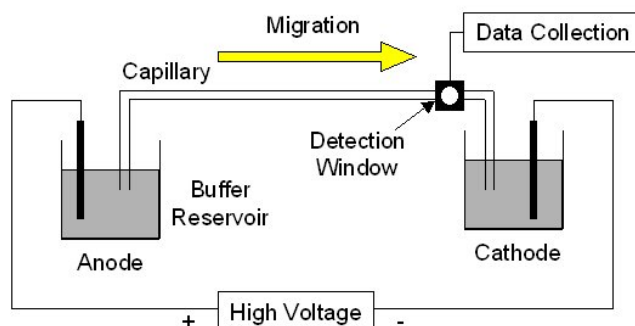


## Determination of Analgesic Formulations Using High-Performance Capillary Zone Electrophoresis

Adapted from: Thompson, L., Veening, H., and Strein, T.G. *J. Chem. Ed.*, 1997, 74, 1117-1121.

### 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.



**Figure 1:** Basic capillary electrophoresis instrumentation.

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* ( $\mu_{ep}$ ). In general small, multiply charged ions have a larger  $\mu_{ep}$  than larger, less charged species, with neutral molecules possessing negligible  $\mu_{ep}$ . In parallel with electrophoretic 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.

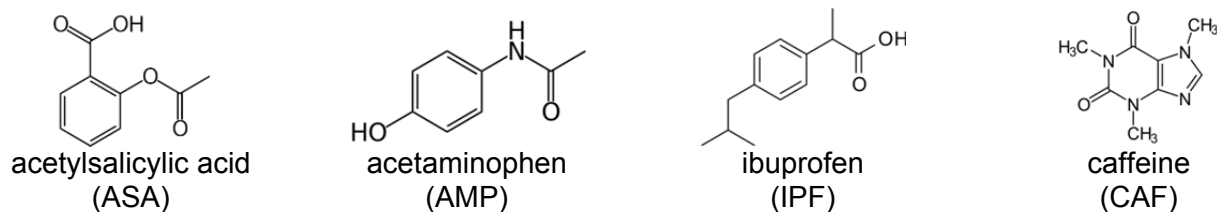
In CZE, the velocity with which a component migrates through the capillary is a function of (1) the applied electric field, (2) the electrophoretic mobility (3) the influence of the electric field on the movement of bulk solution, known as the electroosmotic mobility,  $\mu_{eo}$ . The combination of these factors leads to a characteristic migration time,  $t_m$ , for individual analytes. This relationship is shown in equation 1, where  $L_d$  and  $L_t$  are the length of the capillary from the inlet to the detector ( $L_d$ ) and from the inlet to the outlet ( $L_t$ ).

$$t_m = \frac{L_d L_t}{(\mu_{eo} + \mu_{ep})V} \quad (1)$$

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:

Over-the-counter analgesics contain a variety of active ingredients in a variety of formulations. Ingredients include compounds such as acetylsalicylic acid, acetaminophen, ibuprofen and caffeine, whose structures are shown in Figure 2.



**Figure 2:** Common components of analgesic formulations.

In this experiment you will utilize CE in order to quantify several components in a commercially available analgesic formulation. You will also utilize an internal standard, *p*-aminobenzoic acid (PABA) to help optimize the precision of your separation method.

## 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
- 18 M $\Omega$  ultrapure water
- pH 9.0 borate buffer (15 mM tetraborate)
- acetaminophen (AMP)
- acetylsalicylic acid (ASA)
- salicylic acid (SA) – included because it appears as a degradation product of ASA
- caffeine (CAF)
- *p*-aminobenzoic acid (PABA)
- Sample of commercial analgesic

## Experimental Procedure:

### Buffer Preparation:

1. Electrophoresis buffer is prepared by dissolving 1.43 g sodium tetraborate decahydrate to 200 mL in DDI water, adjusting to pH 9.0 with dilute HCl and diluting to a final volume of 250 mL with DDI water.
2. A small volume (10 mL) of the borate buffer should be filtered and degassed prior to use.

