A SEPARATIONS-BASED SENSOR FOR MONITORING NPK IN GROUND WATER

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INTRODUCTION:

The analysis of inorganic ions in groundwater supplies is an important issue for monitoring water quality. Ideally, *in situ* monitoring would allow fast response and easy tracking of non-point source pollution from species such as nitrate, phosphate, and potassium (NPK). Current analytical methods are not suitable for multi-analyte *in situ* remote sensing. Ion-selective electrodes (ISE) and ion-chromatography (IC) are the most common methods of measuring inorganic ions such as nitrate, phosphate, and potassium (Jackson et al. 2000; Karmarkar 1999; Norkus et al. 1996; Schwarz et al. 2000). Both of these methods are limited. ISE can only measure one species at a time plus it frequently suffers from interference from other species in solution. IC is the most powerful of the two methods for the determination of inorganic ions, providing simultaneous analysis of all inorganic cations or anions. There are limitations to IC. First, the equipment is large and expensive making a field portable unit impractical. Second, two separate analyses must be performed to determine the levels of anions and cations. Therefore, new methods, such as capillary electrophoresis, are being explored as alternative analysis methods.

Capillary Electrophoresis (CE) is a separation method based on the differential rate of migration of charged species in a capillary filled with buffer solution across which a dc field has been applied (Dasilva and Dolago 1998; Fukushi et al. 1999; Kaniansky et al. 1999; Pacakova et al. 1999; Valsecchi et al. 1997). CE uses electroosmotic flow (EOF) that allows cations, anions, and neutral species to be separated simultaneously. EOF results from the movement of a charged interfacial layer under the influence of a large external potential applied across the capillary. Above pH 3, the inside wall of the channel is negatively charged due to ionization of the surface bound silanol groups. Buffer cations congregate in an electrical double layer adjacent to the negative surface. The cations in the outer layer flow toward the cathode, or negative electrode. The cations are solvated thus causing flow of the solvent.

Capillary electrophoresis on microchips has become an important area of research in analytical chemistry (Harrison et al. 1993; Jacobson and Ramsey 1997; Manz et al. 1992). The idea of a lab-on-a-chip, where all separation and analysis steps are accomplished on a single microchip, is becoming a reality. Miniaturization of the components is the limiting factor in the lab-on-a-chip idea. Microchip capillary electrophoresis is less expensive, more durable, much faster than conventional CE and provides more efficient separation.

Several detection methods have been applied to microchip CE. Optical detection methods have been the major detection mode for microchip CE, and laser induced fluorescence (LIF) has been the favored method producing detection limits in the picomolar range (Harrison et al. 1993; Jacobson and Ramsey 1997; Manz et al. 1992). The size and cost of the instrumentation such as lasers required to carry out these detection methods negates some of the benefits of miniaturization. Electrochemistry has been demonstrated as a successful detection method for microchip CE (Henry et al. 1999; Martin et al. 2000; Wang et al. 1999; Woolley et al. 1998). Unlike optical and mass spectrometry detection, it is easily miniaturized to match the microchip size. Furthermore, electrochemistry is extremely sensitive for easily oxidized species.

Conductivity detection methods are thought to be a universal detection method for separation techniques. Conductivity is a bulk solution property unlike electrochemical methods where reduction and oxidation occurs at the surface of the electrodes. Conductivity is based on ionic mobilities unique to each species under observation. Previous reports have shown a limit of detection of sodium to be 0.43 mM (Kaniansky et al. 2000). Here we report the detection of working concentrations of 100 μM of hydroquinone and sodium phosphate separations using microchip CE with in-channel conductivity detection.

THEORY:

For our experiments, the in-channel resistance is the only variable required for conductivity measurement and allows conductivity to be

based simply on the measurement of resistance. Conductivity is the reciprocal of resistivity based on the following equation:

$$\kappa = L/RA$$

Where κ is conductivity, **L** is length between electrodes, **R** is resistance, and **A** represents surface area of the electrodes. In microchip capillary electrophoresis the working electrode is positioned in the channel (fig. 1) in order to measure the resistance between the working electrode and the counter electrode in the waste reservoir. Positioning of the electrode is critical in producing consistent results and data for this reason. The distance of the electrode in the channel must be far enough to detect a change in conductivity measurement of the separated species, yet if the electrode is positioned too far in the channel the resistance becomes so high that any change in the resistance due to an analyte, thus a change in conductivity, will not be detected due to interference and loss of resolution. Electric current in the capillary generates heat called Joule heating at a rate of I^2R joules per second. Joule heating is a current effect. The larger the capillary becomes, the more resistance. This reduces the current at a constant voltage or allows the current to stay constant with increasing voltage. The capillary must be thin enough to dissipate heat rapidly. Temperature gradients disturb the electro-osmotic flow and reduce resolution.

