Microchip Capillary Electrophoresis*

In this experiment you will make a capillary electrophoresis microchip from a low-cost polymer: poly(methyl methacrylate), a.k.a. PMMA or Plexiglas. You will then test the performance of your device in an electrophoretic separation of fluorescently labeled amino acids, using a custom-built instrument. You will also identify the different peaks in your amino acid sample.

Lab Assignment

Equipment

You will use a custom-built laser-induced fluorescence microscope with microchip holder and power supplies. This instrument is interfaced with a computer for data acquisition. Do not touch anything on this setup until either the instructor or TA has briefed you on its usage. There is an oven on the west bench that you will use to bond your PMMA microchips. In the drawer to the right and below the oven, you will find copper blocks, glass microscope slides, tweezers and C-clamps that you will use in device bonding. A micropipettor with disposable tips and a vacuum hose in the drawer below the computer are available for filling and emptying your device. Don't use the micropipettor until you have been instructed on its operation.

Caution: This instrument uses high voltages (up to 5 kV) and laser radiation. You should never put anything reflective or any part of your body in the path of the laser. The high-voltage power supplies have a current limit set for your protection, but you should also take appropriate precautions. Specifically, you must verify that all wires are in the appropriate reservoirs before you turn on the power supplies, and you must always ensure that the power supplies are off before you touch any wires.

Chemicals and Materials

Amino acids have been labeled with fluorescein isothiocyanate (FITC) and diluted for you. You will be given a set of vials containing the following: phosphate buffer with added hydroxypropyl cellulose (to reduce electroosmotic flow), 100 nM fluorescein, 1 μM FITC-Gly, 1 μM FITC-Asp, 1 μM FITC-Arg, 1 μM FITC-Phe, and a solution with 500 nM of each of the four amino acids. ***Note:*** the fluorescent compounds are light sensitive, so you need to store them in the dark (e.g., in a drawer) when you are not using them.

You will be given two PMMA pieces: one has microchannels that have been imprinted from an etched silicon template, and the other has four holes that have been made in it with a laser cutter. You will bond these two pieces together to form your capillary electrophoresis microchip, as outlined below. Contaminants from skin will hinder device bonding and operation, so make sure you wear gloves when you handle the microdevices at all stages of fabrication and use.

Procedure

Your laboratory work will consist of two parts. First, you will prepare a capillary electrophoresis microchip by thermal bonding. Then, you will use this device to carry out rapid separations of fluorescently labeled amino acids and identify the peaks in the mixture. You will also calculate the separation efficiency.

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1. Prepare your microchip device—be sure to wear gloves. First, wash both polymer pieces with soap and rinse them thoroughly with distilled water. You can use the small, flexible hose on the N_2 cylinder to blow the PMMA pieces dry; a pair of tweezers is available to hold your substrates. Align the ends of the imprinted channels with the laser-cut openings in the cover piece. Gently press the pieces together, and put a small water droplet on the edge of the assembly, so surface tension will hold the pieces in alignment. Place the PMMA assembly between two glass microscope slides, and then put the copper blocks outside the microscope slides. Finally, gently tighten the C-clamps to hold the stack together (don't apply excessive force). Place your assembly in the oven, which should be at 110 ºC (230 ºF). After 15 minutes, remove the assembly using leather gloves, gently tighten the C-clamps, and replace the stack in the oven. Repeat the tightening process in another 15 minutes, replace the assembly in the oven, and let it bond for another 15 minutes. Remove your device from the oven and let it cool; loosen the C-clamps a little (1/4 turn or less) once you remove the assembly from the oven. After things have cooled sufficiently, inspect your device for completeness of bonding. If there are unbonded regions along the channels or reservoirs, re-form the PMMA/microscope slide/copper block sandwich and repeat the bonding process.

2. If you think your microchip is ready to be used for capillary electrophoresis, let the TA or instructor inspect it. Once approved, you may proceed to initial testing by filling the device with water. Use a water bottle or the micropipettor to transfer a small volume (<10 μL) of water into one of the three reservoirs at the top of the device. The channels should all fill gradually via capillary action. You may want to place the vacuum hose over the various reservoirs to help in the filling process. If all the channels fill, pipet any liquid from the reservoirs, and then use the vacuum hose to dry the channels. If all your channels do not fill, the instructor or TA can help you try to assess why, by inspecting the microchip with the microscope.

