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Three-compartment electrodialysis device for on-line sample clean-up and enrichment prior to capillary electrophoresis

B.A.P. Buscher, U.R. Tjaden*, J. van der Greef

Division of Analytical Chemistry, Leiden/Amsterdam Center for Drug Research, University of Leiden, P.O. Box 9502, 2300 RA Leiden, Netherlands

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Abstract

An electrodialysis device for on-line coupling to capillary electrophoresis has been developed. The device consists of three compartments that are separated by two membranes with M_r cut-off values of 500 and 30 000, respectively. The selectivity of the method is based on charge, molecular mass and shape. A concentration factor of 40–50 has been achieved. Sample clean-up and analyte enrichment take only ca. 5 min. Optimization and characterization of the device have been performed and electrodialysis has been applied to the analysis of an inositol trisphosphate derivative in a complex matrix. © 1997 Elsevier Science B.V.

Keywords: Electrodialysis; Instrumentation; Sample handling; Inositol phosphates

1. Introduction

Among the separation techniques, capillary electrophoresis (CE) is well known for its high efficiency and short analysis times. Furthermore, CE is selective and simple to use. With respect to environmental considerations, only very small amounts of chemicals and sample are needed. Unfortunately, CE also has certain drawbacks, i.e. the concentration sensitivity, caused by the limited loadability of the capillary and, in case of spectrophotometric detection, the path length of the detection cell. Therefore, attention has been paid to enhancement of the loadability by stacking [1] or field amplification [2], capillary isotachophoresis (cITP) [3–6] and liquid–liquid electroextraction [7]. Also, preconcentration of analytes has been achieved by the insertion of a small bed of a specific [8,9] or non-specific [10–12] adsorptive solid phase at the inlet of the CE capillary [13]. Investigation has also been focused on the improvement of the detection cell geometry [14] and the coupling of detection techniques to CE with very low concentration detection limits, such as laser-induced fluorescence [15].

In general, if CE analysis is performed in a complex matrix, sample pretreatment is required in order to remove compounds that interfere with the characteristics of the analytes or that block the fused-silica capillary. Several sample pretreatment techniques have been combined with CE, either in the off-line or on-line mode, such as (ultra)filtration, centrifugation [16], (micro)dialysis [17–20], solid-phase extraction (SPE) [21,22], liquid–liquid extraction (LLE) [15], supported liquid membranes (SLM) [23], membrane preconcentration [24], cITP [25] and (concentrating) electrodialysis [26,27].

*Corresponding author.

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Some of these techniques enable the combination of both sample clean-up and analyte enrichment, which is very favourable in the case of trace analysis in complex matrices.

Inositol phosphates (IPs) play a very important role in several research areas, among them, biochemistry [28]. IPs are multiply negatively charged sugars with one to six phosphate groups. Even at very low pH values, they are not uncharged. Therefore, most sample preparation methods, based on neutralized analytes, are not suitable. Electrodialysis is a concentrating and purifying technique that can handle charged analytes.

During electrodialysis, analytes migrate from the donor compartment to the acceptor compartment by an electric driving force superimposed on a concentration gradient. Electrodialysis can be performed in the static as well as in the dynamic mode [29]. In the off-line mode, electrodialysis has been used for the enrichment of ions [30] and for the neutralization of acidic [31] or alkaline [32,33] solutions. Furthermore, electrodialysis has been coupled on-line to ion chromatography [34] and to liquid chromatography [35].

Recently, we combined static (concentrating) electrodialysis with capillary electrophoresis [26,27]. Until now, attention was mainly focused on the selectivity of the device. This paper describes the optimization and characterization of the device with respect to sensitivity. An inositol trisphosphate derivative has been used as a model compound.

2. Experimental

2.1. Chemicals

Ammonium acetate (analytical-reagent grade) was obtained from Merck (Darmstadt, Germany). Acetic acid came from J.T. Baker (Deventer, Netherlands). Hydroxypropylmethylcellulose (HPMC), with a viscosity of 4000 cP for a 2% aqueous HPMC solution was purchased from Sigma (St. Louis, MO, USA). Phenylacetate inositol trisphosphate (PIP3) was kindly provided by Perstorp Pharma (Perstorp, Sweden). For the preparation of stock solutions of the analytes and buffer, deionized water was used (Milli-Q system, Millipore, Bedford, MA, USA). The buffer solution was filtered through a 0.2-μm nylon acrodisc syringe (Gelman Sciences, Ann Arbor, MI, USA). The electrophoresis buffer consisted of 10 m*M* ammonium acetate, pH 5, and 0.005% HPMC.

