The capillary used was made of polyamide-coated fused silica, with a total length of 57 cm and a 75 μm internal diameter.

Reagents

All reagent, sample solutions, and all capillary rinses used 18.2 MΩ·cm deionized water prepared with a Millipore purification system of the Milli-RO 6 Plus or Milli-Q UF Plus model (Millipore, Bedford, Mass., U.S.A.).

Standard solutions for cations were based on standard 1,000 ppm solutions supplied by Merck (Darmstadt, Germany) further diluted with water to 10 ppm for Na^{+}, K^{+}, Ca^{+2}, Mg^{+2}, Ni^{+2}, Zn^{+2}, and 50 ppm for Mn^{+2} and Fe^{+2}.

For rinsing the capillary, the liquids used were aqueous solutions of NaOH 1M, prepared from 97%, P.A. quality, from Merck (Darmstadt, Germany) and HCl 1M, prepared from 37%, P.A. quality, from Panreac (Barcelona, Spain).

As electrolyte, aqueous solutions of various concentrations of imidazole of purity in excess of 99% from Merck (Darmstadt, Germany) were used. All other chemicals were of analytic reagent grade.

Sample Preparations

The samples of water from the main water supply and of mineral water for human consumption required no prior treatment. The whey samples were obtained from commercially supplied UHT skimmed milk diluted 1 to 100 with water. The solution was placed in an Ultrafree-MC 30.00 NMWL Filter Unit (Millipore, UFC3 LTK00) and centrifuged at 3,000 g for five minutes.

The blood plasma samples were extracted from rabbit's blood which had been centrifuged and diluted 1 to 10 with water, then ultrafiltered using the Ultrafree-MC at 3,000 g for ten minutes.

Urine samples were diluted 1 to 20 with water.

Electrophoretic Procedures

One of the most crucial factors that lead to high reproducibility is the conditioning of the capillary. To maintain capillary surface always in the same conditions is one of the knottiest problems in CZE.[7,12,15] In order to attain good reproducibility, we performed the following operations:

Prior to first use, the new capillary was given a two-stage wash cycle,[7,19] each stage lasting 15 minutes, with NaOH 1M and with deionized water. Between each analysis and the next, the capillary was rinsed by circulating HCl 1M for one minute, NaOH 1M for two minutes, and finally water for two minutes.

At the start of the work, certain conditions[6,7,9,10,11,21] of buffer concentration, buffer pH, voltage applied, and temperature were selected for the analytical run of the standards for cations under study. Each of these conditions was then systematically adjusted, with the others remaining unchanged, in order to establish variation data for each parameter. The initial values were: imidazole 10 mM electrolyte with a pH of 4.5; voltage applied, 15 kV; thermostat setting, 25°C. The sample was introduced by hydrodynamic injection. Analysis time was eight minutes. Detection was carried out at a distance of 7 cm from the outlet end of the capillary, indirect UV detection at 214 nm being used.

RESULTS AND DISCUSSION

Standard samples of the Na^+, K^+, Ca^{+2}, Mg^{+2}, Ni^{+2}, and Zn^{+2} cations at 10 ppm and Fe^{+2} and Mn^{+2} cations at 50 ppm were initially tested under the following working conditions: imidazole 10 mM electrolyte with a pH of 4.5, voltage 15 kV, temperature 25°C. Each of these parameters was then individually varied so as to establish its effects on the t_m of the cations.

Influence of pH of Electrolyte on Migration Time (t_m)

It is possible to consider pH as one of the most important separation parameters in CZE, [12,19] since it affects the net charge of the cations and also the electro-osmotic flow (EOF).[7, 9, 10, 13, 15, 20] If the pH chosen is incorrect, the