MATERIALS AND METHODS:

Chemicals: The following chemicals were used as received: hydroquinone (Fisher Scientific), NaOH (Fisher Scientific), 2-(N-morpholino) ethane sulfonic acid monohydrate (MES) (Sigma Chemical Co.), N-tris-(hydroxymethyl) methyl-2-amino ethane sulfonic acid (TES) (Sigma Chemical Co.), sodium phosphate (Acros Organics), potassium nitrate (Fisher Scientific) , potassium phosphate (Fisher Scientific), and epoxy (13 TETA: 100 EPON 828).

Flow Injection Analysis: Flow injection analysis was used to test the detection limits of the in-channel conductivity detector. The flow injection system consisted of a pneumatic actuator (Rheodyne Inc. Model 5701), a four-way rotary valve (Rheodyne Inc. Type 50), a Yale apparatus multi-phase syringe pump (Model YA-12), a Hamilton valve (Alltech Inc.), a solenoid valve kit (Rheodyne Model 7163), and a portable air tank at 60 psi. Flow injection methods were chosen to limit the interference and noise that would otherwise be associated with an electrical switch or motor driven valve.

Test electrode fabrication: A gold wire electrode was built in order to use the in-channel conductivity detector with the flow injection system. The purpose of the electrode was to test the DC output signal of the detector with various concentrations of various electrolytes. Two lengths (1 cm) of gold wire (0.1mm diameter) were used as the cathode and anode. A glass microscope slide was carefully scored and broken into halves to serve as a housing for the electrode. An epoxy solution (13 TETA :100 EPON 828) was prepared and applied to one side of the slides. The gold wires needed to be electrically isolated by a thin layer of non-conductive material. Polyethylene terephthalate (PET) film was chosen because its thickness was 6μm and provided an electrical isolation. Each gold wire was folded into a piece of PET film (1cm x .5cm) leaving 0.5 cm of gold wire extending from the film. The gold wires were positioned so that the gold wires crossed at only one point. Then the film and wires were then "sandwiched" between the glass slides with epoxy between all layers and clipped with a binder clip to hold the layers in place. The electrode was allowed to cure for 2 hours at 85°C. After the electrode cured, 20-gauge wire was soldered to the extensions of the gold wire and covered with hot-glue for durability. The electrode was ground down using a sanding wheel with 220 grit wet/dry sandpaper (used wet). The electrode was ground until the distance between the gold wires was near 60 μm. The electrode was then carefully ground with 1200 grit silicon carbide wet/dry paper until the distance was 43 μm. The distance was chosen based on the size of the electrodes in the microchips. The resistance of the electrode was tested using a multimeter to ensure isolation of the gold wires. The electrode was then mounted into the flow injection system in order to measure the conductivity of the solution. The electrode was clamped into a tabletop-soldering vise to ensure stability. The output tube from the flow injection system was mounted to allow the solution to come into contact with the electrode. Both the electrode and output tube were positioned horizontally.

Running buffers and samples: An initial run using Nanopure water was carried out to test the flow injection and electrode system. A sample of Nanopure water was injected into the sample

loop to test for a change in signal from the detector. A series of dilutions were prepared from a 1M potassium chloride (KCl) stock solution. A volume of 1.5 mL of each concentration, beginning with the least concentrated, was injected into the sample loop using water as a "run" buffer. The peak signal was recorded directly from the detector output display.

A strip chart recorder (*LINEAR* model) was the principal means to record the output signal from the detector during flow injection analysis. The strip chart recorder proved to be much more accurate and consistent than the display on the detector. A baseline signal was recorded while the system ran and the sample was injected. Initially, three subsequent injections were run for each concentration with water as the run buffer. Various concentrations of KCl run buffer were used in order to find the optimal concentration of electrolytic solutions to be detected. A series of dilutions ranging from 2.5μM to 250μM KCl were run with distilled water as the buffer. The solutions were diluted each time by a factor of ten and were run from the lowest concentration to the highest.

After many runs using water as the run buffer, KCl solutions were used. A 1mM KCl solution was chosen as the run buffer with sample injections of KCl solutions with concentrations above and below 1mM. Signals from concentrations of 1.5mM, 1.1mM, 1.01mM, 1.025mM, and 0.5mM. The experiment was repeated using solutions with concentrations near the concentration of the run buffer. The solutions included 1.4mM, 1.19mM, 1.095mM, 0.99mM, 0.98mM, and 0.96mM.

