Portable capillary electrophoresis instrument with potentiometric detection

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Portable instrumentation has become of increasing interest in analytical chemistry, because on-site analysis has various advantages. In this report we describe the construction of a field-portable capillary electrophoresis instrument with potentiometric detection. The apparatus is contained in a PVC case of 340 × 175 × 175 mm size and a total weight of 7.5 kg and can therefore be carried easily by one person. Two lead–acid batteries provide the power for a high voltage module capable of supplying up to 30 kV, and for the detection amplifier electronics. As detectors, miniature coated-wire ion-selective electrodes are used and a precise electrode alignment is achieved by a simple electrode holder cell without the need for a microscope. Data acquisition is carried out by a small analog-to-digital converter board, which is integrated into the unit, and a palmtop personal computer. The instrument was tested on the banks of the river Rhine. As examples, alkali and alkaline earth metal cations and inorganic monovalent anions were analysed in the river water.

Introduction

Besides miniaturisation, another major trend in analytical instrumentation design is towards mobile instruments, which are independent of the laboratory infrastructure and can be operated at any place, preferably as close as possible to the point where the sample is taken. The aim of this strategy is, on the one hand, to avoid degradation of the samples during the transport and the storage, as they are often very sensitive to changes in temperature and to contact with the atmosphere or the sample vial. On the other hand, as the data are immediately available, it is possible to decide on the site where to collect the next sample. This reduces the number of the necessary samples, avoids multiple sampling trips and therefore reduces cost and time spent. Mobile analytical instruments are mostly used in environmental applications, such as the survey of water quality, soil contamination or air pollution, but can also be employed in process monitoring, food quality control, industrial hygiene control, warfare agent detection or medical and forensic analysis. In principle, one expects from a mobile instrument results of accuracy and detection limits comparable to conventional instruments, unless the aim is limited to a coarse survey. An important requirement is the robustness of the apparatus and the method, because of increased exposure to movements, mechanical shocks, moisture, dust and temperature changes. A mobile instrument must also include its own power supply in the form of batteries or solar cells. Modern electronic components and portable computers allow powerful data acquisition with a low energy consumption. The size and weight of portable instruments ranges from small, hand-held pH meters of less than 1 kg up to complex systems that have to be transported and operated in land vehicles, on boats or in aircraft. Field-portable instruments have been described for a wide range of analytical techniques, such as gas chromatography,[1] mass spectrometry,[2] X-ray fluorescence,[3] voltammetry,[4] flow-injection analysis[5,6] and liquid chromatography,[7] and some of these instruments are commercially available from various manufacturers.[8]

Capillary electrophoresis has become a widely used method for the separation of ionic species and a large number of research papers have been published in the past 20 years. Interesting features of this technique are the high separation efficiency and the short analysis times that can be achieved, as well as a low consumption of chemicals and sample solution. Initially applied to the analysis of organic molecules and biopolymers, it has also proved to be suitable for the separation of inorganic cations and anions.

A variety of detection methods have been developed, but the most often used ones are the optical methods of UV–visible absorption and fluorescence. However, the miniaturisation of the flow channel is limited for these detectors as their response depends on the pathlength, *i.e.*, on the internal diameter (id) of the capillary. Only laser induced fluorescence achieves good sensitivity with capillaries smaller than 50 μm id. Furthermore, there are many analytes of interest that neither absorb UV nor visible light nor show fluorescence effects, *e.g.*, carbohydrates, amino acids or inorganic ions. In these cases, indirect methods, generally with lower sensitivity, or derivatisation reactions, which imply additional error sources, must be used.

A solution to these restrictions can be the electrochemical methods of conductimetry, amperometry and potentiometry, which have been employed for different types of analytes. The three methods have in common the simplicity of the detector design compared with the optical methods. Moreover, electrochemical detection allows the use of capillaries with an inner diameter smaller than 50 μm, which increases the separation efficiency due to better heat dissipation. Conductimetry[9] is, so far, the only commercially available electrochemical detection method for capillary electrophoresis and represents a rather universal method, which applies to a wide range of analytes. Amperometric detection[10] is more selective and its application is limited to electroactive species. However, it is a very sensitive technique: detection limits lower than 10^{-9} mol l^{-1} have been reported.[11] For the detection of inorganic ions, potentiometry has proved to be a useful method. The first generation of ion-selective electrodes used as CE detectors consisted of drawn-out micropipette electrodes with an internal electrolyte solution and an internal reference electrode.[12–16] These electrodes, however, have a short lifetime and are very fragile. Subsequently, it was found that solid-state coated-wire electrodes which contain a PVC-matrix membrane in direct contact with a platinum wire may also be used. These electrodes impart a longer lifetime and a higher mechanical stability. Such electrodes have been applied to the analysis of alkali and alkaline earth metals and inorganic anions,[17,18] but also to organic ions such as carboxylic acids,[19] sulfonic acids, alkylamines, analgesics and artificial sweeteners.[20] As a further type of a potentiometric detector, a metallic copper electrode has been used for amino acids analysis.[21] Another important development was also the introduction of a fixed cell for alignment of the detector electrode with the capillary end to replace the hitherto employed micromanipulators.[18]