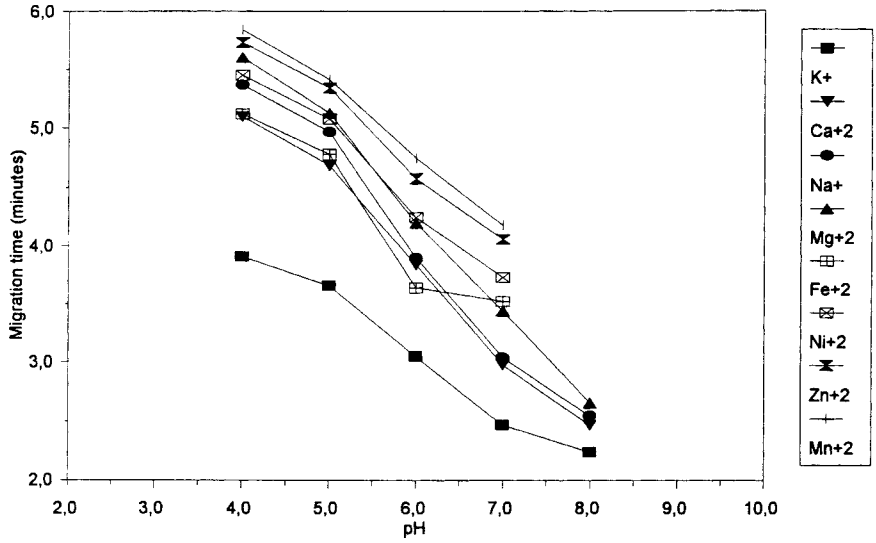

Figure 1. Values of t_m for cations as pH of electrolyte varies. Imidazole electrolyte 10 mM with pH of 4.0, 5.0, 6.0, 7.0, and 8.0; voltage 15 kV; temperature 25°C.

likelihood of a good separation is dramatically reduced. At a high pH value, EOF is very high. The order of migration will be cations, neutrals, anions. At lower pH values, where EOF is greatly reduced, both cations and anions can still be detected, although not in a single run.[20]

The influence of pH on the analyte was determined by performing analyses of the standard cations using the imidazole 10 mM electrolyte at pH values of 4.0, 5.0, 6.0, 7.0, and 8.0, which were attained by the adding appropriate amounts of dilute HCl, and maintaining a voltage of 15 kV and a temperature of 25°C throughout. In Figure 1 a drastic decrease in the t_m of the cations as the pH used is increased may be observed. This is not surprising, as the electro-osmotic flow is increased, dragging them towards the cathode. However, as pH goes up resolution in the peaks obtained is lost. In view of the data recorded, it was decided that the optimum pH for analytic purposes is 4.0.

Influence of Voltage on Migration Time (t_m)

Voltage optimisation can lead to analyses which are at one and the same times faster, more efficient, and better able to resolve electrophoretic peaks. As a general rule it can be stated that t_m decreased as voltage increased.[2] Efficiency

Table 1

Variation in Migration Times (t_m) (in Minutes) of Cations as Voltage is Varied*

Migration Time (t_m) (min)	V = 5kV	V = 10kV	V = 15kV	V = 20kV
K^+	12.84	6.18	3.96	2.89
Ca^{+2}	17.21	8.16	5.19	3.70
Na^+	18.21	8.65	5.48	3.88
Mg^{+2}	19.04	9.04	5.69	4.02
Fe^{+2}	16.61	8.11	4.99	2.91
Ni^{+2}	17.60	8.58	5.27	3.73
Zn^{+2}	18.69	9.17	5.56	3.93
Mn^{+2}	18.68	9.31	5.63	3.98

* Imidazole electrolyte 10mM; pH 4.0; voltage 5.0, 10.0, 15.0, and 20.0 kV; temperature 25°C.

and resolution rose to a maximum and then decreased as voltage was raised further. Increased voltages yielded more plates, faster times, and sharper peaks, but excesive voltage caused pronounced Joule heating, lower theoretical plates, and lessened resolution.[19,22,23]

This work was carried out using the imidazole 10 mM electrolyte at a pH of 4.0 and maintaining a temperature of 25°C, with voltage settings of 5.0, 10.0, 15.0, and 20.0 kV. In all instances a drop in t_m as voltage is raised can be seen (Table 1), and the resolution of peaks was likewise improved in all cases with increasing voltage. However, the higher voltages produced heating as a result of the Joule effect,[3] rendering temperature maintenance difficult, and also led to loss of capacity to separate peaks. With the data obtained and the previously mentioned considerations, 15.0 kV was chosen as the most suitable voltage, as it allows good separation of the cations in a reasonably short timespan.