2.2. Equipment and procedures

The laboratory-made electrodialysis device (Fig. 1) consists of three compartments (1, 2, 3) separated

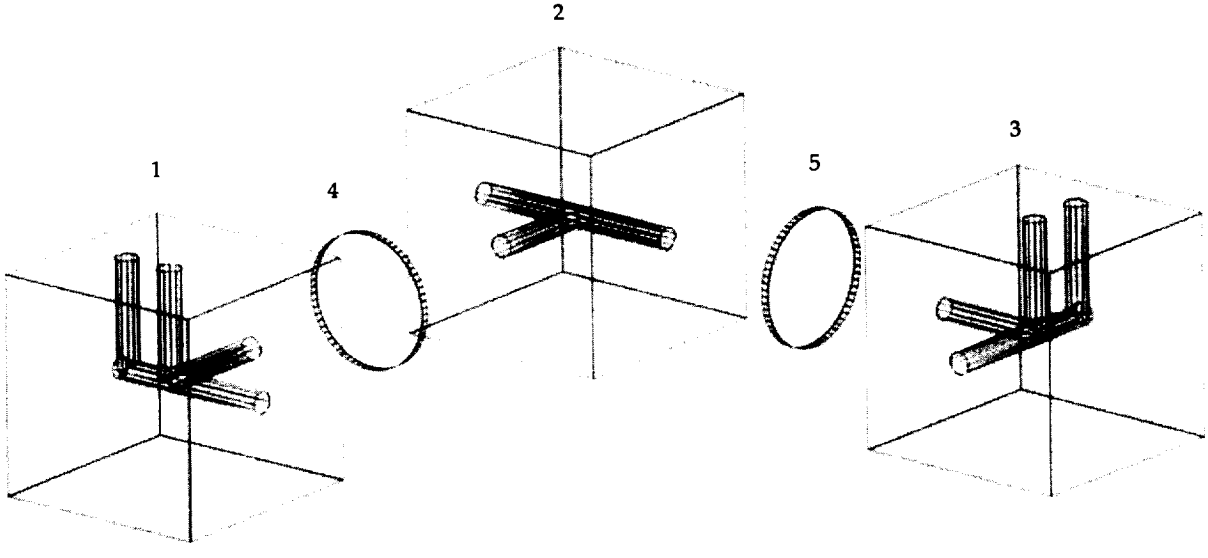

Fig. 1. Three-compartment electrodialysis device (1, 2, 3) including two membranes with M_r cut-off values of 30 000 (4) and 500 (5).

by two porous membranes, made of regenerated cellulose, with M_r cut-off values of 30 000 (4) and 500 (5) (Amicon, Danvers, MA, USA), respectively. The compartments are bores with a diameter of 2 mm in cubes of Perspex ($2\times2\times2$ cm^3). Prior to electrodialysis, the first compartment is filled with sample solution (ca. 50 μl), and the second and third compartments with pure water and 10 m*M* ammonium acetate buffer, pH 5, respectively. All phases are stagnant. The platinum electrodes are positioned in the first (cathode) and the third (anode) compartment. Electrodialysis is performed by applying a voltage (150–600 V) over the electrodes for a few minutes. Subsequently, the fused-silica capillary (S.G.E., Ringwood, Victoria, Australia) (75 μm I.D., 375 μm O.D., l_{tot}=0.80 m, l_{det}=0.55 m) is inserted through the septum (6) onto the membrane in the second compartment and electrokinetic injection is carried out at −10 kV for 15 s, with the cathode in the third compartment and the anode (ground) in the outlet buffer vial. After injection, the capillary inlet is placed in a buffer vial and capillary zone electrophoresis is performed at a voltage of −30 kV (Spellman 1000R, Plainview, NY, USA). UV absorbance detection (CE-adapted Spectroflow 773, Kratos Analytical Instruments, Ramsey, NJ, USA) was at λ=200 nm. For safety reasons, the electrodialysis device (EDD) and the buffer vial containing the high voltage electrode were positioned in a plexiglass box. In between analyses, the EDD is taken to pieces and the channels are rinsed with pure water. In ca. 1 min, the device is cleaned, filled with fresh liquid and, if necessary, provided with a new membrane.