Capillary electrophoresis, especially in combination with electrochemical detection, requires a fairly simple instrumental set-up only and is therefore well suited for the construction of compact portable instruments. An added advantage for a field-instrument is the low reagent consumption of the method.

In this report we describe a field portable capillary electrophoresis instrument. We believe that this is the first report of such an instrument. Detection is carried out by potentiometry using coated-wire ion-selective electrodes. The unit includes a 30 W high voltage power supply module capable of providing 30 kV dc, a signal amplifier and an analog-to-digital converter. Power is provided by two 12 V lead–acid batteries. These allow an independent operation of the unit, which due to the small size and the low weight can easily be carried to the place of interest by one person. The functionality of the instrument was demonstrated by analysing for alkali and alkaline earth metals as well as inorganic anions in Rhine river water.

Experimental

Apparatus

The basic design is very similar to a benchtop instrument for CE with potentiometric detection described previously.[18] The main differences concern the confinement to a small unit, the use of battery power and the portable data acquisition. A simplified block-diagram is given in Fig. 1. Two lead–acid batteries are used in series to provide power to the instrument. The supply voltage is fed to the high voltage unit and to a dc–dc converter module to provide the correct voltage for the electronic circuitry. The electrode signal is first brought to a buffer amplifier and then to an offset stage before analog-to-digital conversion. A personal computer (PC) for data capturing completes the system.

All the components of the capillary electrophoresis instrument, except for the PC, were integrated in a single case, which was built of PVC plates by our university workshop. A schematic representation of the device is shown in Fig. 2. It is divided into two compartments. The injection compartment on the left contains the high voltage module, a sample turntable with six vial positions, a support for the high voltage electrode and a capillary holder for the rolled up separation column. The sample turntable is moved by lowering and turning it manually. Its height is fixed. Injections are carried out electrokinetically. The high voltage electrode consisted of a platinum wire of 0.5 mm diameter. It was found to be important to leave adequate free space in order to prevent electrical arcing. The end of the capillary is fed through a small hole to the detection compartment. This part is lined with aluminium sheet, which is connected to the electrical ground of the system in order to shield the potentiometric detector from electrical interference. The buffer vial containing the detection cell is placed on a height-adjustable support to equalise the liquid level in the two buffer vials. All parts are held very rigidly in place to keep any movements of capillary and electrodes to a minimum. The front of the instrument can be opened over its entire width (a lid folds away to the top) in order to carry out any necessary manipulations and a handle on the top enables it to be carried in one hand.

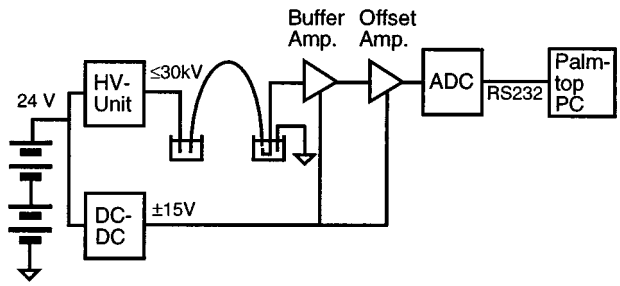

Fig. 1 Block-diagram of the instrument.