Influence of Working Temperature on Migration Time (t_m)

Although the primary purpose of thermostatic control of the capillary temperature is the maintenance of a constant temperature and the removal of Joule heat,[20] this control may also be used as a parameter in optimising a CZE separation.

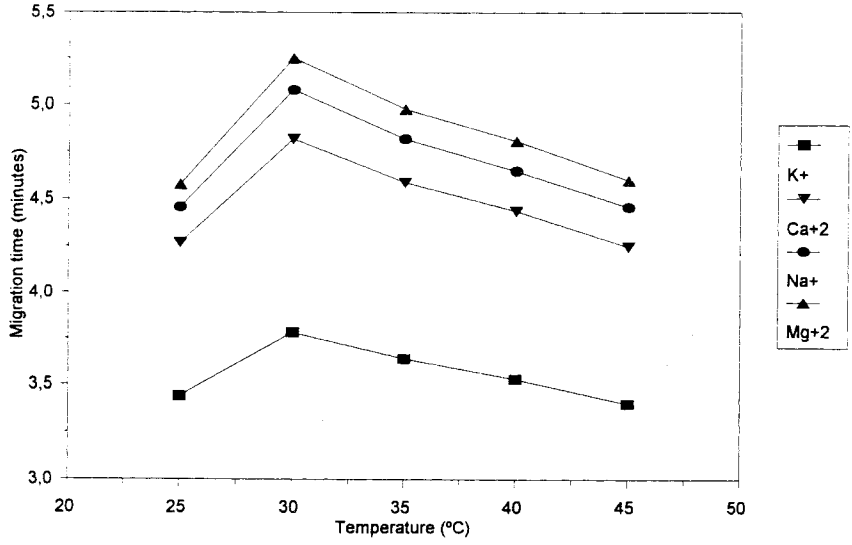

Figure 2. Values of t_m for cations as temperature varies. Imidazole electrolyte 10 mM with pH of 4.0; voltage 15 kV; temperatures 25.0, 30.0, 35.0, 40.0 and 45.0°C.

Higher and lower temperatures affect viscosity, EOF, and analysis time.[4,16,22,33] Working temperatures of 25.0, 30.0, 35.0, 40.0, and 45.0°C were investigated, with voltage kept at a constant 15 kV and the 10 mM imidazole electrolyte at a steady pH of 4.0.

In Figure 2 an increase in t_m in the interval between 25 and 30°C may be observed, together with a drop in t_m for temperatures in excess of 30°C. With these results, and keeping in mind the fact that very high temperatures are undesirable because of the increased heat from the Joule effect, 30°C was deemed to be the most suitable working temperature. At this setting, good resolution of peaks in the electropherogram is obtained, even though the t_m values are the highest in the whole range considered.

Influence of Electrolyte Concentration on Migration Time (t_m)

A wide variety of buffers may be employed as electrolytes in CZE. We used imidazole for this purpose and as the background needed for indirect photometric detection.[7]

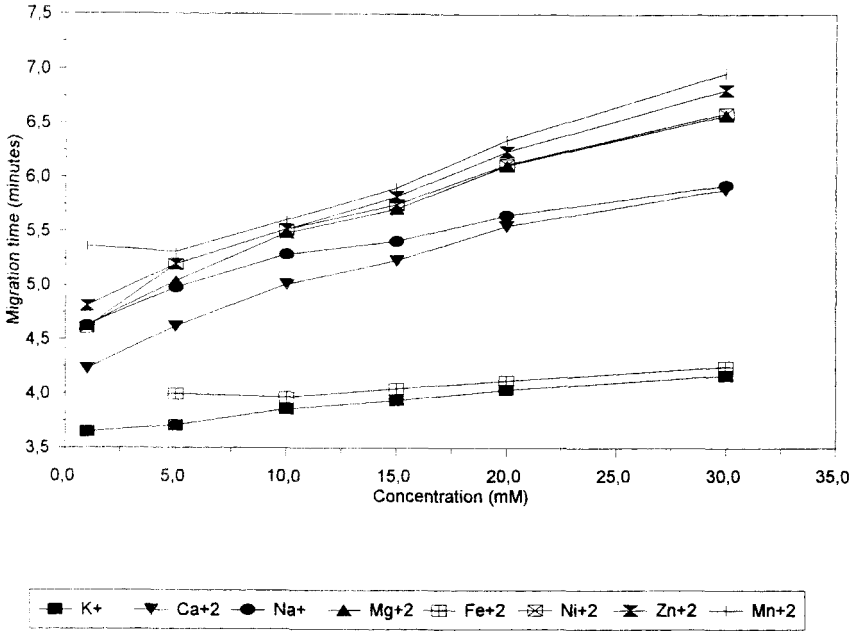

Figure 3. *Influence of electrolyte concentration. Imidazole electrolyte* with concentrations of 1.0, 5.0, 15.0, 20.0 and 30.0 mM; pH 4.0; voltage 15 kV; temperature 30°C.

