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On-line dialysis as a sample preparation technique for ion chromatography

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Abstract

The use of on-line dialysis as a sample preparation technique for ion chromatography is described. A fully automated sample preparation device coupled to an ion chromatographic system for the determination of anions and cations in various matrices is presented. The method was based on stopped-flow dialysis, where the samples were continuously dialyzed for 10 min while the acceptor solution was stationary within the recipient channel. The matrices examined, without additional sample treatment, included milk, untreated wastewater, fruit juice, engine coolant, and a multivitamin tablet. The analyte recoveries for anions and cations in various matrices ranged from 87 to 106%. In addition, multiple sample injections were performed and repeatabilities were found in the range of 0.2 to 4%.

Keywords: Sample preparation; Dialysis; Stopped-flow technique

1. Introduction

Ion chromatography (IC) is a versatile technique that allows for the rapid and precise determination of ions in complex matrices [1]. Since the technique was first described in 1975 [2], it has been used to analyze a wide variety of samples consisting of inorganic and organic anions and cations. Despite its versatility, off-line sample preparation techniques [3] such as solvent or solid-phase extraction, precipitation, digestion, or filtration are still commonly used before a sample is introduced into the IC system. These techniques are often time-consuming and may lead to sample contamination and lower precision. As a result, an increased interest has been directed towards the automation of sample preparation techniques. Sample handling procedures, such as preconcentration, matrix elimination, solid-phase extraction and dialysis, have been successfully combined on-line with IC systems [4,5].

During the last two decades, on-line dialysis has found applicability in a wide variety of samples. A review by van de Merbel [6] chronologically highlights some of the applications for on-line dialysis since 1985. Most of these applications are almost exclusively in the areas of biomedical and food analysis. In these applications, dialysis is commonly applied with two phases, a donor and acceptor phase, separated by a semipermeable membrane. Analytes of lower molecular size are transferred across the membrane into the acceptor phase and thus leaving higher molecular mass solutes in the donor phase [7].

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The most common analytical instrument coupled to dialysis is high-performance liquid chromatography (HPLC). In HPLC, a preconcentration column is commonly employed with on-line dialysis systems [8–14]. These preconcentrators are necessary to trap and possibly enrich the analytes of interest before further analysis without causing dilution by the dialysis procedure. This automated sequential trace enrichment of dialysate (ASTED) system has been successfully applied to the clinical, pharmaceutical, and food industries.

The analysis of inorganic ions using dialysis may also be performed by either a flow injection analysis (FIA) or capillary electrophoresis (CE) system. In FIA, a selective reagent or modifier is sometimes used to enhance the selectivity of the system [15–18]. In addition, dialysis has also been used on-line with CE [19]. Kuban and Karlberg [20] presented an on-line dialysis system coupled to a FIA–CE interface for the treatment of complex matrices. These techniques have demonstrated applicability for the analysis of blood serum, food products (milk, juice) and environmental samples.

Grudpan and co-workers [16] demonstrated the use of a FIA system combined with IC for the analysis of standard anions in natural water samples. They used a manually operated flow injection dialysis (FID) system combined with an automated IC system. The samples were injected into the donor stream where it was combined with a modifier and passed through a mixing coil and finally into the dialysis unit. The analytes of interest were allowed to permeate the membrane where it was injected into the IC system for analysis.

In IC, the most commonly applied version of dialysis uses an ion-exchange membrane where only ions of a specific charge are allowed to pass through the membrane. This technique is useful for samples of extreme pH values (i.e. highly alkaline or acidic), which pose serious problems in IC systems, causing distorted peaks, baseline disturbances, and reduced column life [4]. However, on-line dialysis–IC is also beneficial for the analysis of ions in complex matrices containing high molecular mass components, as those described earlier.

In the present study, on-line dialysis combined with an IC system is described. The dialysis–IC system, based on stopped-flow technique, was examined as a sample preparation device for the removal of macromolecular containing substances. This technique is based on a continuously flowing donor phase and a stationary acceptor phase to yield up to 100% dialysis rates. The study examined samples such as milk, fruit juice, untreated wastewater, and a multivitamin tablet without additional pretreatment. These samples were chosen for the complexity of their matrices containing high molecular mass substances or small particulates that could otherwise damage the analytical column or cause potential interferences.