3. Results and discussion

3.1. Electrodialysis

Commonly, electrodialysis is performed in a device containing two compartments, the donor and acceptor compartments, which are separated by a membrane. The anode and cathode are positioned in the electrode compartments [35]. Electrodialysis thus performed allows the separation of high- from low-molecular mass compounds, determined by the membrane cut-off value. At the same time, positive ions can be separated from negative ions, which is determined by the polarity of the electrodes. Enrichment of ions can be achieved by the use of a flowing donor phase and a stagnant acceptor phase [29]. With such a device, all ions having the same charge and a molecular mass below the membrane cut-off value are concentrated in the acceptor compartment. This method has been combined with ion chromatography [34] and with liquid chromatography [35].

Electrodialysis combined with CE requires another approach. Enrichment of the analytes together with the low-molecular-mass background ions is not very favourable, as this will also increase the sample's conductivity. Electrokinetic injection of high-conductivity samples is disadvantageous in CE as the sample conductivity affects the amount of analyte injected. Whereas a poorly conductive sample leads to stacking/field amplification (high local electric field strength), from a highly conductive sample, less analyte is injected because of a decrease of the local electric field strength. This would diminish the concentrating effect on the analyte(s) achieved during electrodialysis. Therefore, selective analyte enrichment is needed.

So far, two devices have been developed for on-line coupling of electrodialysis to CE. One device consists of two compartments and one membrane and allows sample clean-up in only 10–20 s [26,27]. The other device (Fig. 1) consists of three compartments and two membranes with different M_r cut-off values and enables not only sample purification but also selective analyte enrichment [27]. A schematic representation of this electrodialysis process is shown in Fig. 2. Before electrodialysis, the first compartment is filled with a sample solution, consisting of molecules with different molecular masses. The second and third compartments are filled with water and electrophoresis buffer, respectively. By applying a voltage of ca. 600 V, ions with the appropriate charge, in this case anions, and a molecular mass smaller than the size of the membrane pores will migrate from the first to the second compartment. Anions with a molecular mass below the second membrane cut-off value will eventually migrate into the third compartment, whereas anions larger than the size of the (second) membrane pores are retained at the membrane. Thus, by a proper selection of the membranes, i.e. the size of the first membrane pores (M_r cut-off: $2-100\cdot10^3$) being larger than the analyte and the second membrane

