**Capillary Electrophoresis Distance Learning Program: Operating Procedures**

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# Operating the Custom-built CE System

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# I. Operating the Custom-built CE System 		Return to Menu

The instructions outlined in this section pertain to the parts, equipment, and software (Igor Pro) described in the Fabricating a Homebuilt CE system. If you use different components or parts, which you are not discouraged from doing, then ignore instructions specific to equipment or parts specified in this document.

## 0. Safety Precautions 		Return to Menu

Below are some general safety precautions for your consideration as you begin the fabrication process for the Home-built CE System and working in a research laboratory.

- Consult the safety guidelines and Chemical Hygiene Plan provided by your Institution before beginning any experiment. The safety guidelines of your home Institution supercede any recommendations outlined here.
- Consult the MSDS and the label prior to using a chemical and adjust your laboratory procedures accordingly.
- Personal protective equipment, such as goggles, safety glasses, laboratory coat or apron, gloves, or a respirator, should be used as appropriate for the hazards involved and as recommended on the label and in the MSDS.
- Use chemical fume hoods as advised in the MSDS.
- Store and handle all chemicals appropriately.
- Do not consume anything in the laboratory.
- Do not smoke, chew gum, or use smokeless tobacco in the laboratory.
- Remove your gloves and thoroughly wash your hands before leaving the laboratory.
- Practical advice regarding use of the high voltage power supply:

  There is potential for electrical shock from the high voltage power supply. Typical currents employed in capillary electrophoresis are less than 100 microamps. According to the OSHA tutorial cited below, AC currents of 1mA result in a tingling sensation. However, the degree of danger of such exposure depends upon: (1) if the skin is wet or dry, (2) if the shock may potentially throw the victim away from the electrical connection (for example into an acid bath behind the researcher), or (3) if the exposed person undergoes muscle contraction that does not allow them to let go of the electrical circuit. See the following website for an OSHA tutorial of the risks of electrical shock.

- We recommend the following precautions to prevent electrical shock or minimize the effects in the event of accidental exposure:

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(1) Implement the interlock safety switch outlined in the assembly protocol to facilitate “guarding by location”.

(2) Turn on the voltage only after closing the interlock box with the integrated interlock switch. Turn off the voltage before you intend to open the Plexiglas box with the integrated interlock switch. In doing this, the circuit will never have the potential to be live when you open the Plexiglas box. Should you ever unsafely open the box with the power supply turned on, the interlock switch is the back-up that will prevent electrical exposure. If you press the interlock switch down with the lid to the Plexiglas open, you are no longer protected from accidental exposure to the high voltage. You may further ensure the safety of the systems by wiring an audible alarm to sound when the interlock switch is closed, completing the electrical circuit. This will supplement the visual indicator created with implementation of the interlock switch (power on green button on the front of the high voltage power supply lights up when the circuit is live).

(3) Check that the interlock switch is fully functional, using a voltmeter to measure resistance, every day prior to using the instrument.

(4) Set the current limiting knob so that the power supply can provide a maximum current of 100 microamperes. Use the voltage limiting knob to adjust the applied voltage as necessary.

(5) Be sure your skin is dry, when you are using the instrument. If you, or the device, are sweating, do not operate the instrument.

## 1. Solution Preparation

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When developing a method for performing Capillary Electrophoresis, multiple solutions (aqueous buffers, background electrolytes, flushing solutions) may be required. Aliquots of each solution should be filtered and degassed prior to use. Filtering the solution removes many particulate contaminants while degassing a sample prevents capillary blockage due to bubble formation. The following is common protocol when preparing solution aliquots for use:

1) Refrigerated solutions need to be warmed to room temperature prior to filtering. It is recommended that filtering is performed with a membrane filter with a pore size not exceeding 0.45μm. Syringe filters work best for small volumes, while vacuum filtratration is more adaptable to large volumes. The aliquot should be filtered into a clean beaker or other clean, suitable glass vessel.

