Key Distance Learning Module II: Using Capillary Electrophoresis for Qualitative and Quantitative Analysis

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Materials

The buffer, tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), and analytes, atenolol, mesityl oxide, and naproxen may be replaced. Ideally, the lab should include two positively charged analyte and a neutral marker to require the student to use critical thinking and laboratory skills to qualitatively and quantitatively assess a sample. The cations we chose to work with maintained charge at pH 7. We selected TES as the running buffer because it buffers at pH 7 and provides a low background current. You may choose other systems, for example ascorbic acid and tolmetin (negative charge at pH 7). Before asking students to use different chemicals, we recommend you perform the experiments outlined in Learning Module II with your substitute set.

Procedures: The Module

1. Use the run parameters outlined below to obtain triplicate runs of a standard containing both a neutral marker (for example, dimethylformamide or mesityl oxide) and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run. Calculate the mean, standard deviation (SD) of the mean, and relative standard deviation (RSD) of the mean for each parameter. You may use the table below to report your data.

   Preparation of capillary: Assuming this is the first run of the day, the capillary should be flushed as follows: 30-min, 20 psi flush with 0.1 N NaOH, followed by 15-min, 20 psi flush with deionized water, followed by a 30-min, 20-psi flush with running buffer. This flushing protocol is necessary only at the beginning of the day. If exercises 1-6 are completed within a single lab period, repeating this initial capillary flush should not be necessary.
   Sample: 0.65 millimolar (0.005 % v/v) dimethylformamide, 75 micromolar atenolol in 25 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffered at pH 7.0.
   Injection parameters: 1 second injection at 2 psi pressure
   Run conditions: running buffer 25 mM TES pH 7.0, 25 micron i.d. fused silica capillary, 42.8 cm total length, 32.9 cm to detection window, separation voltage: 15 kV, range 0.001, rise time 0.3 seconds, 220 nm

This material is based upon work supported by the National Science Foundation under Grant No. 0307245.

Injection parameters: 1 second injection at 2 psi pressure				
Atenolol	**Trial 1**	**Trial 2**	**Trial 3**	**Mean**
Peak Height	0.3446	0.3738	0.3495	0.35_6
Migration Time (sec)	77.19	77.55	77.43	77.3_9
σ (sec)	0.620	0.610	0.550	
Plates (x 10^3)	15.500	16.162	19.820	$17._2$
Neutral Marker				
Peak Height	0.5154	0.50626	0.49369	0.50_5
Migration Time (sec)	109.3	109.48	109.27	109.3_5
σ (sec)	1.010	1.010	0.9232	
Plates (x 10^3)	11.711	11.750	14.009	$12._5$

2. Use the run parameters outlined below to obtain triplicate runs of a standard containing both a neutral marker and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run. Calculate the mean, SD and RSD for each parameter. You may use the table below to report your data.

   Sample: 0.65 millimolar (0.005 % v/v) dimethylformamide, 75 micromolar atenolol in 25 mM TES pH 7.0.
   Injection parameters: 1 second injection at 4 psi pressure
   Run conditions: running buffer 25 mM TES pH 7.0, 25 micron i.d. fused silica capillary, 42.8 cm total length, 32.9 cm to detection window, separation voltage: 15 kV, range 0.001, rise time 0.3 seconds, 220 nm.

Injection parameters: 1 second injection at 4 psi pressure				
Atenolol	**Trial 1**	**Trial 2**	**Trial 3**	**Mean**
Peak Height	0.33242	0.4253	0.40625	0.38_8
Migration Time (sec)	75.789	76.7	77.285	76.5_9
σ (sec)	1.1813	1.2279	1.1416	
Plates (x 10^3)	4.116	3.902	4.583	$4._2$
Neutral Marker				
Peak Height	0.59611	0.60641	0.58779	0.596_8
Migration Time (sec)	107.07	107.48	108.75	107.7_7
σ (sec)	1.6278	1.8318	1.7917	
Plates (x 10^3)	4.326	3.443	3.684	3.8_2

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3. Use the run parameters outlined below to obtain triplicate runs of a standard containing both a neutral marker and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run. Calculate the mean, SD and RSD for each parameter. You may use the table below to report your data.

Sample: 0.65 millimolar dimethylformamide, 75 micromolar atenolol in 25 mM TES pH 7.0.
Injection parameters: 1 second injection at 1.5 psi pressure
Run conditions: running buffer 25 mM TES pH 7.0, 25 micron i.d. fused silica capillary, 42.8 cm total length, 32.9 cm to detection window, separation voltage: 15 kV, range 0.001, rise time 0.3 seconds, 220 nm

Injection parameters: 1 second injection at 1.5 psi pressure				
Atenolol	**Trial 1**	**Trial 2**	**Trial 3**	**Mean**
Peak Height	0.34328	0.30514	0.31077	0.32_0
Migration Time (sec)	78.266	77.564	78.339	78.0_6
σ (sec)	0.50504	0.45251	0.49427	
Plates (x 10^3)	24.016	29.381	25.120	$26._2$
Neutral Marker				
Peak Height	0.45072	0.40995	0.45533	0.43_9
Migration Time (sec)	110.36	109.69	110.79	110.2_8
σ (sec)	0.82552	0.76076	0.86963	
Plates (x 10^3)	17.872	20.789	16.231	$18._3$

4. Use the run parameters outlined below to obtain triplicate runs of a standard containing both a neutral marker and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run. Calculate the mean, SD and RSD for each parameter. You may use the table below to report your data.