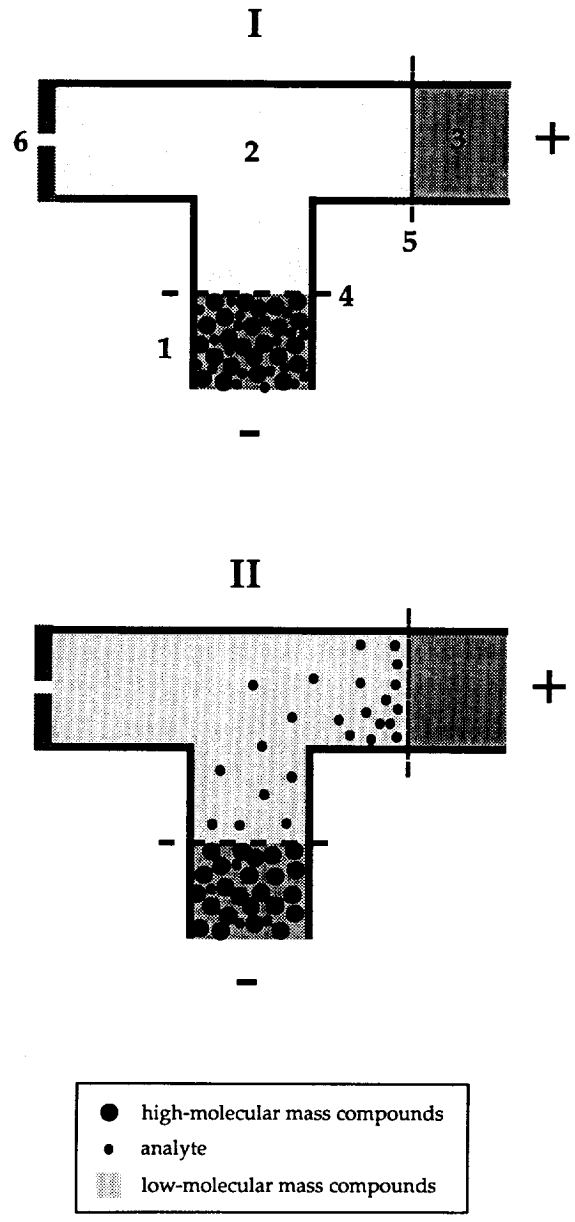

Fig. 2. Electrodialysis process. I=before electrodialysis, II=after electrodialysis. 1, 2, 3=compartments 1, 2 and 3; 4=membrane with a M_r cut-off value of 30 000; 5=membrane with a M_r cut-off value of 500; 6=septum for the fused-silica capillary.

pores (M_r cut-off: 100 or 500) being smaller than the analyte, the analyte can be separated from high-molecular-mass compounds (first compartment) as well as from low-molecular-mass compounds (third compartment). The device also enables the selective enrichment of analytes on the membrane in the second compartment [27]. If the pores of the second membrane are too large, the analyte will migrate through the membrane together with the matrix ions. Consequently, no enrichment of analyte will occur. In this case, the sample is only purified from high-molecular-mass compounds and eventually loss of analyte occurs.

3.2. Optimization and characterization of the device

In order to achieve the best performance, several parameters have been investigated that have an effect on the electrodialysis process. First, attention was focused on the influence of the applied electric field on analyte enrichment. Therefore, compartment 1 was filled with a sample solution consisting of 10 μM PIP3 (M_r=774.1) in electrophoresis buffer. Using an electrodialysis time of 5 min, the electrodialysis voltage was varied from 0 to 600 V. At 600 V, the best result was obtained. At a voltage higher than 600 V, too much heat was generated and gas bubbles were formed. If the applied voltage was zero and, thus, pure dialysis was carried out for 5 min, no PIP3 could be detected. Therefore, the contribution of dialysis to the electrodialysis process in the first 5 min can be neglected.

Next, the electrodialysis time was optimized. Again, compartment 1 was filled with 10 μM PIP3 in electrophoresis buffer. The electrodialysis time was varied from 1 to 10 min at a voltage of 600 V. From 1 to 5 min, the enrichment of PIP3 was improved, which resulted in a non-linear curve (Fig. 3, n=3), whereas at an electrodialysis time of 8 or 10 min, too much heat was generated, leading to the formation of gas bubbles and a reduction of the current. Presumably, the non-linearity of the curve is also due to Joule heating, leading to a decrease in the viscosity and an increase in the electrophoretic mobility of PIP3. Obviously, the dialysis time has to be regulated accurately to achieve reproducible results.

In Fig. 4, two electropherograms are depicted, obtained after electrokinetic injection and capillary zone electrophoresis (CZE) of a solution containing 10 μM PIP3 in electrophoresis buffer, without (A)

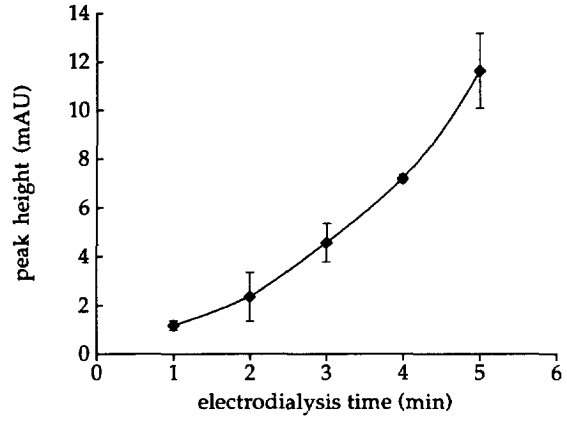

Fig. 3. Peak height of PIP3 versus electrodialysis time ($n=3$). Electrodialysis voltage, 600 V; electrokinetic injection, 15 s, −10 kV; CZE, −30 kV.