2) The filtered solution may be degassed by one of two methods. The first method involves sonicating the solution for approximately 10 minutes. Sonication may be carried out in a water bath if necessary. One must be careful to avoid sonicating too long, causing the aqueous component to evaporate, thus concentrating the solution. The second method involves placing the filtered solution into a vacuum dessicator and vacuum degassing for approximately two minutes. Once again one must avoid degassing for too long due to evaporation concerns.

3) Once removed from the sonicator/vacuum dessicator, the beaker should be covered to prevent interchange with the air, and stored at room temperature until needed. If the filtered

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solution is sufficiently jostled due to rough transport, it may need to be degassed a second time.

4) This process should be repeated for each solution, every day.

## 2. Cutting a New Capillary

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Since most vendors sell bare-fused silica capillaries on spools, it will be necessary to cut a length of capillary for use with the Homebuilt CE system. Cutting a capillary takes practice; therefore it is recommended that smaller lengths (10-15cm) of capillary are used at first. Also be aware of the potential projectile hazard associated with breaking fused silica capillaroes. It is recommended that protective eyeware be worn while cutting a capillary.

1) Measure the desired length of capillary from the spool. Mark this length on the polyimide coating of the fused-silica capillary using a soft-tip marker.

2) Using a ceramic capillary cutter, score the capillary at the mark using a firm, but even pressure at a 45° angle to the capillary. The scoring should be done with one distinct motion. DO NOT use the capillary cutter to saw the capillary. Once scored, the capillary may be snapped from the spool using a gentle bending pressure.

3) Next, measure and mark the location of the detection window. For use with the Homebuilt CE system, the detection window should be located approximately 8 - 9 cm from one end of the capillary. Commercial instruments may require a different location for the detection window.

4) Bend the capillary into a loop with the detection window at the apex. Using a match or a cigarette lighter on its lowest setting, hold the flame to the region of the detection window until the polyimide glows red and chars (about 1 sec). It is important to practice this step on scrap capillary. The detection window should be approximately 1.5-2mm wide. Larger detection windows will have adverse affects on analyte detection.

5) Wet a disposable wipe with deionized water and GENTLY wipe the soot from the window. The exposed bare-fused silica is very fragile, so avoid touching and bending the capillary at the detection window. The capillary is now ready to be placed into the UV-vis absorbance detector.

## 3. Installing a New Capillary

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The content of this section is specific to the UV-vis absorbance detector and optical flow cell used with Homebuilt CE system. Please refer to "Section 5: Integrating the Detector" and the parts list provided with the standard operating procedure for Fabricating a Homebuilt CE system for additional information. If using a different detector or flow cell, please consult the

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respective user's manuals prior to installing the capillary. The detector should not be on at any time during the installation process.

1) Remove the flow cell cover from the detector. Next remove the flow cell. Place the flow cell on a clean surface with the optical lens facing up. Be careful to avoid damaging the lens.

2) Hold the capillary vertically such that the shorter length of polyimide coating is pointing down. Thread the capillary through the first vespel ferrule. The conical end of the ferrule should point towards the detection window.

3) Slide the capillary into the port located on the top side of the optical flow cell for the UV-visible absorbance detector. The capillary should be seen passing through the central channel of the flow cell.

4) Thread the screw into the flow cell on the same side as the vespel ferrule. As the screw is finger tightened, adjust the capillary so that the detection window is aligned with the optical lens of the flow cell.

5) Thread a second ferrule and a second screw onto the opposite end of the capillary. The ferrule should rest inside the port of the flow cell, with its conical end facing the detection window. Finger tighten the second screw.

6) If the detection window is not in line with the lens of the optical flow cell, it can be adjusted by loosening each screw a half of turn and adjusting the capillary. Once properly adjusted, each screw should be finger tightened such that the capillary does not move under gentle tugging.