The variation in molar conductivity and ionic mobility with changes in electrolyte concentration covers a broad field in physical chemistry. Control of electrolyte concentration is a tool that should be kept in mind as of use in improving efficiency, sensitivity, and resolution.[13]

To investigate its influence, aqueous solutions of imidazole with concentrations of 1.0, 5.0, 10.0, 15.0, 20.0, and 30.0 mM were used, with pH=4.0, voltage at 15 kV, and temperature at 30°C in all cases.

As may be seen in Figure 3, in every instance the t_m values increase as higher concentrations of imidazole are used, and the best resolutions are obtained at the intermediate concentrations within the range tested. With the data obtained, it was decided the most suitable concentration for our purposes is 15 mM, as it yields the best resolution.

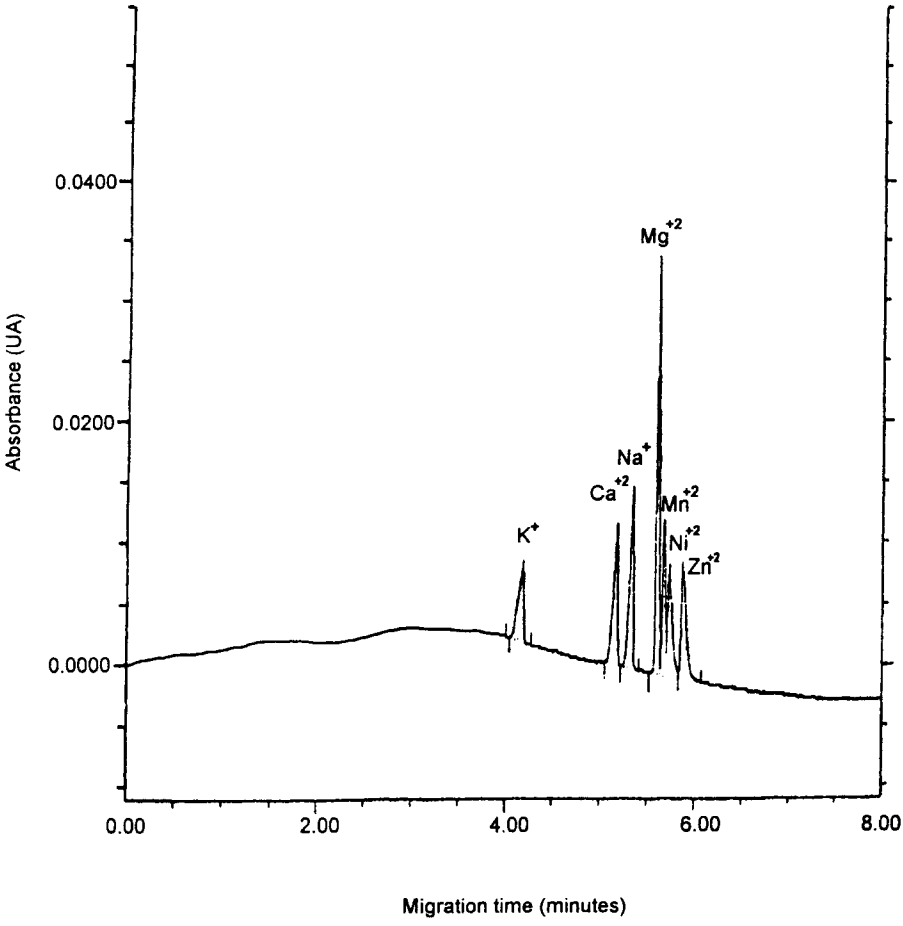

Figure 4. Analysis of all test cations with at 10 ppm in optimal conditions. Imidazole electrolyte 15 mM; pH 4.0; voltage 15 kV; temperature 30°C.