### Standard Preparation:

1. Concentrated standard A: In a 50 mL volumetric flask, accurately weigh to 0.1 mg and dilute ~25 mg each of AMP and CAF and 15 mg of SA with ultrapure water. Calculate the concentration in ppm of each of the compounds in the solution. This solution can be stored in the refrigerator.

2. Concentrated standard B: In a 50 mL volumetric flask, accurately weigh and dilute ~25 mg of ASA with ultrapure water. Calculate the ASA concentration in ppm in the solution. Because ASA hydrolyzes in solution, this solution, and any solutions made from it should be made fresh each day.
3. Concentrated internal standard: Accurately weigh ~50 mg of PABA into a 50 mL volumetric flask and dilute with ultrapure water. Calculate the concentration in ppm of PABA in this solution.
4. Diluted standards: Using volumetric and/or micropipettes, transfer 200, 500, 1000, 1500, and 2000  $\mu\text{L}$  each of concentrated standard A and concentrated standard B to five separate 5 mL volumetric flasks. Add 1000  $\mu\text{L}$  of the concentrated internal standard to each flask. Dilute each flask to the mark with ultrapure water. Calculate the concentration of each compound in each standard.

#### Sample Preparation:

1. Acquire an analgesic tablet and determine its mass to the nearest 0.1 mg.
2. Using a clean mortar and pestle, carefully crush the tablet and quantitatively transfer it to a 500 mL volumetric flask. Dissolve the tablet in ultrapure water and dilute to the mark.
3. Pipet 1 mL of the tablet solution into a 5 mL volumetric flask. Add 1 mL of concentrated internal standard and dilute to the mark with ultrapure water.
4. Filter a small volume (<2 mL) of the homogeneous solution through a 0.45  $\mu\text{m}$  syringe filter into a microcentrifuge tube prior to analysis.
5. Repeat for additional samples if necessary.

#### 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	6.0 min	buffer	buffer	0.2 ramp time
4	1:00	Auto Zero					
5	6:00	Stop Data					
6	6:00	Rinse		0.5 min	water	waste	fwd high
7	6:30	Rinse		1.0 min	NaOH	waste	fwd high
8	7:30	Rinse		0.5 min	water	waste	fwd high
9	8:00	End					

- Make changes to the time program if necessary. A 20 kV separation potential works well for a 75  $\mu\text{m}$  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. To maximize your lab time, you should optimize separation time so that data the separation terminates roughly one minute after the final 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.
- Ensure capillary temperature and detection parameters are appropriate for your

- separation. The default settings are typically reasonable.
3. Document the time program for your records and close the time program window after saving any changes to the program.

#### Analysis:

1. Analyze each of the five diluted standards and all diluted samples. Run triplicate analysis on at least one of the diluted samples.
2. Run standard or sample by click "Run" icon. Wait until the run finishes.
3. Select a detection wavelength that provides optimum sensitivity and record the peak areas and migration times for all components.

#### **Data Analysis and Lab Report:**

1. For each component in each electropherogram, calculate the ratio of peak area to the area of the internal standard peak in the electropherogram.
2. Construct a calibration curve by plotting the peak area ratios determined above as a function of the concentration of each compound in each standard.
3. Calculate all the concentrations in your diluted sample (in ppm) and mass of each component in the original analgesic sample based on your calibration curve.
4. For one of the electropherograms, determine the electrophoretic mobility ( $\mu_{ep}$ ) for each peak. It is reasonable to assume that the electroosmotic mobility ( $\mu_{eo}$ ) is the same for each compound and that the  $\mu_{ep}$  for caffeine is zero since it is uncharged under our separation conditions.
5. Attach at least one example electropherogram you obtained in this experiment to your notebook, and make sure to label all the figures.
6. Discuss possible errors for the HPCE method and suggest any approaches to increase the accuracy of the method.

#### **References:**

Thompson, L., Veening, H., and Strein, T.G. *J. Chem. Ed.*, **1997**, *74*, 1117-1121.

Thorsten, C. "Chemistry of the Borate-Boric Acid Buffer System". CR Scientific LLC. Web. March 2013. <<http://www.crscientific.com/experiment4.html>>.