Samples were prepared and ran in various buffer solutions. The buffers of choice were MES (MES, pK_a= 6.1) and TES (TES, pK_a= 7.5). Buffer solutions of TES and MES were prepared with concentrations of 25mM while the pH of TES was adjusted with 5M NaOH to 7.0, and the pH of MES was adjusted to 5.5.

Serial dilutions of NaH_2PO_4 were prepared in TES with concentrations of 10mM, 15mM, and 20mM. Various concentrations of KNO_3 were prepared in 25mM MES at pH 5.5 to test various analytes in different buffers. Concentrations of all solutions were varied between 1mM and 0.1μM.

MICROCHIP CE: Previously published fabrication and separation techniques were employed in the experiments reported this paper (Liu et al. 2000). The microchips are glass plates with gold electrodes (<100 um wide) deposited and patterned by photolithography. The separation channels are molded into a layer of poly(dimethyl siloxane) (PDMS) using silicon molds. The PDMS layer is positioned on the microchip to allow the electrode to be in-channel, hence the in-channel conductivity detection as shown schematically in Figure 1. Electrical contact to the working electrode was made through microfabricated contact pads on the glass substrate. Samples were injected and separated by previously published methods (Liu et al. 2000). Injection times for microchip CE ranged from only seconds to five seconds.

Initial solutions of hydroquinone (HQ) were made in 25mM TES at pH 7. HQ solutions were made fresh daily because HQ is unstable at pH 7. The output from the conductivity detector was acquired through the use of an analog-to-digital converter controlled by a data acquisition program (Chrom Perfect: Jansen Inc.). Electropherograms were obtained at 600, 900, 1200, and 1500 volts for the separation voltage. NaH_2PO_4 solutions were separated and detected using the same methods prescribed for hydroquinone.

RESULTS AND DISCUSSION:

Flow injection experiments were repeated until enough data was collected to infer a linear relationship between detector signal and concentration. Concentrations less than 10μM appeared to be below the limit of quantitation, but concentrations as low as 2.5μM could be detected using the conductivity detector coupled with the flow injection system. A linear correlation between detector signal and concentration was established using KCl as the analyte proving that conductivity can be quantitatively used as a detection method with electrolytic solutions (Figure 2). Potassium chloride was chosen as an analyte because the electrolytic conductivity of KCl has been well established. A correlation between dc signal output from the detector and the actual conductivity of the solution can be established.

The detector demonstrated an increase in output signal with an injection of KCl when using conventional polarity. The polarity could be

changed and the detector showed a negative change in signal from the detector. This allowed the detection of increased and decreased concentrations with respect to the run buffer. The detector gave an overload signal for concentrations greater than 0.5 M and concentrations above 0.2M increased in a non-linear fashion, most likely due to ion interactions at higher concentrations.

Once the detector had been evaluated using the flow injection system, the microchip CE system was constructed and characterized. Hydroquinone solutions were successfully detected yielding data that can establish the electroosmotic flow. Hydroquinone was chosen as the initial characterization species as it has been studied extensively in our laboratory using amperometric detection with similar microchip systems (Liu et al 2000). The representative electropherogram shown in Figure 3 matches the expected response for the test system. This indicates that the microchip is performing as expected and the conductivity detector has not altered any of the flow properties. This is significant as it allows further testing of phosphate and nitrate systems with no worry of anomalous behavior as a result of the detector.

Solutions of NaH_2PO_4 were separated to evaluate the use of microchip CE with conductivity detection for analysis of inorganic ions. Na^+ is expected to appear in the electropherogram before any of the phosphates species due its higher mobility in the positive electric field. Multiple peaks are expected from phosphate, as it can exist as $H_2PO_4^-$ and HPO_4^{2-} under the solution conditions of the experiment. A representative electropherogram is given in Figure 4. Na^+ gave a very strong signal with faster retention times than the HQ due to the positive charge. Two phosphate signals were seen corresponding to the two expected species. Various concentrations of sodium phosphate were prepared and ran but only three other concentrations of 1.9mM, 190μM, and 50 μM were successful due to faults in the electrodes and microchips. New chips were needed in order to quantify and determine a limit of detection for electrolytic solutions.

Previous publications have reported a limit of detection of sodium in microchip CE with conductivity detection to be 0.43mM. Concentrations of 100uM have successfully been detected in these experiments. It can be inferred that the LOD will be orders of magnitudes smaller than previous experiments with conductivity detection for microchip CE.