Flow Cell (Close-Up)

7) Replace the flow cell cover. Ensure that the screws holding the cover to the flow cell are fully tightened. If this cover is not in place or not completely secure, it will interfere with the UV-visible absorbance readings, and could damage the photomultiplier tube.

## 4. Conditioning a New Capillary

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A new capillary must be conditioned prior to first use. Conditioning charges the exposed silanol groups on the inner walls of the bare fused-silica and allows for a proper electroosmotic flow. To condition a new capillary, it needs to be flushed with 1N NaOH for at least 2 hours at 20 psi. An alternative flush is 0.1N NaOH overnight. After completing the flush procedure, both ends of the capillary should be stored in deionized water until the next use.

1) Place a vial filled with 3.5 ml of filtered 1N NaOH at the injection end of the capillary by screwing the vial into the plastic holder located inside the anode reservoir box. Place a waste

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vial filled with 3.25mL of deionized water on the lab jack beneath the flow cell of the UV-vis absorbance detector.

2) Adjust the height of the lab jack so that the anode and waste vial lids are level. Make sure the ends of the capillary are equally immersed in the anode and cathode vials.

3) Open the main valve on the top of the gas cylinder. Adjust the system pressure by tightening the control valve on the regulator, while monitoring the pressure (psi) on the pressure gauge connected to the regulator by copper pressure tubing. The pressure should be set to 20 psi.

4) Pressurize the capillary by turning the black control switch on the three-way valve towards the gas cylinder. At this point remove the capillary at the cathodic end. A drop of liquid should be visible at the tip of the capillary. Visualizing this drop is useful in ensuring the capillary isn't clogged. Open the interlock box and listen for air leaks. If the septum is leaking air, it will need to be replaced.

5) Time the length of the flush with a stopwatch. After the flush is complete, turn off the pressure to the system by rotating the black control switch back towards the solenoid.

Top: Pressure Regulator. Bottom: Three-Way Valve.

6) Replace the anodic reservoir vial with a vial containing 3.5mL deionized water. Flush the capillary with one volume of deionized water (~60 seconds at 20psi) to remove excess base. Let both ends of the capillary stand in deionized water until next use.

## 5. Daily System Preparation

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The Homebuilt CE system must be prepared for analysis each day. This involves turning on the UV-visible absorbance detector and flushing the capillary to restore the enviroment inside the bare fused-silica. The flushing protocol outlined below is best suited to the experiments outlined in Learning Modules I through IV.

1. Turn on the UV-visible absorbance detector to allow sufficient warm up time. Set the wavelength on the UV-visible absorbance detector to the correct value. The wavelength should correspond to the maximum absorbance value of the analyte. The optimal detection wavelengths for most analytes can be found in the MERCK index or they may be determined experimentally.

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2. To flush the system, first open up the valves on the gas tank by turning the main valve and the secondary valve. The secondary valve on the front of the regulator is useful for fine control of the pressure.

3. Adjust the pressure to the desired value for flushing (for example 20 psi). If the pressure is too high and does not seem to decrease, unscrew the anode vial to allow air pressure to stabilize (you will hear the air escaping from the vial) and then readjust the pressure using the front secondary valve.

4. Flush the system. This is done by filling two 3 mL vials. The first vial will contain filtered 1N NaOH (see Solution Preparation for Details) and is placed at the anodic end of the capillary. The second vial contains deionized water and is placed at the cathodic end of the capillary.

5. Turn on the pressure to the system by turning the black control switch. At this point remove the capillary at the cathodic end. A drop of liquid should be visible at the tip of the capillary (ensuring the capillary isn't clogged).

6. Allow 1N NaOH to run through the system for 20 minutes.

7. Fill a new sample vial with 3.5 mL of deionized water. Replace the anodic vial with this new water vial. Turn the pressure back on and allow the water to run through the system for 10 minutes.

8. After a 10 minute deionized water flush, turn off pressure, remove water vial and replace with vial of run buffer that has been filtered. Adjust the system pressure to 10 psi by using the black valve on the regulator. Flush with buffer for 20 minutes and turn pressure off.