High voltage up to 30 kV is provided from a modular power supply, Model 720A-30, from Bertran (Hicksville, NY, USA) with positive or negative polarity. This unit has to be exchanged to obtain the desired polarity, but this is a simple operation. The dimensions of these units are 150 × 95 × 55 mm. The output voltage is adjusted manually by means of a 15-turn potentiometer and the unit provides a monitoring signal, which allows the indication of the applied high voltage by a standard LCD panel meter with ±199.9 mV input range. The module is driven directly by the 24 V dc supply voltage provided from the two 12 V 2.0 A h lead–acid batteries with the dimensions 150 × 84 × 20 mm and a weight of 700 g (Yuasa NP 2.0-12, Yuasa Battery UK Ltd., Swindon, Wiltshire, UK). The batteries are placed in the back of the instrument. For safety reasons, a microswitch on the front lid interrupts the power supply to the high voltage unit when not closed. Connectors at the back of the case allow charging of the batteries as well as the operation with an external power source such as a mains adapter. A provision for connecting the electrical ground of the system to an external ground is also provided. The detector electronics are located in an aluminium case, which is attached to the instrument on the right side. The total size of the system is 340 × 175 × 175 mm and it weighs 7.5 kg.

Detection

Detection was carried out using coated-wire electrodes and a small holder cell for precise and mechanically stable capillary-electrode alignment, as described in an earlier work.[18] Note that the electrophoretic counter electrode served as a reference for the detector as well. The sensing electrodes were constructed by threading a 90 μm platinum wire (California Fine Wire Co., Grover Beach, CA, USA) through a piece of fused silica capillary (Polymicro Technologies Inc., Phoenix, AZ, USA) of 100 μm id acting as the electrode body. At the front, the wire was cut off flush with the capillary end and coated with the membrane solution. An insulated wire was soldered to the back end and sealed with epoxy glue.

The capillary-electrode holder consists of two Perspex plates of 20 × 10 mm size. In one of the pieces a V-shaped groove was milled to fix the capillary and the coated-wire electrode, which had identical outer diameters, in their positions. In the centre of

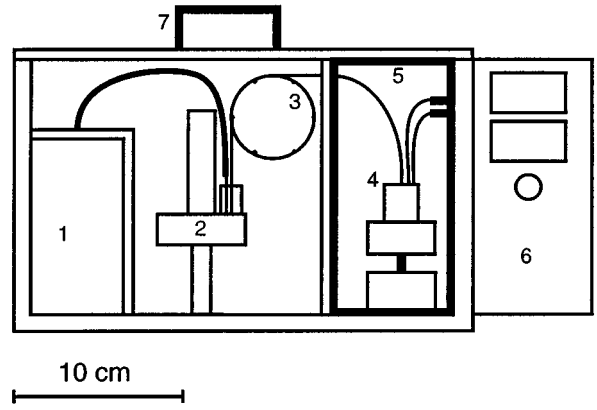

Fig. 2 Front view of the portable capillary electrophoresis instrument (drawn approximately to scale). 1, Compartment for high voltage module with lead to injection end of capillary; 2, turntable for sample vials; 3, separation column rolled on spool; 4, detection cell with electrical connections for detector electrode and ground; 5, Faraday cage; 6, electronics case with two panel meters for signal voltage monitoring on top and knob for offset adjustment; 7, handle for carrying.

both pieces a hole of 3 mm diameter was drilled to allow the buffer solution access. Another V-shaped groove extends from the hole to the long edge. In this groove was placed a grounded Pt-wire with 0.5 mm diameter, which served as counter electrode for the separation voltage and as pseudo-reference electrode for the potentiometric detector. The two plates were clamped together with four stainless steel screws.

A circuit diagram of the detector electronics is given in Fig. 3. The working electrode is connected to an electrometer operational amplifier (OPA121KP from Burr-Brown, Tucson, AZ, USA) in the voltage follower configuration in order to lower the impedance of the measured voltage. In the next stage, a manually regulated dc voltage of inverse polarity is added to the signal by a summing amplifier for zero adjustment. A third operational amplifier is appended as active low pass filter (single pole) with a cut-off frequency of 34 Hz and an amplification factor of 10. For the last two stages operational amplifiers in a dual package integrated circuit (TL072ACP, Texas Instruments, Dallas, TX, USA) are used. Offset and gain is provided in order to make best use (with regard to resolution) of the fixed input span of the data acquisition system.