CONCLUSION:

Capillary electrophoresis is a standard analytical technique used for separations of molecules based on charge-to-mass ratio. Recently, microchip applications have been successful in separations of amino acids, DNA restriction fragments, PCR products, and sequencing ladders (Abramowitz 1999). New detection methods are needed that provide universal detection of ionic species and can be integrated on the chip. The conductivity detector used in these experiments proves is very sensitive as compared to previous microchip CE experiments. Conductivity detection has the potential to be integrated into a small unit with automation of the microchip and requires no derivatization. Conductivity detection can potentially provide a universal detection method that is cost efficient, portable, and disposable. The goal of this project was to test a novel in-channel conductivity detector for microchip CE and to prove that conductivity detection can be successful in microchip CE. While no LOD or quantification was established in these experiments, working concentrations of 100 uM and less were successfully detected proving the applicability and sensitivity of the conductivity measurements to microchip capillary electrophoresis. This report establishes the initial feasibility of coupling conductivity to microchip CE. Future work will focus on improving the consistency of the response, developing more complex separations, and characterizing real world water samples.

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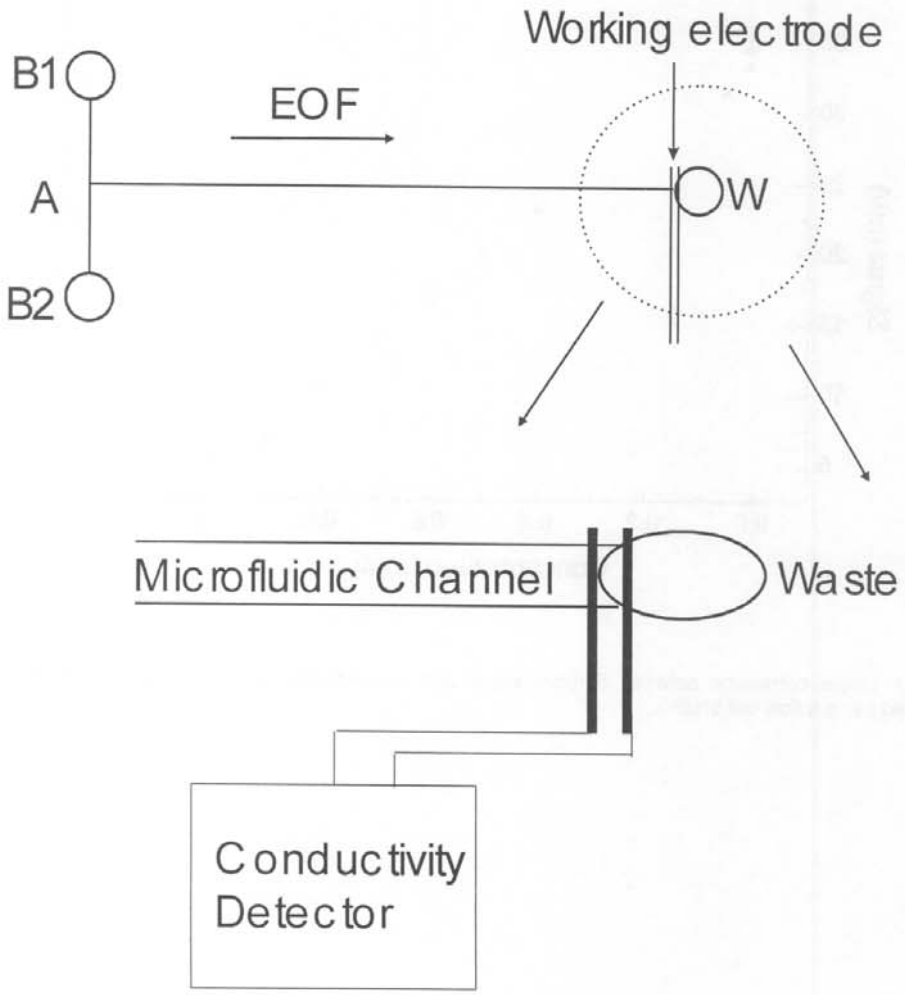

Figure 1: Schematic drawing of the microchip CE-conductivity system. Drawing not to scale.