## 6. Initiating the IGOR Pro Software

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The data collection software used in Learning Modules I – IV is IGOR Pro 5.0 with NIDAQ Tools by Wavemetrics, Inc. All software references in this section pertain to IGOR Pro. If you are using different data collection software, please refer to the user's manuals accompaning that software.

1. Open the data collection software, IGOR Pro, by clicking on the desktop icon or going to the Start Menu, and selecting IGOR Pro from the programs folder.

2. When the program initiates, go the File menu. If this is your first time using IGOR Pro YOU MUST CONSULT THE USER'S MANUAL TO ESTABLISH THE INITIAL DATA COLLECTION PARAMETERS. Otherwise select Recent Experiments and click on the file containing your run parameters. Common run parameters include Channel (wired as #2), Input (same as channel, #2), Gain (1.0), Scan Interval (0.1sec), and Number of Samples. A data table, the channel selector, a graph, and text command window should be visible on the screen.

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3. Click on the channel selector window. Look inside the break-out box to make sure that the input channel in the box is the same channel selected in the software (should be channel #2).

4. Next, click on the <**Scan Control**> button and select the appropriate number of scans for the instrument to make by typing that number into the white box. For example if the instrument scans every 0.1 sec, the number of scans should be ten times the anticipated run time (a 10 minute run is 1200 seconds, or 12000 scans).

5. Click on the graph window. For example if you are using channel 2 to collect data, the graph title should say "Graph0: Input 2". If it does not, close the graph by clicking on the **[X]** button in the upper right corner of the window. Go to the window menu at the top of the tool bar. Select <**New Graph**> and a window should pop up.

6. Select the appropriate channel, for example "Input 2", from the list under "Y Waves" and select "calculated" from the list under "X Waves". Click on the <**Do It**> button at the bottom left corner of the window.

7. To auto scale both the y-axis and the x-axis, enter "setaxis/a" in the text command window. To adjust the y-axis, go to the text command window and enter "setaxis left -1, 1". To adjust the x-axis, enter "setaxis bottom 0, ####" in the text command window, where #### is the desired number of seconds.

## 7. Establishing a Baseline (FIA)

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It is important to establish a baseline prior to undertaking actual experiments because absorbance measurements are used to detect analyte. Establishing a baseline ensures that the UV-vis aborbance detector is working properly and conditions inside the capillary are conducive to successful analyte detection. In this section a baseline will be established for use in Flow Injection Analysis (FIA). Flow Injection Analysis is a technique used to determine the presence of analyte in a sample. After injection, pressure is used to push the injection plug passed the detection window. This is not a separation technique as all sample components reach the detector at the same time. FIA is used solely to ensure analyte is present in the sample and that the response remains onscale with respect to the range setting of the UV-vis absorbance detector.

The data collection software used in Learning Modules I – IV is IGOR Pro 5.0 with NIDAQ Tools by Wavemetrics, Inc. All software references in this section pertain to IGOR Pro. If you are using different data collection software, please refer to the user's manuals accompaning that software.

1. To establish a baseline for FIA, first make sure the black control switch located on the three-way valve is pointed towards the solenoid.

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2. Next examine the pressure reading on the pressure gauge (See Diagram of the Homebuilt CE System). Adjust to desired run pressure by using the black control valve located on the regulator on the gas cylinder.

3. Next pressurize the capillary by turning the black control switch from the solenoid to the gas cylinder while simultaneously clicking on the <**Start**> button located in the Scan Control window of IGOR Pro. If you cannot do this comfortably, click start on IGOR Pro first, and then turn on the pressure and hit the Event button on the lower right corner of the UV-vis detector. This will produce a small peak (~ 0.2) on the graph which will mark the actual start time of the separation.

4. At this point the graph screen should be displaying data real-time. To zero the UV-visible absorbance detector, press the <**Zero**> button located on the front lower right portion of the panel. Return to IGOR Pro and set the y-axis range to -1, 1.