Optimal Conditions

With the data gathered it was deduced that the most suitable conditions for analysing the cations being researched were the following: buffer composed of an aqueous solution of imidazole with concentration 15 mM and pH of 4.0, working voltage 15 kV, and temperature 30°C (see Figure 4).

Table 2

Precision in Units of Absorbance for Each of the Peads Corresponding to the Cations Studied in Several Analyses*

No. Inj.	K^+	Ca^{+2}	Na^+	Mg^{+2}	Mn^{+2}	Ni^{+2}	Zn^{+2}
1	0.136	0.494	0.406	0.737	0.573	5.105	5.453
2	0.267	0.419	0.433	0.808	0.553	0.467	0.014
3	0.145	0.426	0.431	0.789	0.546	0.457	0.014
4	0.144	0.449	0.450	0.813	0.545	0.467	0.014
5	0.147	0.446	0.470	0.843	0.573	0.465	0.014
6	0.147	0.431	0.459	0.830	0.548	0.486	0.013
7	0.159	0.472	0.584	0.930	0.575	0.474	0.013
$\bar{x}$	0.1438	0.4342	0.4486	0.8166	0.5530	0.4618	0.138
σ	0.004	0.011	0.015	0.018	0.010	0.005	0.004
C.V.	**2.83%**	**2.66%**	**3.33%**	**2.27%**	**1.87%**	**1.24%**	**2.8%**

* Statistical parameters: $\bar{x}$, σ, CV. Imidazole electrolyte 15 mM, pH 4.0; voltage 15 kV; temperature 30°C.

Table 3

Equations for the Straight Line Relationships and the Correlation Coefficients Relating to the Cations Studied

Cation	Linear Relationship	Corr. Coeff.
K^+	y = 0.022x - 0.042	0.999
Ca^{2+}	y = 0.066x - 0.144	0.998
Na^+	y = 0.071x - 0.076	0.994
Mg^{2+}	y = 0.141x - 0.563	0.999
Zn^{2+}	y = 0.046x - 0.011	0.996
Ni^{2+}	y = 0.052x - 0.101	0.995
Mn^{2+}	y = 0.057x - 0.369	0.994

Precision, Linearity and Detection Limit

Investigation of the precision of the analytic method was carried out by performing seven determinations, with the working conditions at the previously established optimal settings, on an identical sample containing standards for all