Sample: 0.65 millimolar dimethylformamide, 75 micromolar atenolol in 25 mM TES pH 7.0.
Injection parameters: 2 second injection at 2 psi pressure
Run conditions: running buffer 25 mM TES pH 7.0, 25 micron i.d. fused silica capillary, 42.8 cm total length, 32.9 cm to detection window, separation voltage: 15 kV, range 0.001, rise time 0.3 seconds, 220 nm

Injection parameters: 2 second injection at 2 psi pressure				
Atenolol	**Trial 1**	**Trial 2**	**Trial 3**	**Mean**
Peak Height	0.39943	0.40209	0.40322	0.401_6
Migration Time (sec)	77.054	79.18	78.092	$78._1$
σ (sec)	1.1705	1.1353	1.132	
Plates (x 10^3)	4.334	4.864	4.759	$4._7$
Neutral Marker				
Peak Height	0.62067	0.6374	0.62069	0.626_3
Migration Time (sec)	109.87	111.73	110.4	110.6_7
σ (sec)	1.7754	1.7065	1.8522	
Plates (x 10^3)	3.830	4.287	3.553	3.8_9

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5. Use the run parameters outlined below to obtain triplicate runs of a standard containing both a neutral marker and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run. Calculate the mean, SD and RSD for each parameter. You may use the table below to report your data.
Sample: 0.65 millimolar dimethylformamide, 75 micromolar atenolol in 25 mM TES pH 7.0.
Injection parameters: 4 second injection at 2 psi pressure
Run conditions: running buffer 25 mM TES pH 7.0, 25 micron i.d. fused silica capillary, 42.8 cm total length, 32.9 cm to detection window, separation voltage: 15 kV, range 0.002, rise time 0.3 seconds, 220 nm

Injection parameters: 4 second injection at 2 psi pressure				
Atenolol	**Trial 1**	**Trial 2**	**Trial 3**	**Mean**
Peak Height	0.21345	0.22416	0.2202	0.219_3
Migration Time (sec)	76.682	76.162	75.8220	76.2_2
σ (sec)	2.0975	2.2539	2.2092	
Plates (x 10^3)	1.337	1.142	1.178	$1._2$
Neutral Marker				
Peak Height	0.32948	0.34387	0.34916	0.34_1
Migration Time (sec)	108.39	107.84	107.24	107.8_2
σ (sec)	3.3362	3.5499	3.6671	
Plates (x 10^3)	1.056	0.923	0.855	0.9_4

6. Use the run parameters outlined below to obtain a single run of a standard containing both a neutral marker and atenolol. Report the migration times, peak height, and calculated theoretical plates for the neutral marker and atenolol for each run.
Sample: 0.65 millimolar dimethylformamide, 75 micromolar atenolol in 25 mM TES pH 7.0.
Injection parameters: 2 second injection at 4 kV applied voltage (normal polarity, no pressure!)
Run conditions: running buffer 25 mM TES pH 7.0, separation voltage: 15 kV, range 0.001, rise time 0.3 seconds, 220 nm

2 s, 4 kV	
Atenolol	**Trial 1**
Peak Height	0.21544
Migration Time (sec)	77.304
σ (sec)	0.41192
Plates (x 10^3)	35.219
Neutral Marker	
Peak Height	0.23153
Migration Time (sec)	109.15
σ (sec)	0.64349
Plates (x 10^3)	28.772

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7. Use your findings from the exercises outlined in steps 1-5 to complete the table below. What conclusions can you draw from these studies? What injection protocol is best suited for analyses of 75 micromolar atenolol?

Injection Conditions	4s, 2psi	2s, 2psi	1s, 4psi	1s, 2psi	1s, 1.5psi
Atenolol					
Height, Mean	0.219	0.402	0.39	0.36	0.32
Time (sec), Mean	76.2	78	76.6	77.4	78.1
Plates	1000	5000	4000	17000	26000
Neutral Marker					
Height, Mean	0.34	0.626	0.597	0.50	0.43
Time (sec), Mean	107.8	110.7	107.8	109.4	110.3
Plates	900	3900	3800	12000	18000

The plate count is highest when the total injection volume is low.

8. Independent Excursion: As you can see from this exercise, the injection protocol can have a marked effect on various aspects of the separation. Many other injection protocol, concerns and criteria are important to other applications of capillary electrophoresis. For instance, in addition to hydrodynamic injection (forcing sample on using pressure), injections may also be facilitated by voltage and siphoning. The result produced in step 6 of this lab exercise, might lead you to devise another set of experiment designed to find optimal injection parameters using electrophoretic injection. Difference in the ionic strength of the sample and running buffer can also effect the separation. Below you will find two proposed experiments that might provide insight in these two areas. After you have had time to ponder the experiment performed in steps 1-6, you may attempt the independent experiments below, or design your own experiment.