5. Monitor the baseline. The baseline may drift slightly, but should not go outside of -0.15 to 0.15 on the y-axis, nor should there be any sudden shifts (>0.05) for a period of 5 minutes (300 sec).

6. If the baseline is not steady, performing a quick flush, such as 5 min NaOH, 3 min deionized water, and 5 min run buffer all at 20psi may stabilize the baseline. See System Preparation for more detail on flushing.

7. Once a steady baseline is established proceed to sample injection protocol. If the baseline drifts significantly or there are sudden shifts in the baseline the following troubleshooting steps may be useful:

- Examine the pressure gauge to make sure the pressure is at the desired level. Adjust this value as appropriate.

- Examine the anode and cathode vials to make sure they contain equivalent volumes of buffer and be sure the capillary is equally immersed in both vials. Try to establish a new baseline. If drift continues, try using new vials of room temperature, filtered, degassed buffer.

- Physically jarring or disturbing the lab jack, anode reservoir box, anode vial holder, capillary or UV-visible absorbance detector while conducting a run may result in sudden shifts of the baseline. It is possible to cause vibrations, which changes the orientation of the capillary with respect to the optics. If this occurs, consider tightening or realigning the capillary in the optical flow cell.

- If the range on the UV-visible absorbance detector is set to a very sensitive setting (<0.001), and the baseline drift will appear to be greater.

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## 8. Establishing Baseline (CE)

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It is important to establish a baseline prior to undertaking actual experiments because absorbance measurements are used to detect analyte. Establishing a baseline ensures that the UV-vis aborbance detector is working properly and conditions inside the capillary are conducive to successful analyte detection. In this section a baseline will be established for use in Capillary Electrophoresis (CE). Because the HV Power Supply will be used during this process, please review the Safety Precautions for using high voltage prior to turning on the power supply.

The data collection software used in Learning Modules I – IV is IGOR Pro 5.0 with NIDAQ Tools by Wavemetrics, Inc. All software references in this section pertain to IGOR Pro. If you are using different data collection software, please refer to the user's manuals accompaning that software.

1. Make sure the black control switch located on the three-way valve is turned towards the solenoid (pressure only introduced via the electronically actuated solenoid valve) while establishing a baseline.

2. Check to see that the power source is properly grounded. Make sure the anode and cathode vials contain equivalent volumes of the same buffer, and the electrodes and capillary are adequately immersed in buffer.

3. Turn on the voltmeter and set it to read the resistance. You will convert resistance readings to current (v = iR). Adjust the current and voltage control knobs so that they both read zero initially.

4. Turn on the power supply (the anode reservoir box lid needs to be closed to complete the circuit). Set the voltmeter to read current. Adjust the current control knob on the power supply so that it reads 100µA. Peg this value as the maximum allowed current by throwing the black switch on the current control knob. Next, carefully adjust the voltage knob until the voltage dial and scale reads the correct separation voltage (typically above 10 kV, but not to exceed 20 kV).

Power Supply (Front View)

5. The separation current should be between 3 and 30 µA when the power is on. For standard free zone capillary electrophoresis, the polarity switch located next to the voltage control knob should be set to "positive" or "+". Turn off the power supply. The power source configuration is now set for capillary electrophoresis analyses.

6. To being establing a baseline, simultaneously turn on the power source and click <**Start**> on the Scan Control window of IGOR Pro. If you cannot do this comfortably, click start on

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IGOR Pro first, and then turn on the power supply and hit the Event button on the lower right corner of the UV-vis detector. This will produce a small peak (~ 0.2) on the graph which will mark the actual start time of the separation.

7. After approximately 3 seconds, hit the <**Zero**> button on the UV-visible absorbance detector. Return to IGOR Pro and set the y-axis range to -1, 1.

8. Monitor the baseline. The baseline may drift slightly, but should not go outside of -0.15 to 0.15 on the y-axis, nor should any sudden shifts (> 0.05) appear for a period of 5 minutes (300 sec).