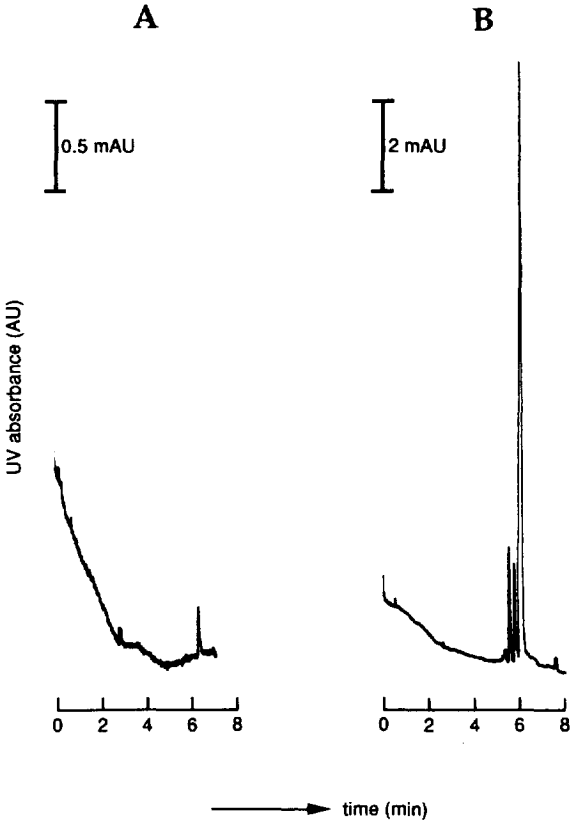

Fig. 4. Electropherograms of 10 μ*M* PIP3 after electrokinetic injection–CZE without (A) and with (B) electrodialysis pretreatment. UV detection was at λ=200 nm.

and with (B) electrodialysis pretreatment. In this figure, the enrichment is clearly shown by the enormous increase in peak height. The concentration factor for PIP3 was ca. 50 in only 5 min of electrodialysis time. However, two analyte impurities with higher electrophoretic mobilities than that of PIP3 can be seen in the electropherogram. The impurities were also concentrated in the electrodialysis device due to their molecular masses, 656 and 756, respectively, which were determined with CZE coupled to mass spectrometric detection [36]. The concentrating effect on the impurities was higher than on PIP3, due to their higher electrophoretic mobilities.

In order to investigate the effect of sample conductivity on analyte enrichment, several sample solutions, all containing 10 μ*M* PIP3, were electrodialyzed. The sample conductivity was varied by changing the ammonium acetate concentration. As reference experiments, electrokinetic injection followed by CZE was performed with identical sample solutions. As has been described by several researchers, analyte stacking can be achieved during electrokinetic injection by the use of poorly conductive matrices by the use of a poorly conductive matrix and a highly conductive electrophoresis buffer in the fused-silica capillary [37]. Using a CZE buffer of 10 m*M* ammonium acetate, the effect of sample conductivity on the electrokinetic injection is shown in Fig. 5 (diamonds). A sample matrix of 10 m*M* ammonium acetate did not cause any stacking or dilution. However, a decrease of the conductivity in the sample leads to a higher peak height of PIP3, and vice versa.

A similar relationship between matrix conductivity and peak height was observed after electrodialysis–electrokinetic injection–CZE (Fig. 5, triangles). However, the PIP3 peak height in this curve is much higher, due to the concentrating effect of electrodialysis. Also, for this curve, a highly conductive sample solution appeared to be disadvantageous for PIP3 peak height. Acetate ions, which are smaller than the membrane pores, migrate freely through the device from the first to the third compartment. After 5 min of electrodialysis, the acetate ion concentration in the second compartment equals that of the first compartment before electrodialysis. Consequently, the initial acetate concentration in the sample has an

