Determination of Benzalkonium Salts in Household Cleaners
Using High-Performance Capillary Zone Electrophoresis

Adapted from: Gardner, W.P., Girard, J.E. *J. Chem. Ed.*, **2000**, *77*, 1335-1338.

Introduction:

Capillary electrophoresis (CE) is a 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.

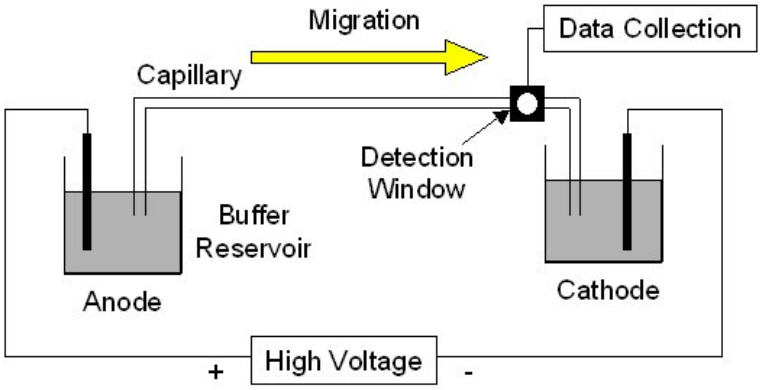


Figure 1

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:

Many popular household cleaning products contain alkyl benzyl dimethyl ammonium (BAK) salts (generic structure shown in Figure 2) as active ingredients. These compounds serve to act as antimicrobial agents and surfactants.

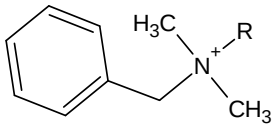
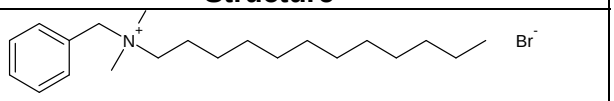
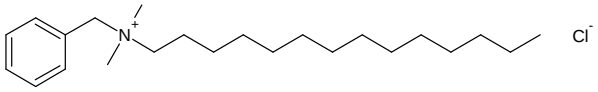
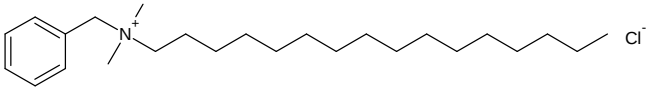
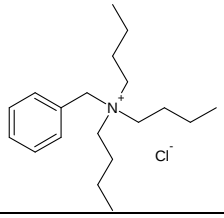


Figure 2

In this experiment you will utilize CE in order to quantify the three BAK compounds below in a household product. One of the key challenges in the determination of BAK compounds is their surfactant character. If the concentration of these compounds is large enough, the BAK compounds aggregate to form micelles, spherical clusters of molecules with a hydrophobic core and a hydrophyllic surface. The presence of micelles leads to diminished separation quality. In order to minimize (and hopefully eliminate) the formation of micelles, an organic solvent will be added to the CE buffer. The presence of this solvent helps to solubilize the BAK compounds, making micelle formation less favorable. You will also utilize an internal standard (BTK) to help optimize the precision of your separation method.

Compound	Structure
Dodecyl benzyl dimethyl ammonium bromide (C_{12}-BAK)	
Tetradecyl benzyl dimethyl ammonium chloride (C_{14}-BAK)	
Hexadecyl benzyl dimethyl ammonium chloride (C_{16}-BAK)	
Benzyl tributyl ammonium chloride (BTK)	

Safety Precautions:

- High voltage is used during the CE separation. During a run, take care not to open the autosampler cover.

Instrument, Apparatus, and Chemicals:

- CE System with UV Detector and Separation Capillary
- Degas and filtration apparatus
- Syringe and syringe filter
- Micropipettes and tips
- Sample of commercial household cleaner
- Acetonitrile
- Methanol

- 18 MΩ Water
- pH 3.0 phosphate buffer (200 mM phosphate). This buffer is prepared by adding 6.8 mL of concentrated phosphoric acid to 300 mL of DDI water, adjusting to pH 3.0 with 6M NaOH and diluting to a final volume of 500 mL with DDI water.
- Benzyl tributyl ammonium chloride (BTK)
- Dodecyl benzyl dimethyl ammonium bromide (C_{12}-BAK)
- Tetradecyl benzyl dimethyl ammonium chloride (C_{14}-BAK)
- Hexadecyl benzyl dimethyl ammonium chloride (C_{16}-BAK)

Experimental Procedure:

The instrument must be turned on and allowed to warm up for about 30 minutes.