9. Make note of the voltage reading on the voltmeter (that you convert to current), it should not fluctuate more than +/- 0.001V during the 5 minute period. Once a steady baseline is established, it is time for sample injection.

Notes: If the baseline drifts outside the prescribed range, or shifts suddenly and does not, turn off the power supply and click <**Stop**> on the Scan Control window of IGOR Pro. Some trouble shooting tips are provided below.

- Check to make sure the anode and cathode vials contain equal volumes of buffer and that the electrodes are equally immersed in buffer. If extreme baseline drift continues or sudden baseline shifting reoccurs, try using new vials of room temperature, filtered, degassed buffer.

- Disturbing the lab jack, anode reservoir box, anode vial holder, capillary or UV-visible absorbance detector while conducting a run may result in sudden shifts of the baseline.

- If the Range on the UV detector is set to a very sensitive setting (<0.001), the baseline drift will appear to be greater, but should not exceed a range of -0.2 to 0.2 on the y-axis.

- If the baseline seems to oscillate, you may have a ground loop in your system. The power source may need to be grounded using a ground connection to a cold-water tap. Make sure than random metal objects (pens, paper clips, spare parts, etc.) are not in contact with any of the electrical wiring connecting the power source to the voltmeter or the anode reservoir box.

## 9. FIA: Flow Injection Analysis (pressure only, NO voltage)

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Flow Injection Analysis is a technique used to determine the presence of analyte in a sample. After injection, pressure is used to push the injection plug passed the detection window. This is not a separation technique as all sample components reach the detector at the same time. FIA is used solely to ensure analyte is present in the sample and that the response remains onscale with respect to the range setting of the UV-vis absorbance detector. When first learning Capillary Electrophoresis it is a useful practice to perform a FIA prior to performing CE.

The data collection software used in Learning Modules I – IV is IGOR Pro 5.0 with NIDAQ Tools by Wavemetrics, Inc. All software references in this section pertain to IGOR Pro.

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If you are using different data collection software, please refer to the user's manuals accompaning that software.

1. From the start menu find IGOR pro under programs. Under IGOR pro, click on the Igor icon. Once the application starts, go to the file menu. Open the file with your preferences. The most recently used file will be under recent experiments. This will bring up a table for the number of samples to collect, the channel selector, and the graph for the data collected.

2. Check that the channel selector is set to the correct channel. The channel you should select depends on what inputs you have used and how they are configured in the break-out box.

3. Set the numbers of data points that you need to collect. Remember that the scan control panel is set to scan at intervals of every 0.1 sec. So if you want to let a sample run for 6000 sec, which is 10 minutes, you need to have number of samples set to 60000.

4. With the buffer vial in place at the injection end (anode), turn on the pressure and hit the <**Start**> button on the bottom of the scan control on the computer program. Let the buffer run through the capillary until you have a steady baseline. If the absorbance on the UV-visible absorbance detector is not at zero, hit the <**Zero**> button on the UV-visible absorbance detector.

5. Once baseline is steady, turn off pressure. Remove the waste vial at the detection end of the capillary (cathode) and replace it with a vial containing run buffer. Remove the vial at the injection end (anode) and replace it with sample.

6. Set the timer box to the correct injection time and turn it on. Make sure that the capillary is submerged in the sample (but not the Teflon-pressure tubing).

7. Remove the sample vial and replace it with a vial containing running buffer.

8. Turn on pressure and simultaneously hit the <**Start**> button to collect data. If you cannot do this comfortably, click start on IGOR Pro first, then turn on the pressure and hit the Event button on the lower right corner of the UV-vis detector. This will produce a small peak (~ 0.2) on the graph which will mark the actual start time of the FIA.

9. To adjust the data graph, for example to expand the first 500 data points, click the cursor on the bottom line and type "setaxis bottom 0, 500". You can also do the same with the left axis, for example by typing, "setaxis left –1, 1".