2. Experimental

2.1. Reagents and standards

All solutions were prepared with house-distilled water deionized through a Milli-Q water purification system with a specific resistivity of 18.2 MΩ·cm (Millipore, Milford, MA, USA). Standards were prepared from 1000 ppm stock solutions (Ricca, Arlington, TX, USA). Stock solutions of 0.5 *M* Na_2CO_3 and 0.5 *M* $NaHCO_3$ were prepared from analytical grade reagents (Sigma, St. Louis, MO, USA) and used to prepare final eluent concentrations. The eluent for the anion analysis was 2.4 m*M* $NaHCO_3$/2.5 m*M* Na_2CO_3 (except as stated). The cation eluent (except as stated) was prepared by combining 0.6 g of L-tartaric acid (Aldrich, Milwaukee, WI, USA) and 0.167 g of 2,6-pyridinedicarboxylic acid (PDCA) (Aldrich) in water then diluting to 1 l to make a final concentration of 4 m*M* tartaric acid/1 m*M* PDCA. A flow-rate of 0.5 ml/min (except as stated) was used for the anion system and 1.0 ml/min for the cation system.

2.2. Equipment

A Metrohm Modular IC system (Metrohm Ltd., Herisau, Switzerland) included a 709 pump, 732 conductivity detector, 753 suppressor module, 762 IC interface, and 754 dialysis unit. Sample injection and dialysis flow was used with dual six-port injection valves equipped with a 20 μl sample loop, a conductivity detector, a microcapillary packed bed suppressor, and chromatographic column. The sup-

pressor operates from a three-port switching valve that allows the use of a freshly regenerated (with 100 m*M* H_2SO_4) suppressor prior to each chromatographic analysis. The Metrosep Anion Dual 1 column (150×3 mm, I.D.), including the guard column (30×3 mm, I.D.), was used for the anion analysis and is based on a hydroxyethyl methacrylate resin. The Metrosep Cation 1–2 column (125×4 mm, I.D.) was used for cation analysis and consists of a silica gel resin. Cations were detected by direct conductivity and anions by suppressed conductivity. Data was acquired with Metrohm IC Net 2.0 chromatography workstation.

2.3. 754 dialysis unit

A schematic diagram of the 754 dialysis unit coupled to the IC system is shown in Fig. 1. The dialysis device consisted of two Tygon peristaltic pump tubings for acceptor (400×0.51 mm, I.D.) and donor (400×0.76 mm, I.D.) phase flow and a dialysis cell with equal donor and acceptor volumes of 130 μl. Valve B was connected to the acceptor channel of the dialysis cell and was used to direct the flow in the dialysis procedure. Fig. 2 illustrates a cross-sectional diagram of the dialysis cell. The cell was made of Plexiglas material and contained a cellulose acetate membrane with the following dimensions: pore size=0.2 μm, diameter=4.7 cm, thickness=115 μm. A timed-event program, controlled by the chromatography software, allowed complete automation of the dialysis unit by sequencing through the following four steps.

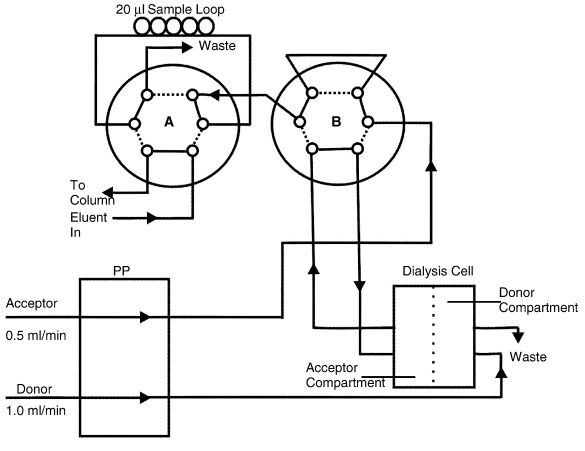

Fig. 1. Schematic flow diagram of the 754 dialysis unit and dual injection valves. See text for description of timed-event programs for the dialysis procedure. ———, fill position; ·····, inject position; PP, peristaltic pump.

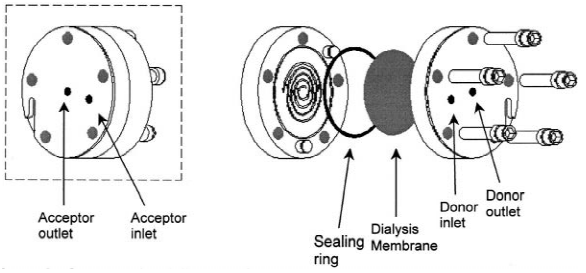

Fig. 2. Cross-sectional diagram of the dialysis cell. The cellulose acetate membrane contains the following dimensions: pore size=0.2 μm, diameter=4.7 cm, thickness=115 μm.