Background Electrolyte Preparation:

Prepare 50 mM phosphate buffer in 50% acetonitrile by diluting 12.5 mL of 200 mM phosphate buffer and 25 mL acetonitrile to 50 mL with deionized water. Filter and degas the buffer before use.

Standard Preparation:

1. Concentrated standard: In a 25 mL volumetric flask, accurately weigh and dilute ~25 mg each of C_{12}-BAK, C_{14}-BAK and C_{16}-BAK with 60:40 (%v/v) methanol:water. Calculate the concentration in ppm of each of the BAK compounds in the solution.
2. Concentrated internal standard: Accurately weigh ~20 mg of BTK into a 50 mL volumetric flask and dilute with 60:40 methanol:water. Calculate the concentration in ppm of BTK in this solution.
3. Diluted standards: Using micropipettes, transfer 50, 150, 250, 500, and 800 μL of concentrated BAK standard to separate 10 mL volumetric flasks. Add 1 mL of the concentrated internal standard to each flask. Dilute each flask to the mark with 60:40 methanol:water. Calculate the concentration of BTK and each BAK in each standard.

Sample Preparation:

1. Based on the information provided on the product label, calculate the volume of cleaning solution required to prepare 10.0 mL of a solution whose BAK concentration falls near the middle of your calibration range. Pipet this volume of solution into a 10.0 mL volumetric flask, add 1.0 mL of concentrated internal standard and dilute with 60:40 methanol:water. Calculate the BTK concentration in this sample.

   NOTE: If you use the Formula 409 Antibacterial Kitchen Cleaner, the product label reads as follows:

Active Ingredients:	
Akyl (C_{12} 40%, C_{14} 50%, C_{16} 10%) dimethyl benzyl ammonium chloride	0.3%
Other Ingredients	99.7%
Total Ingredients	100.0%

2. Repeat for additional samples.

HPCE Instrument operation:

1. Follow appropriate procedures for powering up the instrument and loading sample and buffer solutions. This is typically described in the Standard Operating Procedure (SOP) for the instrument.
2. Program the instrument so that the following steps are executed.

#	Time	Function	Value	Duration	Inlet Vial	Outlet Vial	Summary
1		Rinse		2.0 min	buffer	waste	fwd high
2		Inject-Pressure		5.00 sec	sample	waste	
3	0:00	Separate-Voltage	20 kV	11.0 min	buffer	buffer	0.2 ramp time
4	1:00	Auto Zero					
5	11:00	Stop Data					
6	11:00	Rinse		0.5 min	water	waste	fwd high
7	11:30	Rinse		1.0 min	NaOH	waste	fwd high
8	12:30	Rinse		0.5 min	water	waste	fwd high
9	13:00	End					

   - Make changes to the time program if necessary. A 20 kV separation potential works well for a 75 um x ~40 cm capillary. Under these conditions, your separations should be complete in less than 10 minutes. Consult your instructor for a reasonable potential for other capillary dimensions. You should optimize separation time so that data the separation terminates roughly one minute after the final BAK peak emerges. *This may be as short as three minutes depending on capillary and conditions.* Once you have optimized your separation time, adjust the duration of the Voltage step and the timing of subsequent steps appropriately.
   - From this window, check to make sure capillary temperature and detection parameters are appropriate for your separation. The default settings are typically reasonable.
3. Print the time program for your records and close the time program window after saving any changes to the program.
4. Run standard or sample by click “Run” icon. Wait until the run finishes.
5. Record the migration time and peak area and/or peak height for each component.
6. Print at least one representative electropherogram.

Analysis:

1. Analyze each of the five diluted standards and all diluted samples. Run triplicate analysis on at least one of the diluted samples.

Data Analysis and Lab Report:

1. For each BAK in each electropherogram, calculate the ratio of peak area to the area of the BTK peak in the electropherogram.
2. Construct a calibration curve by plotting the peak area ratios determined above as a function of the BAK:BTK concentration ration in each standard.
3. Calculate all the BAK concentrations in your diluted samples (in ppm) and in the original sample based on your calibration curve.
4. Attach at least one example of the electropherograms you obtained in this experiment to your notebook, and make sure to label all the figures.
5. Discuss possible errors for the HPCE method and suggest any approaches to increase the accuracy of the method.