Soil Cation Analysis Using High-Performance
Capillary Zone Electrophoresis
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Introduction:

Capillary electrophoresis (CE) is a relatively new, but rapidly growing separation technique that has numerous applications for chemical and biochemical analysis. Capillary electrophoresis techniques separate molecules or ions in a number of ways depending on the type of capillary and buffer solution which are used. Capillary zone electrophoresis (CZE) is the most common mode of CE and separates mixtures based on the overall mobility of analytes in solution under the influence of an electric field. A schematic of a basic capillary electrophoresis experiment is shown in Figure 1. When a high voltage is applied between the anode and cathode, ionic species in solution will begin to migrate based on their mass and charge, with cations migrating to the cathode, and anions to the anode. The tendency for species to move in such a field is called the *electrophoretic mobility (μ_{ep})*. In general small, multiply charged ions have a larger μ_{ep} than larger, less charged species, with neutral molecules possessing negligible μ_{ep}. In parallel with elecrophoretic movement is a net motion of all species in solution toward the cathode as a result of *electroosmosis*. The presence of an electrical double-layer on the walls of the capillary aids in the general motion of solvent (often containing water) toward the cathode compartment. As a result of interactions with this moving solvent, solutes are also drug toward the cathode. In CZE, the separation is the result of the combination of electrophoretic and electroosmotic flow.
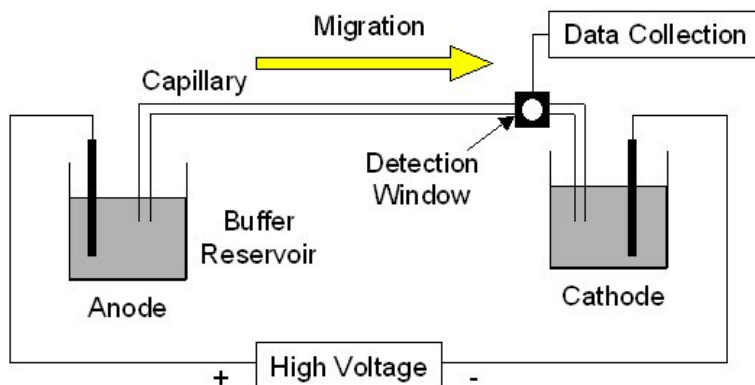

Figure 1

As in any separations technique, the separation step must be coupled with an appropriate detector to convert the presence of components eluting from the separation into an analytically useful signal. Several kinds of detectors are available for use with CE, including optical detectors involving UV and Visible absorbance or fluorescence, electrochemical detectors, and mass spectrometers. Further description of the modes of CE separation and CE instrument components can be found in your textbook.

Experiment Scenario:

In this experiment, six different cations (Ca^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+, and Mn^{2+}) in a soil sample will be detected simultaneously by indirect absorption HPCE method. The instrument employed is a Beckman P/ACE 5500 capillary electrophoresis system with an UV absorption detector operating at 214 nm. Detection occurs indirectly by using a background electrolyte (BGE) of imidazole/H_2SO_4/HCl/ 18-crown-6(ether). Imidazole absorbs strongly in UV region (max. 214 nm). Since the analyzed cations do not absorb at this wavelength, the absorbance decreases when packets of these cations flow past the detector. After the cations pass, the absorbance rises back to its original position creating a negative peak. The area of each peak is proportional to the amount of cations in the sample. The BGE also includes 18-crown-6 ether for separating K and NH_4^+ that normally have the same migration time. A typical electropherogram is shown in Figure 2.
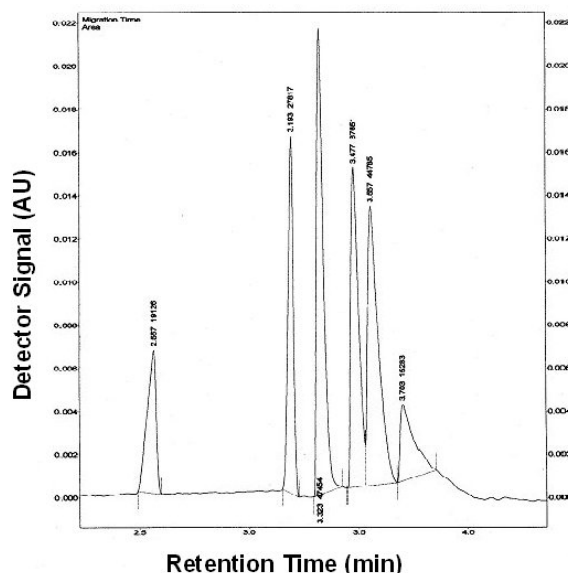

Figure 2.