Two panel meters, which indicate the measured voltage before and after applying the offset, facilitate the adjustment and allow easy monitoring of the signal. The analog detector signal is available on a socket for use of external data acquisition systems if so desired. Power for the electronic circuitry is regulated to ±15 V by a dual output dc-to-dc converter (Model TED 2422, Traco Electronic AG, Zurich, Switzerland). The temperature in the detection compartment is monitored using an LM35 temperature sensor (National Semiconductors, Santa Clara, CA, USA). Data acquisition is accomplished with a Pico ADC-16 analog-to-digital converter system (Pico Technology Ltd., Cambridge, UK), which could be incorporated into the electronics case because of its small size. The Pico ADC-16 has 8 input channels and a fixed input voltage range of ±2.5 V. The resolution can be adjusted to between 8 and 16 bits, but a compromise has to be made between resolution and sampling rate. Three channels are used to allow monitoring of the temperature and the battery voltage besides the potentiometric signal. The analog-to-digital converter is connected *via* an RS-232 serial port to a PC. Power to the converter unit is provided *via* the serial line. Any PC (preferably portable) operating under MS-DOS is suitable. We used the HP 1000CX palmtop personal computer (Hewlett-Packard, Palo Alto, CA, USA) because of its small dimensions and weight. The control of the ADC-16 and the data acquisition is carried out with the program PICOLOG, which is supplied with the data acquisition unit and which was running on the palmtop computer. The measured data were subsequently transferred to a Macintosh computer for preparation of the figures.

Reagents and methods

The cocktails for the PVC-based sensor membrane consisted, for the cation-selective electrode, of 1.2% *N*,*N*,*N*′,*N*′-tetracyclohexyloxybis(*o*-phenyleneoxy)diacetamide (V163), 0.8% potassium tetrakis(4-chlorophenyl)borate, 65.3% *o*-nitrophenyl octyl ether and 32.7% PVC dissolved in tetrahydrofuran.[22] For the detection of anions, the liquid membrane cocktail used by Kondo *et al.* was modified and contained 5.0% 5,10,15,20-tetraphenyl-21*H*,23*H*-porphin manganese(III) chloride (MnTPP), 1.0% tetradodecylammonium tetrakis(*p*-chlorophenyl)borate, 4.0% decan-1-ol, 60.0% *o*-nitrophenyl octyl ether and 30.0% PVC dissolved in THF.[23] All membrane components were purchased from Fluka (Buchs, Switzerland), except for the anion ionophore MnTPP, which was from Aldrich (Milwaukee, WI, USA).

For cation analysis, the background electrolyte contained 10 mmol l^{-1} magnesium acetate, 2 mmol l^{-1} 18-crown-6 and 1 mmol l^{-1} tartaric acid. The pH was adjusted to 4.7 with acetic acid. An unbuffered 10 mmol l^{-1} potassium sulfate solution was used for the analysis of the inorganic anions. The Rhine water samples were diluted (1 + 1) with background electrolyte in order to avoid electrostacking. Standard analyte solutions and background electrolytes were prepared from analytical grade reagents purchased from Merck (Darmstadt, Germany) and water purified by a Milli-Q (Millipore, Bedford, MA, USA) purifying system. All solutions were filtered with 0.2 μm Nylon filters before use.

An uncoated fused silica capillary (Polymicro Technologies Inc., Phoenix, AZ, USA) with an internal diameter of 25 μm was used. The capillary length was 90 cm. The separation voltage was +30 kV for the cations and −30 kV for the anions. Injection was carried out electrokinetically at +5 kV and −5 kV, respectively, during 7 s. The capillaries can readily be flushed manually, in the field if required, by use of a special syringe adapter that is commercially available (Supelco, Buchs, Switzerland).

Results and discussion

Power supply

The use of high voltages in a small portable package presents a certain hazard to the operator. Two essential security features have been integrated in order to guarantee the safe operation of the instrument. Firstly, the power supply for the high voltage module is interrupted when the lid is opened, so that direct contact with the charged electrode is avoided. Secondly, it was found that the entire unit showed a tendency to charge itself up