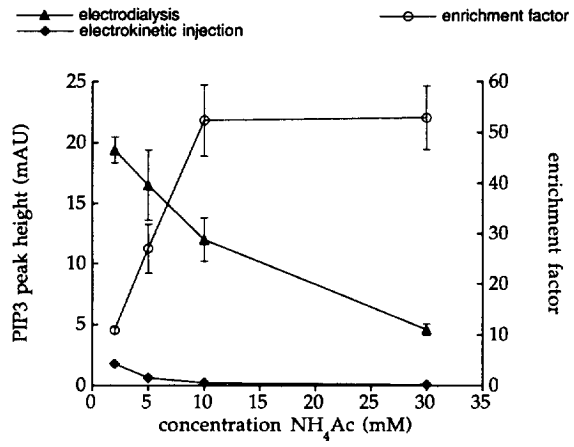

Fig. 5. Effect of the conductivity of the sample matrix (ammonium acetate concentration) on the peak height of PIP3 (left axis) and the enrichment factor (right axis). A comparison between electrokinetic injection with (triangles) and without (diamonds) electrodialysis pretreatment is shown. The bullets indicate the enrichment factor of PIP3 as a function of sample conductivity.

effect on the electrokinetic injection performed after electrodialysis. PIP3, however, has been selectively concentrated on the second membrane, as its molecular mass is larger than the pore size.

Furthermore, in Fig. 5, the enrichment curve (bullets) is constructed as the quotient of the electrodialysis curve and the electrokinetic curve. For PIP3 samples with an ammonium acetate concentration of 10 or 30 m*M*, the enrichment factor was more than 50. For a poorly conductive sample, however, the extra enrichment by electrodialysis, on top of the stacking achieved during electrokinetic injection, is rather low. This is probably the result of the buffer solution (10 m*M* ammonium acetate) in the third compartment, which increases the conductivity in the second compartment during electrodialysis, thus affecting electrokinetic injection after electrodialysis. Fig. 5 clearly demonstrates that sample pretreatment using electrodialysis and electrokinetic injection prior to CZE is influenced by the conductivity of the sample. Calibration of the system is recommended for accurate quantitative analysis.

Because the electrodialysis device has been developed for trace analysis in complex matrices, its performance was tested at the submicromolar level. Unexpectedly, the concentration factor of ca. 50 could not be achieved at PIP3 concentrations below 5 μ*M*. This phenomenon appeared to be caused by adsorption of PIP3 to the Perspex electrodialysis device. By a competitive mechanism between PIP3 and another inositol phosphate, inositol hexakisphosphate (IP6), adsorption of PIP3 could be reduced. In Fig. 6, the PIP3 peak height is plotted versus the concentration of IP6 that was added to the sample ($n=3$). The peak height of PIP3 is actually the result of two effects. First, IP6 reduces the wall adsorption of PIP3 during electrodialysis, leading to a higher PIP3 signal (Fig. 6). Second, IP6, having a molecular mass of 660, is also concentrated during electrodialysis. Due to this increase of the conductivity, IP6 has a negative effect on the electrokinetic injection of PIP3, performed after electrodialysis (Fig. 6). An optimum of both effects was obtained at 50 μ*M* IP6.

3.3. Quantitative aspects

For quantitative purposes, a comparison has been made between electrokinetic injection with and without electrodialysis pretreatment. The following parameters were compared: The reproducibility, expressed as the relative standard deviation (R.S.D.), the linearity, characterized by the correlation coefficient, and the concentration limit of detection (CLOD). All experiments were carried out for standard samples of PIP3 in electrophoresis buffer and for the electrodialysis experiments, 50 μ*M* IP6

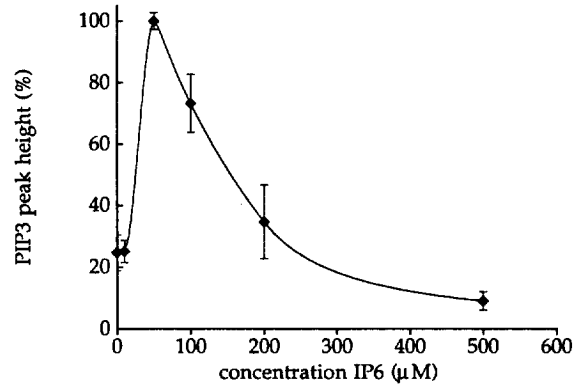

Fig. 6. PIP3 peak height after electrodialysis–electrokinetic injection–CZE versus the concentration of IP6 added to the sample ($n=3$). PIP3 concentration, 1 μ*M* in electrophoresis buffer.

was added to the sample. Reproducibility data showed that electrokinetic injection without electrodialysis could be performed more precisely (R.S.D.=7.8, n=5) than with electrodialysis (R.S.D.=13.8, n=5). In order to investigate the linearity of the method as well as the CLOD, a calibration plot was constructed. It showed that, without electrodialysis pretreatment, the correlation coefficient was 0.996 in the concentration range 5–500 μM (n=3). With electrodialysis pretreatment, the correlation coefficient was 0.989 in the concentration range 0.1–2 μM (n=3). The CLOD was 100 nM with electrodialysis compared to 5 μM without electrodialysis.