Safety Precautions:

- 18-crown-6-ether and imidazole are toxic. Be cautious to avoid contacting these chemicals and their solutions on your skin. The waste of these two chemicals should be put in a hazardous waste container
- High voltage is used during the CE separation. During a run, take care not to open the autosampler cover.

Instrument, Apparatus, and Chemicals:

- Beckman P/ACE 5500 System with UV Detector
- Separation capillary
- Degas and filtration apparatus
- Hot-stir plate and stir bar
- Centrifuge and tubes
- pH meter
- 25-ml volumetric flask
- 100-ml beaker
- Syringe and syringe filter
- Micropipets and tips
- Soil sample
- HNO_3, H_2SO_4, HCl. NaOH, KNO_3, NH_4NO_3, $NaNO_3$, $Mg(NO_3)_2$, $Mn(NO_3)_2$, $Ca(NO_3)_2$
- imidazole, 18-crown-6(ether)

Experimental Procedure:

The instrument must be turned on and allowed to warm up for about 30 minutes. You may start to prepare and run your standards during soil sample preparation. Plan accordingly so that you can finish the experiment on time.

Background Electrolyte Preparation:

About 0.272g imidazole and 0.053g 18-crown-6(ether) are dissolved in about 150ml DI water. The pH of this solution first is adjusted to 7.2 with 5% H_2SO_4, then to 6.0 with 0.1M HCl. The final volume is brought to 200ml with DI water (imidazole concentration is 20mM, 18-crown-6(ether) concentration is 1 mM). Filter and degas this solution before using for HPCE.

Soil Sample Preparation:

1. In a 100-ml beaker, accurately weigh out about 1 - 1.5g of soil (make sure to record the exact mass).
2. In a hood, add 10 ml of concentrated nitric acid to the soil sample and stir the mixture well
3. Put this beaker on a stirring hot plate in the hood. Heat the solution slowly with stirring until boiling. Continue to heat with stirring to boil off excess nitric acid. The nitric acid should be boiled off completely in about 20 min. Remove the beaker from the hot plate and allow it to cool. (This step is critical for your sample separation. You need to make sure that you get rid of all the HNO_3 in the sample.)
4. Add about 5 ml of 0.01 M HCl in the sample. Mix well and transfer the mixture into a centrifuge tube. Wash the beaker twice with 5ml of 0.01M HCl each time and combine the washes with the sample in the centrifuge tube.
5. Centrifuge for 5 min (make sure to use second centrifuge tube with an equal amount of water as balance).
6. Transfer all the supernatant to a beaker, add about 5ml of 0.01M HCl to the residue, mix well and centrifuge again. Combine all the supernatant and filter the supernatant with a syringe filter into a 25-ml volumetric flask. Bring to 25ml with DI water.

Standard Preparation:

1. Make standard stock solutions: standard stock solution of each cation is prepared from its nitrate salt. Standard aqueous stock solutions should be prepared with following concentrations: Ca^{2+} 100mM, Na^+ 50mM, K^+ 50mM, Mn^{2+} 50mM, Mg^{2+} 50mM, and NH_4^+ 10mM.
2. Make standard mixture A: contains 1 mM of each cation from the standard stock solutions. (Total of 1ml in a microcentrifuge tube is recommended)
3. Make standard mixture B: contains 10mM Ca^{2+}, 5mM Na^+, 5mM K^+, 5mM Mn^{2+}, 5mM Mg^{2+}, and 1mM NH_4^+ (Total of 1ml in a microcentrifuge tube is recommended)

HPCE Instrument Operation:

1. Turn on the nitrogen gas cylinder and adjust the outlet pressure to 80 psi.
2. Turn on the computer and the HPCE instrument.
3. Double-click the Pace Station icon, click *OK* to get the Beckman Menu
4. Click "Instrument Ito get in the operation screen.
5. From "file" drop down list, choose 'open method" and click "INST.MET" method.