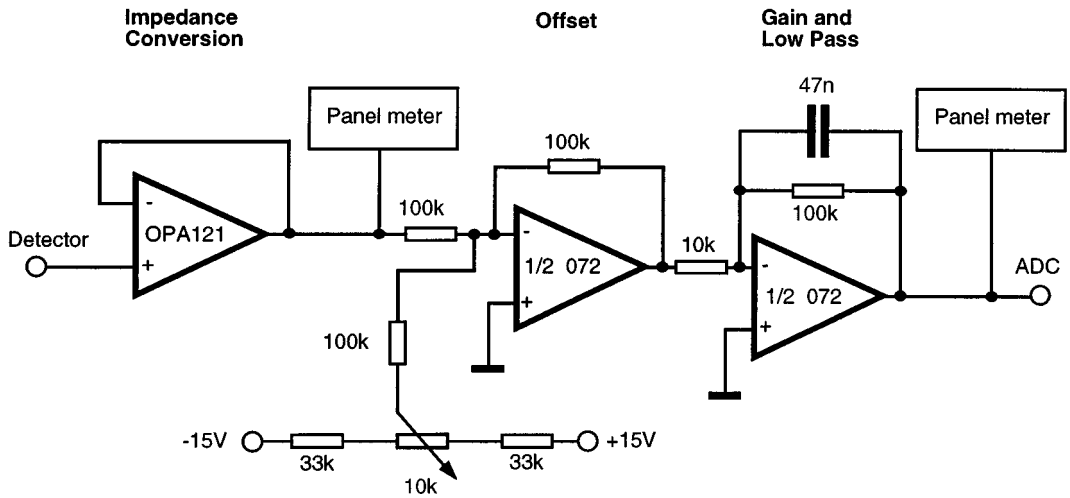

Fig. 3 Circuit diagram for the detector electronics.

due to leakage from the high voltage supply. This problem does not occur with commercial electrophoresis instruments as these are inherently connected to mains earth. Special care has to be taken therefore. It was found that charging can be avoided by tying the system ground to an external earth using the socket provided. In the field, effective earthing can be accomplished by using a metallic spike which is pushed into the soil.

The two lead–acid batteries have a capacity of 2.0 A h each, which corresponds to an available energy at 24 V of about 48 W h. This was found to be sufficient to run the high voltage source and the detection electronics for about 5 h under the conditions employed. They may alternatively be operated independently from an external supply capable of delivering 24 V dc. The high voltage module has a maximum output voltage of 30 kV and can provide up to 120 μA current, which is adequate to drive an electrophoretic system with 25 μm id capillaries.

Data acquisition

Potentiometric detection, which is an application of the Nernstian equation (59 mV per decade of activity), is particularly sensitive to electronic noise. In our earlier laboratory system extensive means for noise reduction could be employed in the form of a carefully designed Faraday cage and different forms of analog and digital filtering built into the data acquisition system. On the other hand, battery operated systems are intrinsically free of mains interference. The measures to reduce the electrical noise taken for our portable system were, on the one hand, shielding of the detection compartment by a metallic cage, and on the other hand, an active filter stage with a cut-off frequency of 34 Hz. The integrating data acquisition system was set to 14 bit resolution. This allowed a maximum sampling rate of 5 samples s^{-1}, which is adequate for resolution of the peaks in the capillary electrophoresis system. Thanks to an amplification of the signal by a factor of 10, the resolution of the measured voltage was 30 μV. The typical noise level was found to be 60 μV (standard deviation, n = 10), which is comparable with the results obtained with the earlier laboratory system.

Field analysis

The instrument was tested by the analysis of alkali and alkaline earth cations and monovalent inorganic anions in the Rhine river water on the site. For the cation separation, the electropherograms of a standard solution and Rhine water are shown in Fig. 4 A and B. The addition of the crown ether to the background buffer enables the separation of K^+ and NH_4^+ and leads further to a sensitivity enhancement, as shown in a previous publication.[18] Because of the strong tailing of the Ca^{2+} peak, tartaric acid was added as a second complexing agent in the buffer, which caused a reversal of the Na^+ and Ca^{2+} peaks.