2.3.1. Washing

Valve A was switched to the fill position and valve B to the inject position. The sample loop and dialysis cell were allowed to rinse for approximately 2 min.

2.3.2. Dialysis with stopped-flow

Valve B was switched from inject to the fill position. This allowed the acceptor solution to remain static while 10 ml (1.0 ml/min) of the sample solution was continuously pumped through the donor channel allowing equilibration of the sample analytes between the donor and acceptor phase.

2.3.3. Transfer of dialysate to sample loop

Valve B was switched from the fill to inject position. This allowed 0.15 ml of the acceptor solution (0.5 ml/min) to pass through the acceptor compartment and transfer the sample ions to the injection loop.

2.3.4. Injection of sample

Valve A was switched from the fill to inject position allowing the eluent to carry the ions from the sample loop to the analytical column for analysis.

3. Results and discussion

3.1. Determination of transfer and dialysis times

The influence of the time required for dialyzing the sample and transferring the dialyzed ions from the acceptor channel to the injection loop was investigated. The transfer time was determined by dialyzing an anion standard of known concentration and calculating the recovery of each anion as a function of time (results not shown) while the dialysis time remained constant at 10 min. At 0.3 min, the recoveries of each anion reached a maximum value of nearly 100% and this time was recorded as the transfer time. In addition, the dialysis time was also examined and is defined as the time for the ions in the sample solution to permeate through the membrane and allow equilibration between the acceptor and donor phase. The dialysis time was determined by using a similar experiment as just described (results not shown). However, in this case, the standard was dialyzed at variable times while the transfer time remained constant at 0.3 min. The percent recovery of each anion was then calculated as a function of time. The dialysis time was determined when the recoveries increased up to a constant value of nearly 100%. This experiment revealed a dialysis time of 5 min, however, 10 min was used throughout these experiments to insure the absolute maximum recovery of all inorganic ions present in the sample.

3.2. Dialysis–IC system

Using the system illustrated in Fig. 1, a mixture of standard anions or cations were used to calibrate the system to allow accurate quantitation of sample matrices. Standards and samples were analyzed using the same procedure as previously outlined. An acceptor stream of deionized water was used for the analysis of anions and 2 m*M* HNO_3 was used for cation analysis. The acidic cation acceptor solution was used to minimize any potential complexing between the analytes and organic solutes present in the sample matrix. The standards were calibrated with different concentration ranges due to diversity of the analyte concentrations present in the matrices. The calibration linearity for each ion resulted in an r^2 value of 0.9997 or better. The total analytical time required was approximately 30 min. However, this may be significantly decreased by choosing an alternative column with shorter run times and by performing dialysis while a sample is being chromatographed.

3.3. Analysis of anions in real samples

The dialysis–IC system was applied to the analysis of real samples, such as milk, untreated wastewater, engine coolant, and a multivitamin tablet. In general, most common anions are present in these matrices in significant quantities (mg/l to g/l). Therefore, the addition of a preconcentration column is not necessary for the on-line dialysis system. As a result of these high concentrations, most samples were diluted prior to dialysis with the exception of wastewater, which was analyzed without further treatment. Fig. 3 illustrates the separation of nitrate and *o*-phosphate in a dialyzed engine coolant sample.

A common sample analyzed with dialysis is milk that usually consists of large amounts of proteins and other macromolecules that could potentially damage

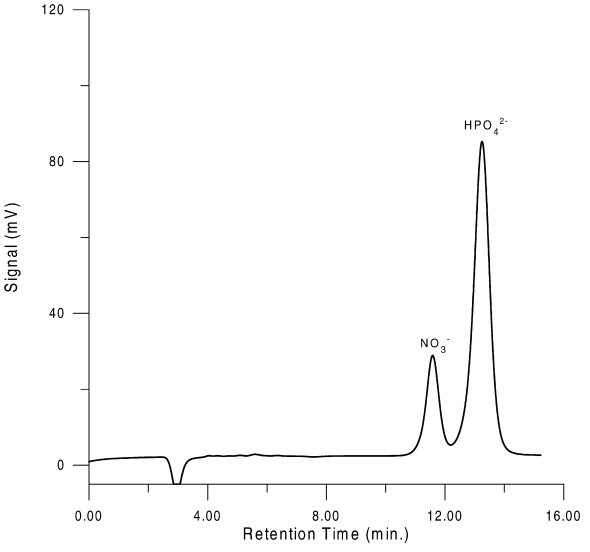

Fig. 3. Chromatogram of common anions found in engine coolant using stopped-flow dialysis as a sample preparation technique (Metrosep anion dual 1 column (150×3 mm, I.D.)+guard (30×3 mm, I.D.), 2.4 m*M* $NaHCO_3$/2.5 m*M* Na_2CO_3, 0.5 ml/min, 20 μl sample). See Table 1 for quantitative results.