6. From "Method" drop down list, click "Time program" to open the time program screen. The time program should shown as below:

#	Time	Function	Value	Duration	Inlet Vial	Outlet Vial	Summary
1		Rinse		2.00 min	33	10	fwd high
2		Inject-Pressure		5.00 sec	21	10	
3	0:00	Separate-Voltage	28 kV	4.00 min	11	1	0.2 ramp time
4	4:00	Stop Data					
5	4:00	Rinse		1.00 min	31	10	fwd high
6	5:00	Rinse		1.00 min	32	10	fwd high
7	6:00	End					

- Make changes to the time program if necessary.
- From this window, check to make sure capillary temperature is 15°C, detector is UV detector, detection wavelength is 214nm, and signal is indirect. Otherwise, click "change" to modify the parameters. By using "indirect" signal, the negative peaks will be flipped to positive peaks so that the integration of the peak areas will be automatically done.

7. Print the time program for your records.
8. Close the time program window after saving any changes to the program.
9. Filling Vials: Press "Vial down" key in frond panel of the instrument to lower the vials from the capillary. Press "inlet tray" or "outlet tray" to rotate the autosampler trays in order to access the vials. Fill the vials up to the neck and arrange the vials in the autosampler tray based on your time program. Recommended locations: Running buffer in #1, #11, and #33; 0.1 N NaOH in #31; DI. water in #32; Waste vial in #10; Sample in #21. The sample is contained in a 0 6-ml microcentrifuge tube (top part of the vial must be cut off) and placed in the special vial with a spring.
10. Run standard or sample by click "Run single" icon. Wait until the run finishes.
11. Press "Ctrl" and "Z" keys simultaneously to autoscale your electropherogram. You may also use click and drag to change the scale.
12. Under "Analysis" drop down list, click "analyze" to integrate the electropherogram.
13. Print "electropherogram channel A" to get print out of the electropherogram

   To save time, is recommended that you process your data using "Offline Method edit" or "Instrument 2"

Peak Identification:

- In this section, you will run a standard mixture contains six cations. Then two of six peaks will be identified by adding a standard cation into the mixture one by one.

1. Inject standard mixture A. You should get an electropherogram with all six peaks.
2. Choose one of the cation standard stock solutions, add small volume (about 10% of the volume of standard mixture A) of this stock solution into standard mixture A. Mix well and inject for CE. Use the results of this run and that of the unspiked run to identify the peak corresponding to the spiked cation.
3. Choose second cation standard stock solution and repeat step 2.
4. After identifying your two cations, ask your instructor for confirmation and for other peak identification.

Analysis of Soil Sample by Standard Addition:

1. Inject your soil sample and get an electropherogram.
2. Mix small volume (about $20\mu L$) of standard solution B with your soil sample (about $200\mu L$). Make sure to record the exact volume of standard solution B you used and the volume of soil sample you used (Why?). Inject this mixture for HPCE analysis.
3. If Ca^{2+} peak in your soil sample is too big to be analyzed, you might need to dilute your soil sample and repeat the steps 1 and 2 to detect Ca^{2+}. In this step, you need to use only appropriate concentration of Ca^{2+} standard for quantifying the Ca^{2+} in your soil sample since other ions have been analyzed in above steps.

Data Analysis and Lab Report:

1. Make a table to show all the peak areas for soil sample before standard addition and after standard addition.
2. Calculate all the cation concentrations in the soil sample (in ppm) based on the single standard addition equation you learned in class.
3. Attach all the electropherograms you obtained in this experiment to your notebook, and make sure to label all the figures.
4. Discuss possible errors for the HPCE method for soil analysis and suggest any approaches to increase the accuracy of the method.