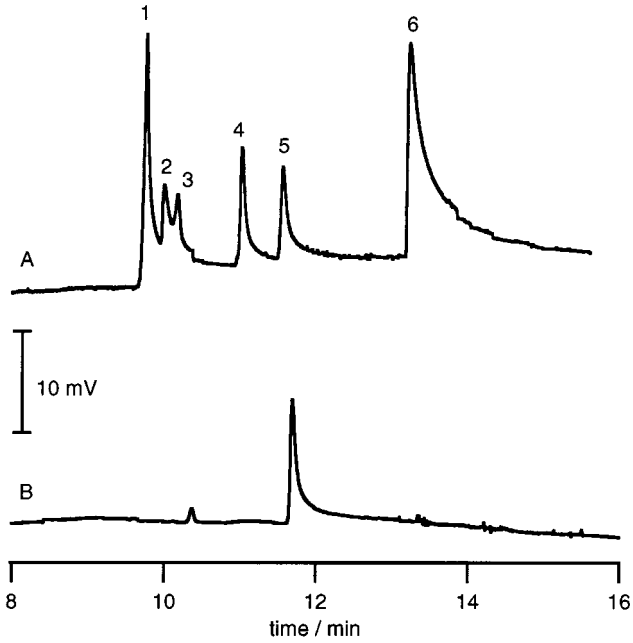

Fig. 5 Electropherograms of monovalent inorganic anions. A, Standard solution containing 10^{-3} mol l^{-1} Cl^- (3), Br^- (12), NO_2^- (4), ClO_4^- (6) and 10^{-4} mol l^{-1} I^- (2), NO_3^- (5). B, Rhine river water. Background electrolyte: potassium sulfate 10 mmol l^{-1}. Samples were diluted 1 + 1 with background electrolyte solution.

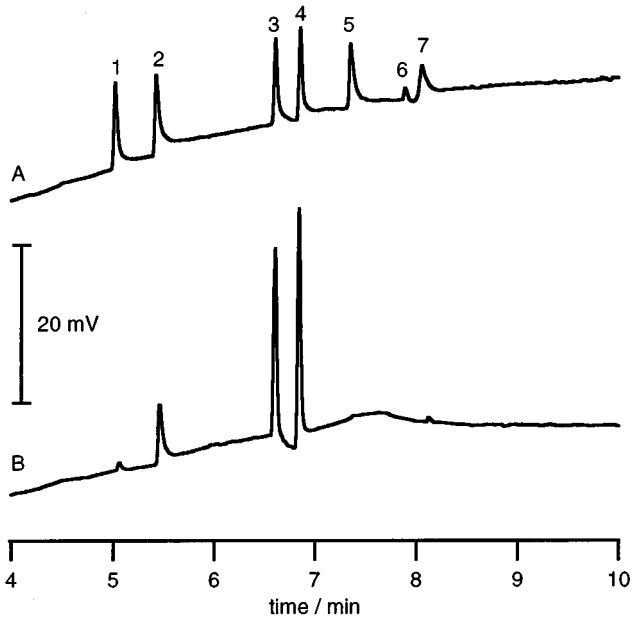

Fig. 4 Electropherograms of alkali and alkaline earth metal cations. A, Standard solution containing 5×10^{-4} mol l^{-1} Li^+ (6), 10^{-4} mol l^{-1} Na^+ (3), Ca^{2+} (4) and 5×10^{-5} mol l^{-1} K^+ (2), NH_4^+ (1), Sr^{2+} (5) and Ba^{2+}(7). B, Rhine river water. Background electrolyte: magnesium acetate 10 mmol l^{-1}, 18-crown-6 2 mmol l^{-1}, tartaric acid 1 mmol l^{-1}, pH 4.7. Samples were diluted 1 + 1 with background electrolyte solution.

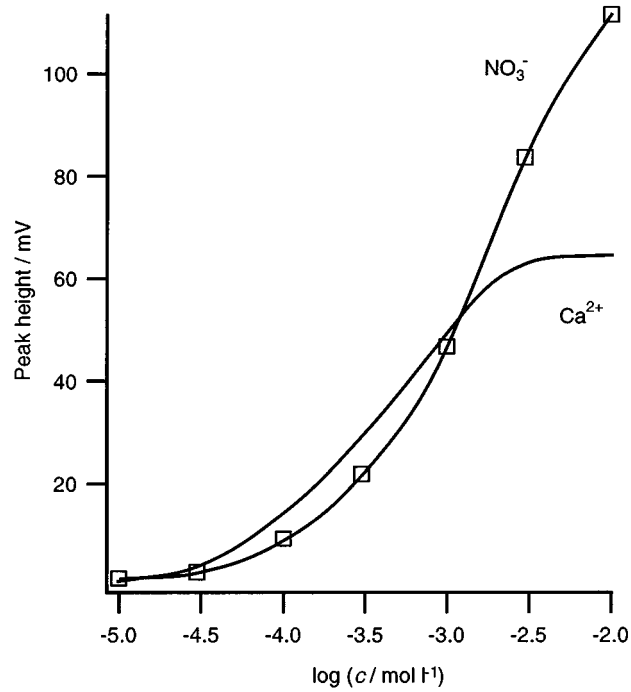

Fig. 6 Calibration curves. Standard solutions diluted 1 + 1 with background electrolyte solution. Other conditions as for Fig. 4 and 5.