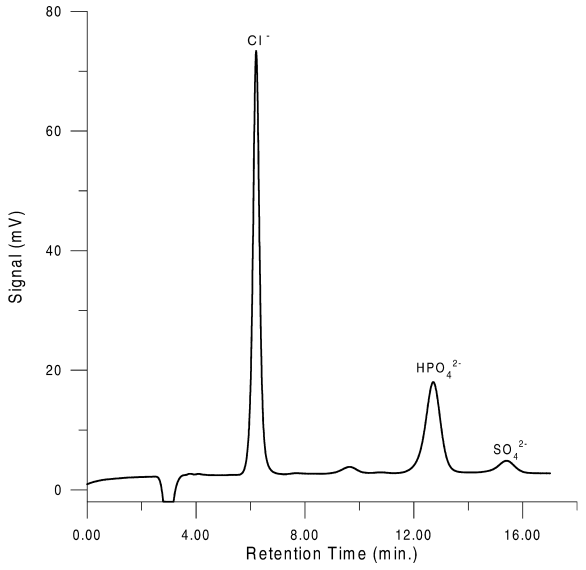

Fig. 4. Chromatogram of common anions found in milk using stopped-flow dialysis as a sample preparation technique. Same conditions as Fig. 3. See Table 1 for quantitative results.

the analytical column. Fig. 4 shows a chromatogram of a dialyzed milk sample indicating the presence of high concentrations of chloride. Previously reported FIA studies [17] showed similar chloride concentrations in milk as those found with the present dialysis–IC system. A comparison of the results in the milk samples was also attempted by manual filtration techniques. However, these experiments proved difficult and results could not be easily obtained. As a result of the large amounts macromolecular constituents present in the matrix, the filter was easily obstructed and could not be analyzed by direct injection. Table 1 summarizes the results of the concentrations found in each sample matrix and the repeatabilities of a multiple injection analysis. As shown, repeatabilities ranged from 0.3 to 4% RSD. Also, the recoveries of each analyte was determined by spiking known concentrations of the analyte in the sample matrix resulting in average recoveries $>87\%$.

In addition, the performance of the column was monitored over a period of several weeks to determine if any macromolecular substances permeated the dialysis membrane and reached the analytical column. This was determined by monitoring the column pressure and standard chromatograms on a daily basis after approximately 70 sample injections were performed during this time. The observed pressure remained stable and no influence was present on any of the chromatograms examined. This indicated that the analysis of these samples did not influence the lifetime of the column and macromolecular components remained on the donor side of the dialysis membrane.

Table 1
Quantitative results for anions found in real samples using stopped-flow dialysis for sample preparation

Sample	Anion	*n*	Concentration measured (mg/l)	RSD (%)	Recovery (%)
Milk	Cl^-	3	920	0.28	106
	HPO_4^{2-}	3	1760	0.28	100
	SO_4^{2-}	3	160	0.53	90
Untreated waste-water	NO_2^-	4	0.90	1.90	87
	HPO_4^{2-}	5	9.7	0.66	91
Engine coolant	NO_3^-	4	1400	1.23	102
	HPO_4^{2-}	4	11000	0.45	99
Multivitamin tablet[a]	Cl^-	5	320	0.59	–[b]
	NO_3^-	5	1.50	0.67	–
	HPO_4^{2-}	5	39.0	3.99	–
	SO_4^{2-}	5	18.0	1.91	–

Metrosep anion dual 1 column (150×3 mm, I.D.)+guard (30×3 mm, I.D.), 2.4 m*M* $NaHCO_3$/2.5 m*M* Na_2CO_3, 0.5 ml/min, 20 μl sample volume.

[a] Metrosep anion dual 2 column (75×4.6 mm, I.D.) with 2.0 m*M* $NaHCO_3$/1.3 m*M* Na_2CO_3 at a flow-rate of 0.8 ml/min.