10. After observing the analyte signal, stop data collection by clicking the <**Stop**> button of the scan control window. Turn off the pressure.

11. Click on the graph and then go to <**Graph**> on the menu bar. Click <**Show info**> and <**Show tools**>. The graph will now have two icons in the upper left corner. Click on the bottom icon and select the "T" icon, which stands for text. Then click on the graph. This will allow you

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to type information about the run (buffer, sample, run name, injection time, sample name, pressure, etc) directly on the graph.

12. Next, you will need to click on the top icon (graph icon) and select the circle cursor at the bottom of the graph. Drag this circle cursor to the left of where the peak starts. Then drag the square cursor to the end of where the peak stops. Both of these cursors need to be placed on the baseline before and after the peak you want to fit.

13. On the menu, click analysis and go to curve fittings. Select "gauss" under the "Function and Data" tab and select cursors under Data Options. Click <**DO IT**>. The peak will be fit with a Gaussian curve at the peak cursors. Following the fit, the program will report a "w-coefficient", which will provide the following information: baseline (k0), peak height (k1), migration time (k2), peak width (k3). It is important that the peak height falls between 0.1 and 1.0 on the full scale due to sensitivity issues with the UV-vis abosorbance detector. To bring the signal onscale adjust the range of the detector or change the sample injection conditions.

14. Down at the bottom of the screen is preferences box. This box will contain the w-coefficient. Copy the w coefficient numbers and paste them into the data graph.

15. SAVING DATA: After each run you need to save the data. Click on "Data" tab on the menu bar. Go to <**save waves**> and then <**save Igor binary**>. Click on the input that is associated with the channel connection (for example, input 2 is recorded at channel 2) and then click <**DO IT**>. Select <**new folder**> and name the folder for the date you are collecting data. Then name the run (for example "100µM ibuprofen FIA run 1").

## 10. Capillary Electrophoresis (voltage only, no pressure)

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The remaining sections describe how to perform a single Capillary Electrophoresis (CE) analysis on a single sample. This section is a review of Establishing a Baseline for CE. Because the HV Power Supply will be used during this process, please review the Safety Precautions for using high voltage prior to turning on the power supply.

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1. To get a baseline, screw the buffer sample vial on to the platform and close the lid. There is a safety switch on the plexi-glass box that prevents current flow in the system if the lid is not closed. Turn on the power supply. Remember that the pressure should be turned off.

2. Turn on the voltmeter by pressing button labeled "power". Set the voltmeter to read current. Next, set the separation voltage by turning the knob on the right front of the power supply.

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3. Hit <**Start**> on the Igor program and monitor the baseline. Look at the voltmeter on top of the power supply to see if the current reading is steady. It should not deviate more than 0.2 mV (2 μA) for a period of 120 seconds.

4. Turn off the power supply. Hit <**Stop**> on the Igor program. The base line information does not need to be saved.

## 11. Sample Injection

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The following descibes the process for using pressure to inject a sample plug into the separation capillary. The process of sample injection should be practiced with non-critical samples until a high level of consistency is obtained. Inconsistant sample injection makes quantitative analysis impossible.

1. Make sure the black control switch located on the three-way valve is pointed towards the solenoid. In this position, pressure is only introduced via the electronically actuated solenoid valve. Adjust the injection pressure with the black control valve on the regulator to the desired pressure.

2. Turn on the timer box. Adjust the injection time using the gears located underneath the injection time display window.

3. Replace the anode buffer vial with the sample vial containing the sample needing to be analyzed. It is important that the sample vial is fully screwed into the sample vial holder; otherwise a partial injection may result. Close the anode reservoir box lid.

4. Inject a sample by actuating the timer box. There should be a click followed by the sound of pressurized gas entering the sample vial and then another click. During the injection, monitor the pressure gauge to make sure that the injection pressure remains steady (changes not to exceed 0.2psi)

5. Turn off the timer box and replace the sample vial with the anode buffer vial. Close the anode reservoir box lid. If a partial injection occurs, the sample plug needs to be removed from the capillary by a pressurized flush.

