Distance Learning Module IV: Determination of Nonsteroidal Anti-Inflammatory Drugs Using Micelle Electrokinetic Capillary Chromatography

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Objective:
To apply experimental practices and knowledge of separation science established in Learning Modules I-III to analyze a series of nonsteroidal anti-inflammatory drugs (NSAIDs) by MEKC using a capillary electrophoresis instrument.

Learning Outcomes
Upon successful completion of Learning Module IV, researchers will be able to:
  (1) design and implement the separations necessary to determine capacity factors for several compounds;
  (2) perform quantitative and qualitative analysis of an unknown using capillary electrophoresis;
  (3) establish standard protocol for future MEKC experiments.
Introduction

This learning module is written for undergraduate chemistry researchers who are already familiar with fundamentals of separation mechanisms, separation efficiency and figures of merit for free zone capillary electrophoresis and MEKC, as well as basic aspects of operation of a capillary electrophoresis system including sample introduction, the development of operating protocol, and anticipation of experimental outcome. These concepts are covered in Learning Modules I-III. The experiment outlined in this Module may be accomplished using a commercial or custom-built capillary electrophoresis system. The first step of this learning module requires the user to develop and document experimental procedures that will lead to the determination of retention factor of MEKC separations of four NSAIDs. Step 2 requires the user to determine retention factors experimentally for four NSAIDs using the protocol formulated in Step 1. The last step (3) requires the user to perform qualitative and quantitative analyses of an unknown sample using MEKC. This exercise outlined in step 3 provides the user an opportunity to assess her or his laboratory skill and knowledge of the operation of MEKC. The experiment outlined here is performed with recommended chemicals (n-decanophenone, dimethylformamide, flurbiprofen, naproxen, sulindac, tolmetin). The experiment may be completed with other compounds, and alternative chemicals are outlined in the instructor’s manual accompanying this material. Successful completion of Learning Module IV assists the user in acquiring the skills necessary to apply MEKC to determination of samples.

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Background

Fundamentals. In Learning Module III we discussed the separative transport in MEKC and demonstrated how retention factor is derived. In addition, you performed the separations necessary to experimentally determine the capacity factor of a single NSAID. Retention factor can be quite useful for qualitative analysis of an unknown if the experimental procedures result in reproducible data. Quantitative analysis further requires the peak height, or area, is reproducible. The only way to assess this is to participate in a practical exercise, where the composition and concentration of a sample is unknown to you, but is provided following the submission of your results. Gauging your success using a sample for which you can compare your results with known values is critical to developing separation based assays using MEKC.

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Materials

In order to complete Learning Module IV you will need the materials listed below.
(1) A capillary electrophoresis system that includes the five components (injection, capillary, high voltage, detection, analog-to-digital converter). We recommend you use a bare fused silica capillary with an inner diameter of ~25 microns.
(2) Chemicals: 3-[cyclohexylamino]-1-propanesulfonic acid (CAPs), deionized water, n-decanophenone, dimethylformamide, flurbiprofen, naproxen, sodium hydroxide, sulindac, tolmetin
(3) Standard laboratory equipment: electronic balance, pH meter, volumetric pipets, sonicator (for degassing running buffer).

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This material is based upon work supported by the National Science Foundation under Grant No. 0307245.
**Safety Precautions**
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: [http://www.osha.gov/SLTC/etools/construction/electrical_incidents/eleccurrent.html](http://www.osha.gov/SLTC/etools/construction/electrical_incidents/eleccurrent.html)

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

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.

Consult the safety guidelines provided by your Institution before beginning any experiment. The safety guidelines of your home Institution supercede any recommendations outlined here.

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**Procedures: The Module**

1. Outline the experiments you must do to determine the retention factor (capacity factor) for 4 NSAIDs.

2. Determine the retention factor (capacity factor) for flurbiprofen, naproxen, sulindac, and tolmetin. These values should be reported as mean values determined from triplicate runs ($n = 3$). For these runs, the background BGE is 25 mM CAPs, 100 mM SDS buffered to pH 10. You must use a ~25 micron inner diameter fused silica capillary ~42 cm in total length, ~32 cm to the window, 20,000 V.

### Free Zone Data

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### MEKC DATA

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3. Lab Practical

Through a faculty mentor or collaborator, you should arrange to receive an unknown solution containing some combination of flurbiprofen, naproxen, sulindac, and tolmetin diluted in BGE at a concentration above the limit of quantification for your CE 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.

Follow-up Activities

Upon completing Learning Module IV, you have documented procedures for applying MEKC for qualitative and quantitative analysis using capillary electrophoresis. Following the separation you completed in step 3, consider whether you would revise any of the protocol you developed in this Learning Module. Now that you are familiar with the parameters necessary to determine retention factor consider how you might design an experiment to determine the retention factors for a series of similar analyte, perhaps cationic compounds. Take a look at the answer key we have provided for Learning Module IV. If you are in contact with other researchers who have completed this Learning Module, you should consider sharing your responses with others. You may find subtle differences or explanations that you find useful.

Conclusions and Future Direction

If you have mastered the learning outcomes for Learning Module IV, congratulations! MEKC is a flexible separation technique with a host of applications [1]. You have completed several self-guided exercises designed to expand your skill at performing qualitative and quantitative analyses using MEKC or capillary electrophoresis. This will assist you in devising separation strategies for future MEKC analyses that have not previously been performed. As you expand your use and knowledge of capillary electrophoresis, you will undoubtedly learn about, and hopefully apply, other modes of capillary electrophoresis such as capillary gel electrophoresis, affinity capillary electrophoresis, capillary isotachophoresis, and capillary electrochromatography.

References