[b] –, not determined.

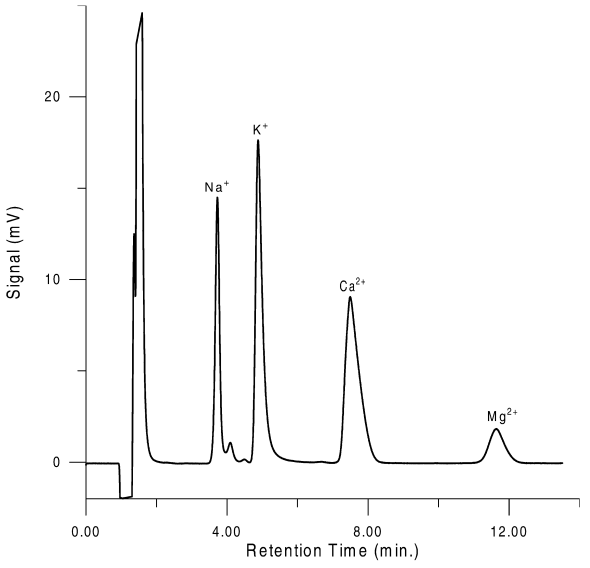

Fig. 5. Chromatogram of common cations found in milk using stopped-flow dialysis as a sample preparation technique (Metrosep cation 1–2 column (125×4 mm, I.D.), 4 m*M* tartaric acid/1 m*M* PDCA, 1 ml/min, 20 μl sample). See Table 2 for quantitative results.

3.4. Analysis of cations in real samples

The same experiments were performed with cations in complex matrices. In these studies, the Metrosep Cation 1–2 column was used where common cations elute in the following order: $Li^+ < Na^+ < NH_4^+ < K^+ < Ca^{2+} < Mg^{2+}$ when a tartaric acid/PDCA eluent is used. However, when PDCA is replaced with oxalic acid, the selectivity of Ca^{2+} and Mg^{2+} are reversed. In our studies, we were not interested in determining any levels of Li^+ or NH_4^+ since these are generally absent from the types of samples in our investigation. Common cations, such as Na^+, K^+, Ca^{2+}, and Mg^{2+}, were examined in milk and fruit juice. Fig. 5 shows a chromatogram of cations present in milk after dialysis. As shown, high levels of potassium and calcium were determined in the sample as expected. In addition, common cations and transition metals were also determined in a multivitamin tablet. Table 2 summarizes the results obtained from these studies. As shown, the repeatabilities of the samples were evaluated and ranged from 0.2 to 3.6% RSD with average recoveries >87%.

4. Conclusion

On-line dialysis combined with IC is a viable technique for the treatment of samples in complex matrices. The present system represents an excellent tool for the routine monitoring of a variety of

Table 2
Quantitative results for cations found in real samples using stopped-flow dialysis for sample preparation

Sample	Cation	*n*	Concentration measured (mg/l)	RSD (%)	Recovery (%)
Milk	Na^+	4	390	0.17	93
	K^+	4	1500	0.56	98
	Ca^{2+}	4	910	0.99	104
	Mg^{2+}	4	110	1.02	87
Fruit juice	Na^+	5	35.0	0.70	93
	K^+	5	880	0.74	99
	Ca^{2+}	5	86.0	0.72	95
	Mg^{2+}	5	49.0	0.96	95
Multivitamin tablet[a]	K^+	5	3.40	1.01	–[b]
	Ni^{2+}	5	0.20	3.55	–
	Zn^{2+}	5	0.70	3.08	–
	Ca^{2+}	5	6.20	0.82	–
	Mg^{2+}	5	4.00	1.10	–

Metrosep cation 1–2 column (125×4 mm, I.D.), 4 m*M* tartaric acid/1 m*M* PDCA, 1.0 ml/min, 20 μl sample volume.
[a] The eluent for this study was 3 m*M* tartaric acid/0.5 m*M* oxalic acid.
[b] –, not determined.

aqueous samples containing proteins, particles, fats, and other macromolecules and eliminating conventional off-line sample treatment. In addition, the column life is preserved by preventing the macromolecular-containing constituents from passing through the membrane. These sample types have wide applicability in the food, environmental, and the pharmaceutical industries that demonstrates a large potential for process analysis. Experiments are currently being performed to further evaluate additional sample types for the present dialysis–IC system.

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