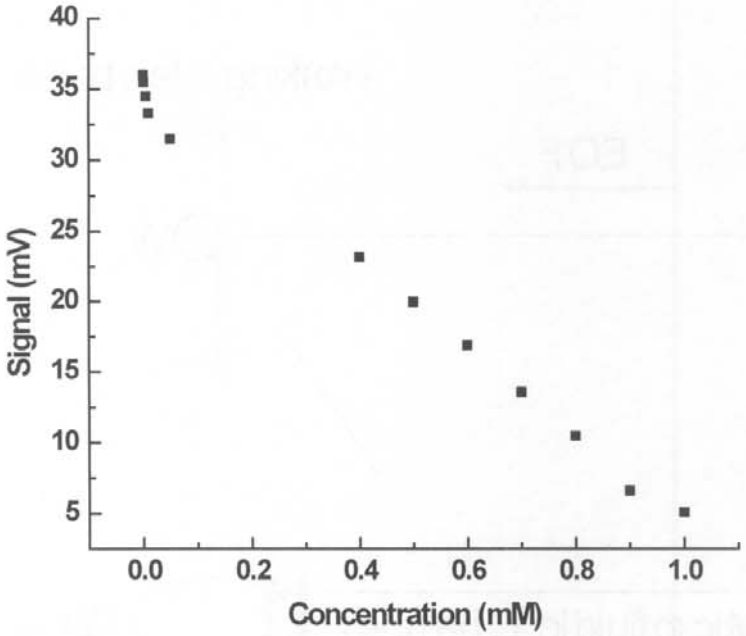

Figure 2: Linear correlation between detector signal and concentration of KCl in the solution. Test performed using a flow cell system.

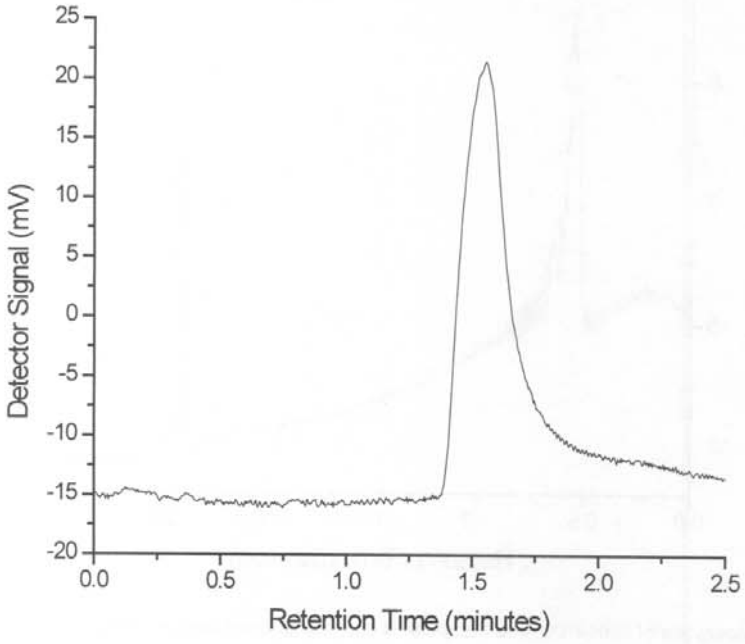

Figure 3: Electropherogram of hydroquinone. Hydroquinone was chosen as the initial marker to test the performance of the system.

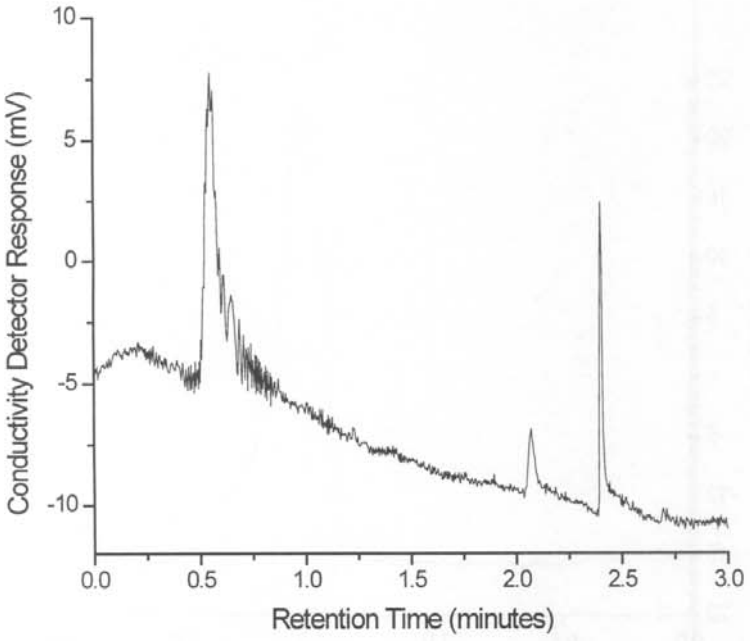

Figure 4: Separation of NaH_2PO_4. The first peak is Na^+, the second peak is $H_2PO_4^-$, and the third peak is HPO_4^{2-}.