Injection facilitated by siphoning:

Elevate the anodic buffer reservoir and the separation capillary such that the liquid line (top of vial) and capillary end in are 8 cm higher in the anodic reservoir relative to the cathodic reservoir. Determine the mean value for migration time (n=2) for both a neutral marker and tolmetin. Repeat this exercise with the liquid line (top of vial) and capillary end are at identical heights in both the anodic and cathodic reservoirs.

Determination of analyte in matrix of ionic strength different from running buffer*

Use identical pressure-based injection and run conditions for the samples outlined below. Determine the mean values for migration time, height and theoretical plate count for triplicate runs of each of the solutions listed below. Be sure to report the uncertainty in your answers.

(A) neutral marker and 100 micromolar mesityl oxide, and 300 micromolar tolmetin dissolved in deionized water

(B) neutral marker and 300 micromolar tolmetin dissolved in background electrolyte (25 mM TES pH 7.0)

(C) neutral marker and 300 micromolar tolmetin dissolved in background electrolyte (25 mM TES pH 7.0) that also contains 500 mM sodium chloride

*Note: this exercise may be attempted using voltage-based injections (electrophoretic) or siphoning.

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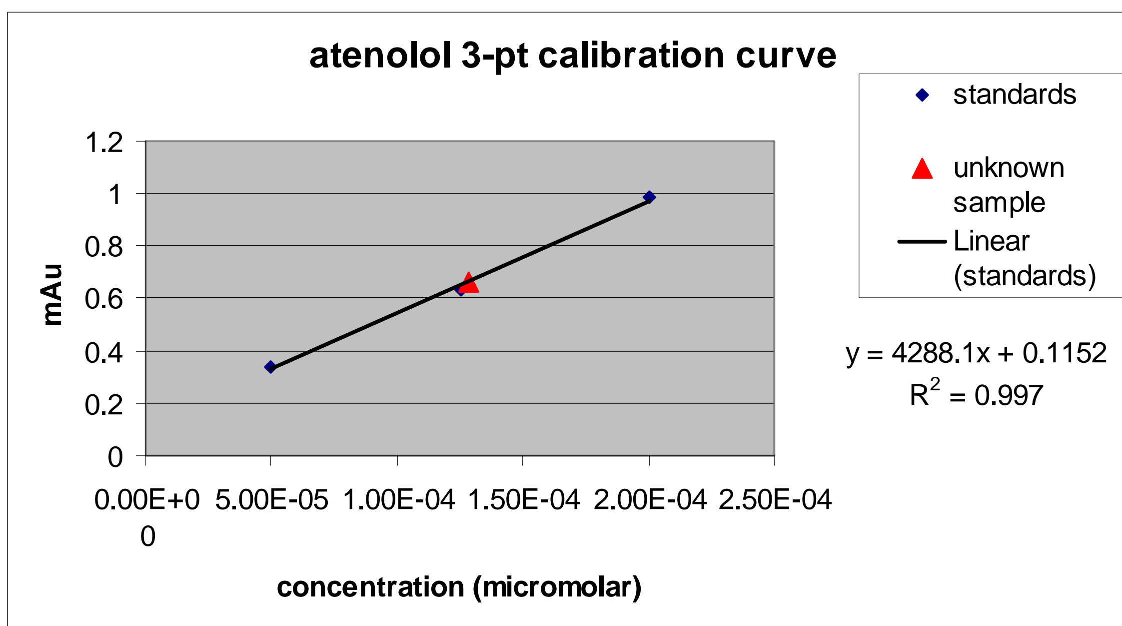
9. Outline the experimental strategy to determine the concentration of atenolol in an aqueous solution.

The concentration of atenolol in an aqueous solution can be determined by using a calibration curve. A calibration curve can be generated using a set of atenolol standards that relates the absorbance versus peak height or absorbance versus peak area. The calibration curve will only be valid for the experimental conditions under which the measurements were made (range, wavelength, rise time, injection time, injection pressure, separation voltage). If one of the parameters is changed, such as the wavelength, then a new calibration curve would have to be generated before measuring the concentration of atenolol in an unknown sample. If the curve is fit using linear regression, then the concentration range must be within the linear range of the method. You may have to determine the linear range of the method before making your first calibration curve. Finally, due to drifting of the lamp source and because the fused silica capillary must be flushed periodically, the measurements of the standards and the unknown sample should occur as timely as possible to prevent the introduction of time-dependent error. Below is a 3-point calibration curve for atenolol. In this case the sample contained 125 micromolar atenolol. Based on the response for the unknown (0.66536 mAu) and error in the calibration curve, the concentration is 130 ± 7 micromolar.



10. Lab Practical

Through a faculty mentor or collaborator, you should arrange to receive an unknown solution containing either naproxen or atenolol diluted in background electrolyte at a concentration above the limit of quantification for your capillary electrophoresis system. Once you receive the unknown solution, you are to determine the analyte composition and concentration in the solution. Your final report should include pertinent data and a clear explanation of your results. When you report your final value, be sure to include uncertainty. You should have access to standard solutions as necessary.

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