3. A device that filled with water is ready to be loaded with buffer solution; however, if it is near the end of the lab period, wait until next time to try. You should use the same procedure that worked for filling with water. Once all the channels are full of buffer, fill all four buffer reservoirs to be level with the top of the device.

4. Turn on the detection system as described in the Operating Instructions.

5. Make sure that buffer in all reservoirs is still level with the top of the device. Then, pipet out the contents of the top left (sample) reservoir, and add ~5 μL of sample. Place the device on the chip holder on the microscope stage, making sure that the injection intersection is just to the right of the left edge of the circular opening in the chip holder. Secure the device in position with 2-3 pieces of tape. Verify that the high-voltage power supplies are off, and then carefully insert the four electrodes into their respective reservoirs.

6. Follow the Operating Instructions for initial alignment of the microchip in the optical detection system, focusing the laser in the injection channel near the intersection region.

7. Check that the electrodes are still in position, and verify that the inject switch is in the "inject" position. Then, turn on the high-voltage power supplies, following the Operating Instructions for trial injection and fine alignment. If you have any questions about whether or not you are doing this properly, check with the instructor or TA first. After a time, the fluorescent sample will flow through the injection channel, and the PMT will detect the fluorescence signal. Align the pinhole and adjust the fine focus, then turn off the high voltage and the power supplies.

8. Move the detection spot ~1 cm down the separation channel and adjust the Z-position the distance you optimized above. Then, carry out an injection/separation, following the Operating Instructions. Be sure to hit the "record" button on the VI at the same time you switch the box from "inject" to "run".

9. Obtain as many replicate separations as you desire. You should also explore the effects on separation of parameters such as injection time and detection position. When you are ready to switch samples, follow the procedures in the Operating Instructions.

10. Identify which peak corresponds to which analyte in your unknown.

11. At the end of each lab period, flush your chip with water, vacuum the channels dry, and follow the shutdown procedures for the instrument in the Operating Instructions.

Some things to include in your report (along with whatever other information is needed)

Experimental
List any instrumental settings that differed from the recommended ones for the experiment.

Describe the approach you used to identify the peaks in your sample.

Results and Discussion
Discuss (and be quantitative about) the reproducibility of peak migration times and theoretical plate counts.

How did the injection time and separation distance affect the number of theoretical plates and peak height? Did the number of theoretical plates scale as expected with separation distance?

Show the electropherograms you used to identify the peaks, and identify the amino acid peaks on an electropherogram of the sample. Also, explain why the analytes migrate in the order observed.

Discuss any strengths and weaknesses of microchip capillary electrophoresis.

Address all other questions and issues raised in the lab procedure.

Discuss any other conclusions you can draw from your experiments. Talk about what you would do differently, if you had to do the experiment over again, or what you would do additionally, if you had more time.

Appendix–calculations
Show representative calculations for theoretical plate count. Show other calculations only as they aid your discussion.

OPERATING INSTRUCTIONS

Note: you MUST NOT TOUCH the optical components; doing so could misalign the laser and make the experiment difficult to complete on time.

Turning on the detection system

On the laser power supply, make sure the key is in the "1" position and press the green "laser on" button. If the laser head does not emit blue light within a few seconds, notify the TA or instructor. Visually inspect that the laser spot is centered on the back aperture of the microscope. Turn on the PMT power supply and increase the reading to 0.500 by turning the dial clockwise. Turn on the preamplifier. Also, you will need to close the window blinds to reduce background light interference.

Log in to the data acquisition computer. Double click the "523 Microchip Lab" icon on the desktop to open LabView and the VI that will record your electropherograms. Click on the right-facing arrow in the upper left corner to start the VI. You will be prompted for the number of data points to be recorded per second; a good initial value is 20. The VI will start displaying data from the PMT, but will not write it to a file unless you tell it to. Thus, create a folder for your team's data and enter that path in the box in the VI, along with a filename. To write your electrophoretic data to the file, press the "record" button. When your separation is done, press "stop". Be sure to change the filename before you hit "record" again.