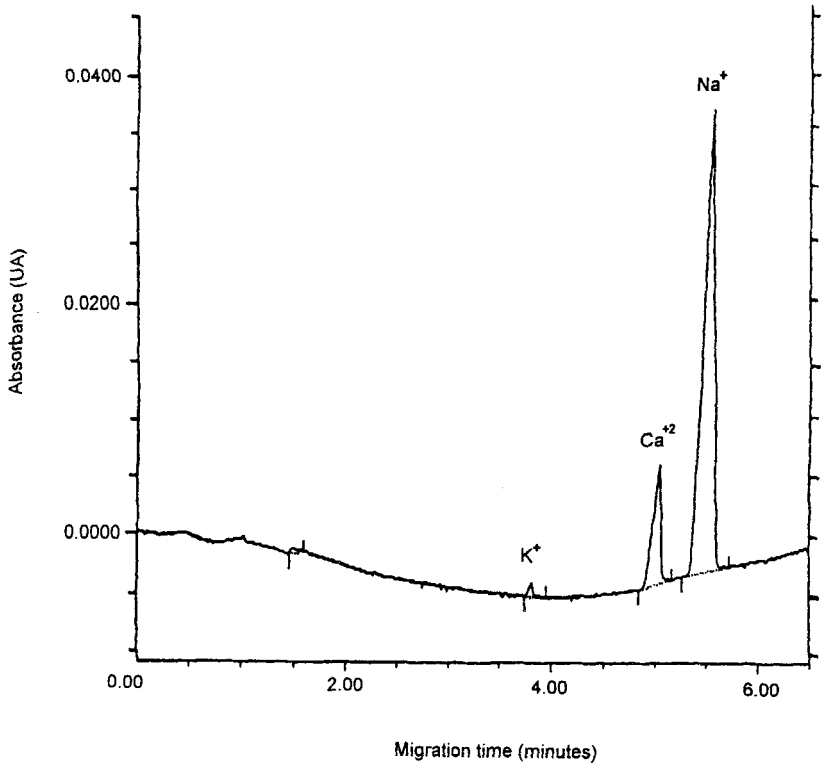

Figure 5. Analysis of commercially sold mineral water. Imidazole electrolyte 15 mM; pH 4.0; voltage 15 kV; temperature 30°C. K^+ = 4.54 ppm, Ca^{+2} = 15.48 ppm and Na^+ = 69.07 ppm.

of the cations. Table 2 shows that the results relate to the area of the electrophoretic peaks in units of absorbance (UA) corresponding to each of the cations under study, as well as the statistical parameters, arithmetical mean ($\bar{x}$), standard deviation (σ) and coefficient of variance (CV) for each cation investigated. All the results yield a relative standard deviation of 2% to 3%, which is acceptable for this type of study. With the data obtained, it may be stated that this method is precise in the results, that it gives for the analysis of standards of cations, and that its reproducibility is acceptable within the concentration range that was adopted for the research.

Linearity was investigated for the concentration range from 10 ppm to 100 ppm, and for this purpose standards of each of the cations at known concentrations of 10.0, 30.0, 70.0, and 100.0 ppm were subjected to analysis.

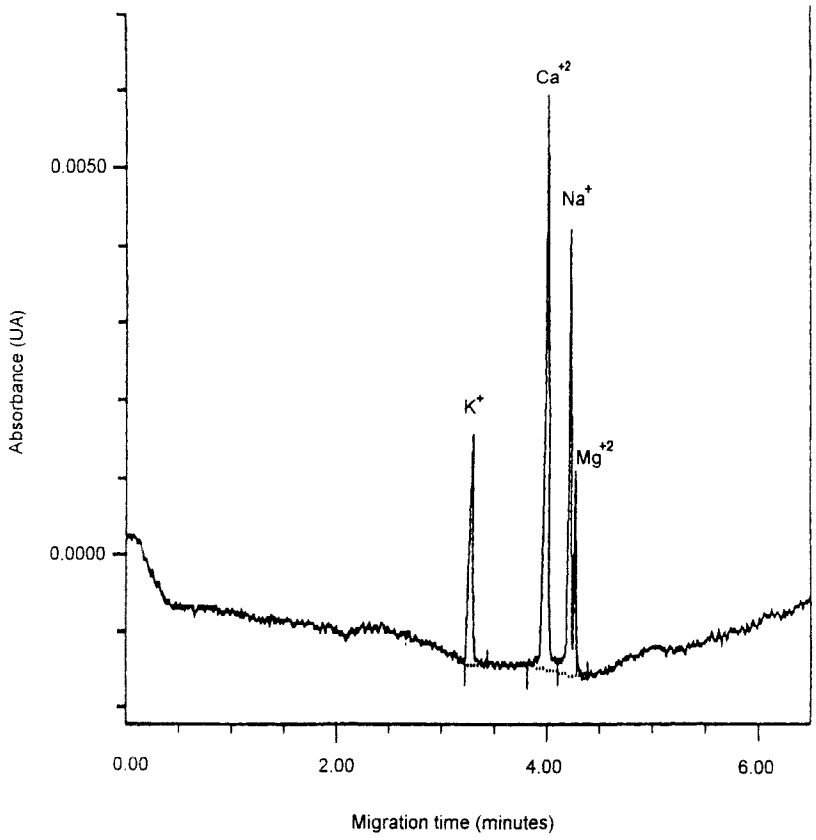

Figure 6. Analysis of whey diluted 1 to 100. Imidazole electrolyte 15 mM; pH 4.0, voltage 15 kV; temperature 30 °C. K^{+} = 612.10 ppm, Ca^{+2} = 1,827.00 ppm, Na^{+} = 684.50 ppm, and Mg^{+2} = 498.60 ppm.

The straight line relationships and coefficients of correlation for this are shown in Table 3. On the basis of the data obtained, it may be stated that the procedure has a linear response in the results it yields for the concentration range that was investigated.