This facilitates the separation of these two ions, which are, besides K^+, the major cations present in Rhine water as well as in tap water. Small amounts of ammonium, strontium and barium could also be detected. The peak shape is typical for ion-selective electrode detection and a feature of the logarithmic response of the detector which accentuates the low concentrations.

After exchange of the high voltage module, the ion-selective electrode and the background electrolyte, monovalent inorganic anions were analysed in the Rhine water and compared with a standard sample. The results are shown in Fig. 5. Because of the very similar electrophoretic mobilities of the halide anions, they could not be completely separated under these conditions. For the Rhine water analysis, this was not significant, because the concentrations of bromide and iodide are low and below the detection limit of the method applied. Chloride gave a small peak due to the low sensitivity of the ion-selective electrode towards this poorly lipophilic ion. The highest sensitivity, however, was obtained for nitrate, which is an interesting analyte in water and soil analysis.

Calibration curves obtained in the field for nitrate and calcium ions are given as examples in Fig. 6. Dynamic ranges extend over more than two orders of magnitude. The relative standard deviations ($n = 5$) of the peak heights for solutions of 10^{-4} mol l^{-1} were 1.7% for nitrate and 1.3% for calcium. The detection limits were determined as 8×10^{-6} mol l^{-1} for nitrate and 9×10^{-6} mol l^{-1} for calcium ($3 \times$ standard deviation).

Conclusions

The capillary electrophoresis instrument described in this work represents a compact, robust and portable system, which can be operated independently at any location. Analysis can therefore be carried out in the field immediately after the sample collection. This allows a very flexible strategy, for example, for environmental studies.

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References

1 E. B. Overton and K. R. Carney, *Trends Anal. Chem*., 1994, **13**, 252
2 G. Baykut and J. Franzen, *Trends Anal. Chem*., 1994, **13**, 267.
3 S. Piorek, *Trends Anal. Chem*., 1994, **13**, 281.
4 A. M. Bond, H. A. Hudson, S. N. Tan and F. L. Walter, *Trends Anal. Chem*. 1998, **7**, 159.
5 K. N. Andrew, N. J. Blundell, D. Price and P. J. Worsfold, *Anal. Chem*., 1994, **66**, 917A.
6 P. W. Alexander, L. T. Di Benedetto, T. Dimitrakopoulos, D. B. Hibbert, J. C. Ngila, M. Sequeira and D. Shiels, *Talanta*, 1996, **43**, 915.
7 G. I. Baram, *J. Chromatogr. A.*, 1996, **728**, 387.
8 C. Henry, *Anal. Chem*., 1997, **69**, 195A.
9 X. Huang, T. J. Pang, M. J. Gordon, R. N. Zare, *Anal. Chem*., 1987, **59**, 2747.
10 R. A. Wallingford and A. G. Ewing, *Anal. Chem*., 1987, **59**, 1762.
11 W. Lu, R. M. Cassidy and A. S. Baranski, *J. Chromatogr*., 1993, **366**, 433.
12 C. Haber, I. Silvestri, S. Röösli and W. Simon, *Chimia*, 1991, **45**, 117.
13 A. Nann and W. Simon, *J. Chromatogr*., 1993, **633**, 207.
14 A. Nann, I. Silvestri and W. Simon, *Anal. Chem*., 1993, **65**, 1662.
15 A. Nann and E. Pretsch, *J. Chromatogr. A*, 1994, **676**, 437.
16 P. C. Hauser, N. D. Renner and A. P. C. Hong, *Anal. Chim. Acta*, 1994, **295**, 181.
17 T. Kappes, P. Schnierle and P. C. Hauser, *Anal. Chim. Acta*, 1997, **350**, 141.
18 T. Kappes and P. C. Hauser, *Anal. Chem*., 1998, **70**, 2487.
19 B. L. De Backer and L. J. Nagels, *Anal. Chem*., 1996, **68**, 4441.
20 P. Schnierle, T. Kappes and P. C. Hauser, *Anal. Chem*., 1998, **70**, 3585.
21 T. Kappes and P. C. Hauser, *Anal. Chim. Acta*, 1997, **354**, 129.
22 T. Kleiner, F. Bongardt, F. Vögtle, M. W. Läubli, O. Dinten and W. Simon, *Chem. Ber*., 1985, **118**, 1071.
23 M. Huser, W. E. Morf, K. Fluri, K. Seiler, P. Schulthess and W. Simon, *Helv. Chim. Acta*, 1990, **73**, 1481.

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