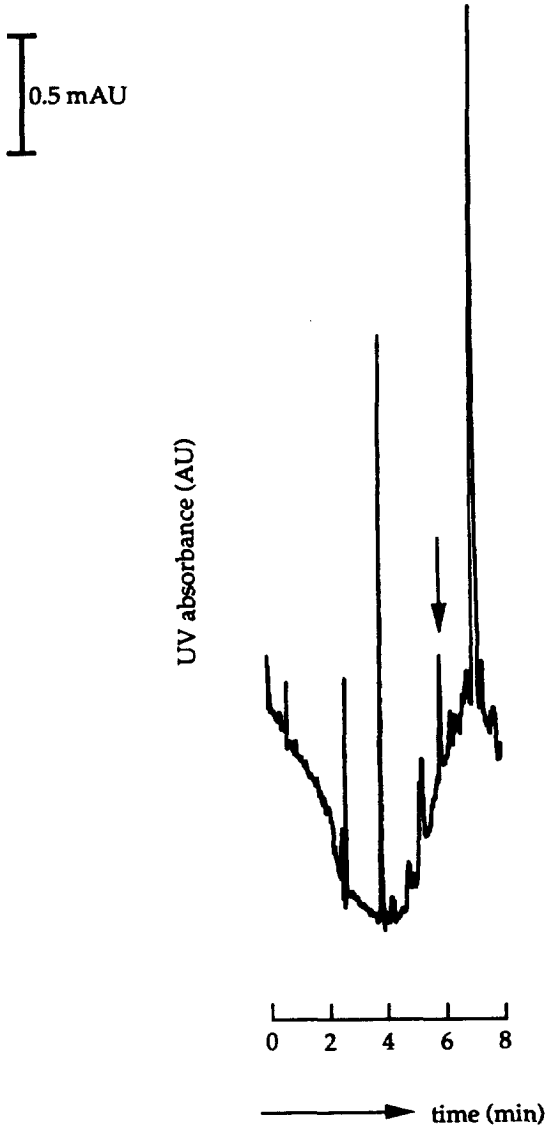

Fig. 7. Determination of 500 nM PIP3 in a complex matrix using electrodialysis–electrokinetic injection–CZE. Membrane M_r cut-off values, 2000 and 500. UV detection was at λ=200 nm.

3.4. Determination of IP3 derivative in a complex matrix

Fig. 7 demonstrates the usefulness of electrodialysis pretreatment for the analysis of PIP3 in a complex matrix. The yeast cell matrix, containing high- as well as low-molecular-mass (unknown) compounds, was spiked with PIP3 and IP6 to concentrations of 500 nM and 50 μM, respectively. In this complex matrix, the addition of 50 μM IP6 had the same effect as in a standard solution of PIP3. The M_r cut-off values of the membranes were 30 000 and 500. Under these conditions, however, too many compounds from the matrix were still being captured in the second compartment. Therefore, the selectivity was improved by using a membrane with a M_r cut-off value of 2000. The result is shown in Fig. 7. Only negatively charged compounds with molecular masses of between 500 and 2000 are trapped and concentrated. Thus, the selectivity of sample purification can be modified without affecting the concentrating effect.

4. Conclusions

An electrodialysis device has been developed and described for on-line coupling to capillary electrophoresis. It allows selective analyte enrichment and sample clean-up at the same time. The electrodialysis time was only 5 min and a concentration factor of 40–50 has been achieved. In addition to the speed, the developed technique is relatively cheap, uses a small amount of sample (ca. 50 μl), a small volume of other chemicals, i.e. electrophoresis buffer, and does not use any organic modifiers.

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