To determine the limits of detection, standard samples of cations were analysed at decreasing concentrations until the minimum concentration detectable by the apparatus was reached, this being taken as five times greater than the background noise of the electropherogram. The results obtained were the following: Ca^{+2} and Mg^{+2} = 0.01 ppm, Na^{+} = 0.05 ppm, K^{+} = 0.25 ppm, Ni^{+2} and Zn^{+2} = 1.00 ppm, Fe^{+2} and Mn^{+2} = 5.00 ppm.

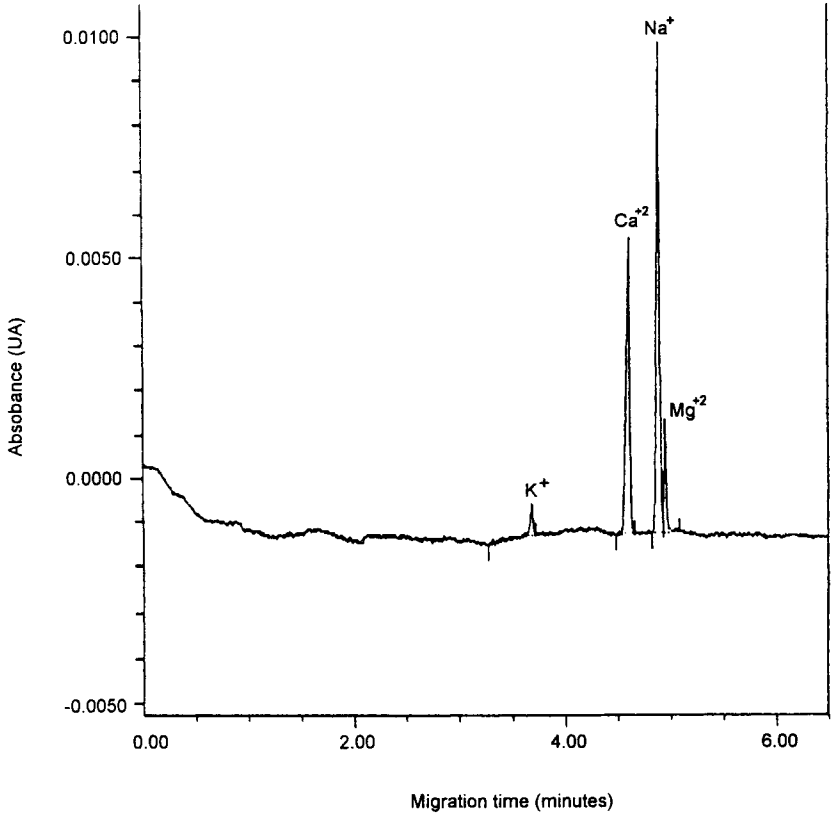

Figure 7. Analysis of plasma from rabbit blood diluted 1 to 100. Imidazole electrolyte 15 mM; pH 4.0; voltage 15 kV; temperature 30°C. K^+ = 93.18 ppm, Ca^{+2} = 71.21 ppm, Na^+ = 1,148.03 ppm and Mg^{+2} = 60.28 ppm.

Analysis of Samples

Once the conditions for analysis had been optimised and the reliability of the analytical procedure checked, analyses were performed on the samples in which the concentrations of the cations which it was desired to determine were unknown.

In the analysis of water from the town's main supply, Ca^{+2}, Na^+, and Mg^{+2} were detected at concentrations of 22.48 ppm, 1.90 ppm, and 6.20 ppm, respectively.