Initial alignment of the microchip in the optical detection system

Verify that the front knob on the right side of the microscope is in the "eye" position. Then, turn on the green switch on the white box to the right of the microscope; visible light should shine onto the microscope stage. The microscope has 4X and 20X objectives directly under the stage. Rotate the objective ring until the 20X objective is in position directly beneath the microchip. The stage can be moved in the X- and Y-directions using the wand on the right side; focusing is accomplished using the knob on the microscope body. Adjust the position of your microchip using X-Y translation until you find the injection channel, which should cross the viewing area vertically. Then, adjust the Z position so the bottom of the channel is in focus. Note the number on the microscope focusing knob. Turn off the green switch that controls the stage illumination. You should see a faint greenish spot where the laser is focused. Adjust the X-Y stage so the laser spot is in the middle of the channel. Have the TA or instructor verify that you are correctly positioned before you continue. Next, adjust the microscope stage about 30 units higher—this is approximately where the maximum signal will reach the PMT. Check that the green switch to control stage illumination is off, and turn the front knob on the right side of the microscope from "eye" to "side" to direct 80% of the light collected from the objective to the PMT attached on the left port. Look at the trace from the LabView VI (you may want to rotate the computer monitor so you can view it from the microscope); the overall signal should be about 0.5. If the signal is more than a factor of 2 higher, consult the TA or instructor before continuing.

Trial injection and fine alignment

Before you turn on the high-voltage power supplies, make sure that the electrodes are still in the reservoirs. Push in the on/off switch on the bottom right of the power supplies to turn them on. The settings should be 1000 V for the separation (top) power supply and 400 V for the injection (bottom) power supply. Move the switch on the inject box to the "inject" position. Turn on the voltage for both power supplies by pressing the top half of the black switch on the left of the power supplies (to turn off the voltage, press the bottom half of the black switch). The voltage on the display should increase to the set value. The voltage will drive your sample electrophoretically through the injection channel, and you

should see a gradually increasing signal at the detector. When the signal levels off, align the pinhole by turning the black knobs on the side and top to maximize signal, then optimize the Z-position by changing the focus to maximize signal; note the value on the focus knob relative to where you were focused on the channel bottom. You will need to adjust the focus knob (after focusing on the channel bottom) by this difference to maximize signal. Any time you are not actively detecting, make sure that you rotate the right knob on the microscope to the "eye" position.

Carrying out separations

Once you have moved the laser spot to the desired position in the separation channel and adjusted the Z position, turn off the stage illumination light and turn the right knob to the "side" position. Switch the inject box to the "inject" position, turn on the high voltage on both power supplies, and wait for the desired time period (40 seconds is a good trial value). At the end of the injection time, switch the inject box to the "run" position, and at the same time press the "record" button on the VI on the computer. When your run is over, press the stop button and make sure to change the filename before you hit "record" again. When you are done with a set of separations, turn off the high voltage and shut off the power supplies. Then, switch the side knob to the "eye" position.

Switching samples in the microchip

After you have shut off the power supplies and switched the side knob to the "eye" position, carefully remove the electrodes from the reservoirs and pull off the tape securing your device. If your device was working fine, you can just pipet old buffer from each reservoir and replace it with new buffer. Then, pipet your old sample from the reservoir, rinse the reservoir with buffer, and pipet the new sample into the reservoir. Your device is now ready for additional separations.

If your device was having problems, it is best to flush it out and refill with buffer. Spray each reservoir with a wash bottle and then pipet the liquid out. Repeat as needed. Apply vacuum to each reservoir to remove the liquid from the channels. Then, follow the process in steps 3 and 5 of the write-up to fill the microchip with buffer and load sample.

Equipment shutdown

When you are finished for the day, switch off the high voltage, turn off the power supplies, and follow the procedure listed above for removing the microchip and flushing out the channels. You will want to rinse your microchip and channels with water and then store it with the channels dry.

Switch the right knob to the "eye" position, turn the dial on the PMT power supply counterclockwise until the voltage is as low as it goes (~0.24), and then switch off the power button. Turn off the preamplifier, and press the red 'laser off' button on the laser power supply. Close the LabView program (do NOT say "yes" to save changes), transfer your data from the computer, and then shut it down. Rotate the microscope objectives so they are not directly under the microchip viewing region. Turn off the stage illumination light on the microscope and put away any samples, supplies or materials you used during the lab period.