6. Replace the sample vial with the anode buffer vial. Close the lid of the interlock box.

7. Silmultaneous turn on the high voltage power supply and click start on IGOR Pro. If you cannot do this comfortably, click start on IGOR Pro first, and then turn on the power supply and hit the Event button on the lower right corner of the UV-vis detector. This will produce a small peak (~ 0.2) on the graph which will mark the actual start time of the separation.

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## 12. Data Analysis

Return to Menu

The following section descibes how to analyze peaks obtained from a CE run. The peaks will be analyzed using a Gaussian fit. Peak height, migration time, and sigma value will be the primary data values determined.

The data collection software used in Learning Modules I – IV is IGOR Pro 5.0 with NIDAQ Tools by Wavemetrics, Inc. All software references in this section pertain to IGOR Pro. If you are using different data collection software, please refer to the user's manuals accompaning that software.

1. The first step after collecting data is to save the run data. This is done by first clicking on the graph window. Next click Data from the toolbar at the top of the IGOR Pro window. Select Save Waves. Select Save IGOR Binary. Select the appropriate input, for example Input 2, from the list under Waves. Click <**Do It**>.

2. A file directory screen should appear. Select the appropriate data folder to store your run or create a new one by moving the cursor to the directory window, right clicking with the mouse, and selecting New and the selecting Folder.

3. Double left click on the name of the folder to change the folder name. Double left click on the folder icon to open the folder. Click on the appropriate wave, for example Input 2, in the file name line and change to desired file name to something more informative. Click on <**Save**>.

4. After saving the data, return to the main IGOR Pro window. Click on the graph. Now begin analyzing the data. First select Graph from the toolbar, and then select <**Show Info**>. Select Graph again from the toolbar and then select <**Show Tools**>.

5. Two icons will appear in the upper left corner of the graph window. Click the [T] icon, which stands for text. Next, click on the graph. A screen should appear that will allow you to type the run parameters (such as buffer, run name, injection time, sample, pressure, etc.) directly onto the graph.

6. After typing this information, click on the graph icon located next to the [T] icon in the graph window. Two lines should appear at the bottom of the graph window. These contain movable cursors to designate the region of the graph to be fit. First, expand the region of interest in the graph, by moving the cursor to the graph, holding down the left mouse button and drawing a box around the peak. Right click and select Expand.

7. Select the circle cursor from the bottom two lines appearing under the graph. Drag the circle cursor to the graph and place it on the baseline as close to the beginning of the peak as possible.

8. Next, drag the square cursor to the graph and place it on the baseline as close to the end of the peak as possible.

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9. Select Analysis from the toolbar. Select Curve Fitting. A new window should open. Select <**Function and Data**>. Make sure "Gauss" is selected from the Function list and the appropriate wave is selected from Y-Data list, for example Input 2, and "_Calculated_" from the X-Data list. Select <**Data Options**>. Click the <**Cursors**> button from under the Range box. This should set the peak range to the square and circle cursors placed on the peak. Click <**Do It**>.

10. A fitted curve should appear on the graph. A rectangular box should open that provides four parameters (peak height, sigma, time, and y-initial). Click <**Ok**> to close the window. Next go the text command box.

11. The curve fitting data should automatically be displayed as the last entry. Scroll up in the text command box to find the w-coefficient line. The four values listed are (from left to right): y-initial, peak height, migration time, and sigma (4σ defined as width of the peak base).

12. After the values are recorded or printed, close the graph by hitting the [X] in the upper right corner of the graph window. For a new run, go to the menu at the top of the tool bar. Select <**New Graph**> and a window should pop up. Select the appropriate wave, for example "Input 2", from the list under Y Waves and select "calculated" from the list under X Waves. Click on the <**Do It**> button at the bottom left corner of the window.

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