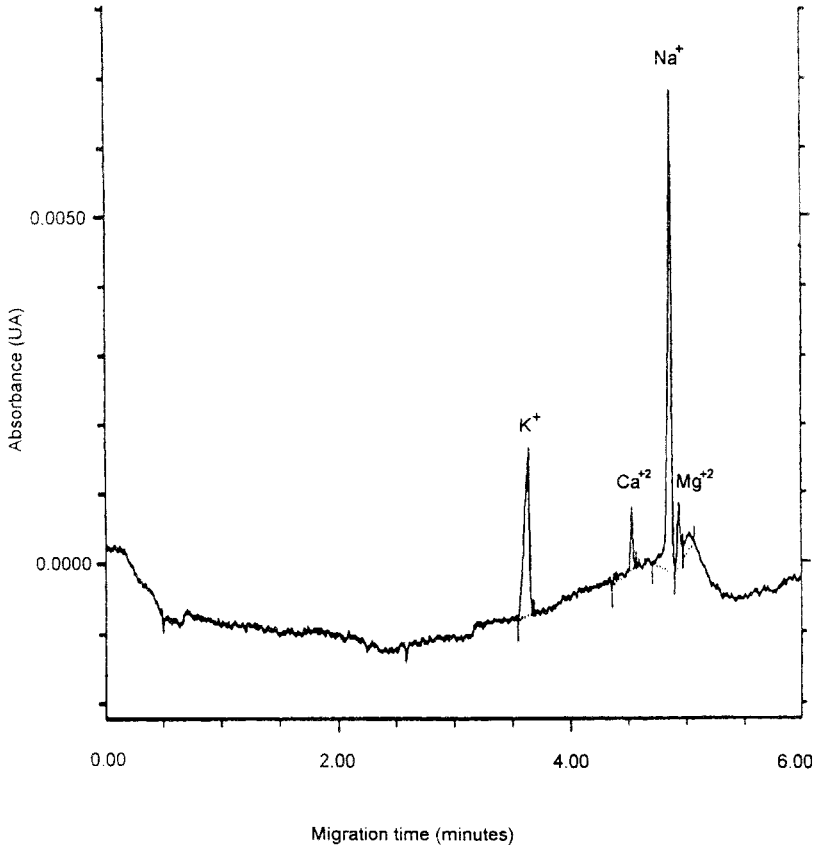

Figure 8. Analysis of urine diluted 1 to 20. Imidazole electrolyte 15 mM; pH 4.0, voltage 15 kV; temperature 30°C. K^+ = 2,738.00 ppm, Ca^{+2} = 482.40 ppm.

The analysis of lightly mineralised commercially sold mineral water yielded the electropherogram shown as Figure 5, indicating the presence of K^+, Ca^{+2}, and Na^+ at concentrations of 4.54 ppm, 15.48 ppm, and 69.07 ppm, respectively.

It was the whey, or lactoserum, of the milk that was analysed, as it contains the soluble cations. These are K^+, Ca^{+2}, Na^+, and Mg^{+2}, at concentrations of 612.10 ppm, 1,827.00 ppm, 684.50 ppm, and 498.60 ppm, respectively, as is shown in Figure 6, which relates to the analysis of milk diluted 1 to 100.

In blood plasma the cations which are to be found in free forms were detected. As is shown in Figure 7, K^+, Ca^{+2}, Na^+, and Mg^{+2} are present in concentrations of 93.18 ppm, 71.21 ppm, 1,148.03 ppm, and 60.28 ppm, respectively.

In the electropherogram given as Figure 8, the electrophoretic analysis of the urine diluted 1 to 20 is shown. The levels obtained were: K^+ = 2,738.00 ppm, Ca^{+2} = 482.40 ppm, Na^+ = 3,229.80 ppm, and Mg^{+2} = 166.38 ppm.

CONCLUSIONS

The influence of the parameters of greatest importance in the process of Capillary Zone Electrophoresis upon the migration times (t_m) of the following cations: Na^+, K^+, Ca^{+2}, Mg^{+2}, Ni^{+2}, Zn^{+2}, Fe^{+2}, and Mn^{+2} were studied. It was observed that as buffer pH rises there is a very important reduction in the t_m values for all these cations. Increasing the working voltage likewise reduces the t_m values for all of the cations. As the working temperature rises there is a slight increase in migration times, but then, from 30°C upwards, they all decrease. Greater concentrations of the buffer cause the migration times for all the cations to grow longer.

It is of interest to obtain data on the behaviour of cations in CZE, because this is a very recent technique, and it is crucial to provide information allowing analytical procedures to be established for the various cations in different types of sample. On the basis of the results of our research, we have worked out the settings that we see as the most suitable for analysing these cations in water, milk, blood plasma, and urine. The procedure proposed gives good precision, linearity, and low detection limits, as well as short analysis time. This analytical method constitutes a valid alternative to Ion Chromatography, and offers the advantages of being somewhat faster and not needing the samples to be so clean, in the instances researched.

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