

Distance Learning Module III: the Separation of Neutral Compounds Using Micelle Electrokinetic Capillary Chromatography

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Abstract

This learning module outlines basic concepts of micelle electrokinetic capillary chromatography (MEKC) separations of neutral molecules. The fundamental equation used to calculate retention factor is derived. The learner is instructed to perform an MEKC separation of neutral compounds. The module teaches the learner how to calculate capacity factor using *cis*- and *trans*-1,2,4,-trimethoxy-5-(1-propenyl)benzene in an MEKC separation.

*Note regarding improvements in this module:

This learning module is a derivative work of the original learning module III and includes improvements based on student feedback. The improvements enhance the learning experience significantly and we therefore recommend using the new module only. The new module centers on the application of a simpler MEKC separation of neutral molecules as an introduction to MEKC. This approach allows the learner to perform MEKC using fewer runs and simpler calculations. The test analytes we have selected are structurally similar and related to other on-going applications in the Holland group. Users of this module should be aware that *cis*-1,2,4,-trimethoxy-5-(1-propenyl)benzene is a known carcinogen.

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Objective:

To become familiar with fundamentals, experimental practices, and figures of merit necessary for micellar electrokinetic capillary chromatography (MEKC) by implementing the technique with a capillary electrophoresis instrument.

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Learning Outcomes

Upon successful completion of Learning Module III, researchers will be able to:

- (1) translate fundamental concepts of MEKC into anticipated experimental outcome;
- (2) determine pertinent figures of merit from MEKC data;
- (3) establish standard protocol for future MEKC experiments.

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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, 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 and II. The experiment outlined in this module may be completed using a commercial or custom-built capillary electrophoresis instrument. The first 2 steps of this learning module require the user to describe fundamentals of MEKC separations. Step 3 provides the user an opportunity to apply her or his laboratory skill and knowledge of the operation of MEKC. The experiment outlined here is performed with recommended chemicals (asarone, dimethylformamide, n-decanophenone). The experiment may be completed with other compounds. Successful completion of Learning Module III assists the user in acquiring the skills necessary for a new practitioner of MEKC.

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Background

Fundamentals. In Learning Module I we discussed the two mechanisms of separative transport in free zone capillary electrophoresis: electrophoresis and electroosmosis. In evaluating the order of migration we noted that neutral analyte comigrate in a band. It turns out that free zone capillary electrophoresis can be modified with certain additives that impart an additional selectivity based on hydrophobic partitioning. MEKC is a popular mode of capillary electrophoresis first reported in 1984 by Shigeru Terabe et. al. [1] and requires the addition of a micellar pseudo-stationary phase in capillary electrophoresis. A micelle is formed when amphiphilic molecules that contain distinct hydrophilic and hydrophobic regions self-assemble to form liquid crystal aggregates. A common surfactant used in MEKC is sodium dodecyl sulfate, SDS, which is comprised of a 12-carbon hydrophobic chain and charged sulfate head group. The hydrophobic alkyl chains collect or assemble in the core of the aggregate to reduce the contact of alkyl and water. The sulfated head groups are presented on the portions of the aggregate with greatest exposure to the aqueous running buffer. These aggregates are classically represented as rigid spheres with a well-

defined core region and a well-defined outer region, although, the surfactant molecules are constantly undergoing exchange.

An SDS micelle has a net negative charge, since SDS itself is anionic. As a result, an SDS micelle has a characteristic electrophoretic mobility when it is a component of the running buffer. Additionally, an SDS micelle contains a hydrophobic region, into which analyte molecules may partition. A highly hydrophobic analyte, such as n-decanophenone, or Sudan III, may partition completely into an SDS micelle, in which case, the migration time of this analyte is identical to the migration time of the SDS micelle. A neutral analyte with an intermediate hydrophobicity will exchange between micelle and aqueous running buffer. During the time the neutral analyte associates with the micelle, it will assume the velocity of the micelle. When the neutral analyte is not associated with the micelle, it will assume a velocity dictated by electroosmotic flow. The migration time of the analyte will be a function of the amount of time it assumes the electroosmotic velocity and the amount of time it assumes the micelle velocity. Thus a series of neutral analyte with varying hydrophobicity will have different migration time. This is demonstrated pictorially in Figures 3.1A, B.

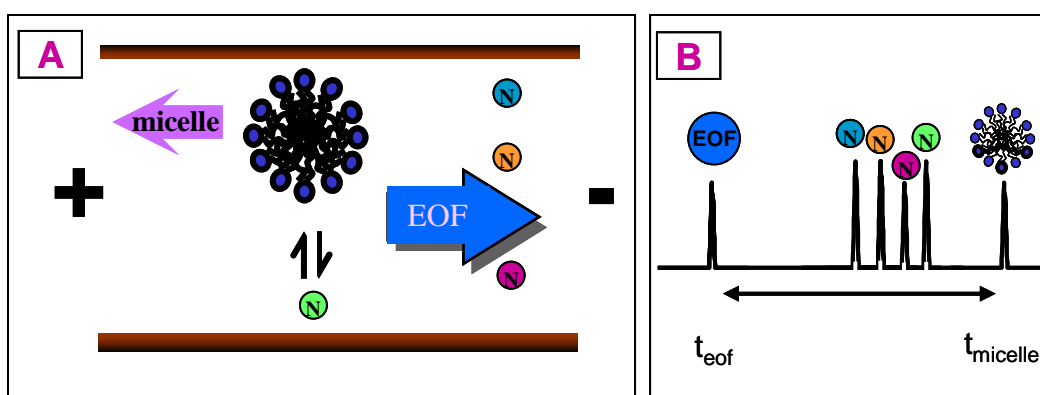


Figure 3.1a is a pictorial representation of the separative transport in MEKC. Figure 3.1b describes the components of the resulting hypothetical electropherogram.

MEKC Figure of Merit. Retention factor, sometimes called capacity factor, is used to evaluate the relative hydrophobicity of the analyte. The term for retention factor is written k' , and is the ratio of moles analyte in the micelle phase-to-moles of analyte in the aqueous component of running buffer (see equation 3.1). Recall that when neutral analyte associates with micelle it assumes the micelle velocity and when neutral analyte is not associated with micelle it has a velocity equal to that of the bulk electroosmotic flow. Therefore, the velocity of neutral analyte in MEKC, v_{apparent} , is the sum of the mole fraction of neutral analyte in the aqueous phase times the velocity of electroosmotic flow, v_{eof} , and the mole fraction of neutral analyte in the micelle phase times the velocity of the micelle, v_{micelle} , (see equation 3.2). Equation 3.2 can be rearranged in order that capacity factor may be expressed in terms of parameters that are easily measured in an MEKC experiment, shown in equation 3.14. Thus capacity factor of a neutral molecule may be determined by measuring the time of the electroosmotic flow, t_{eof} , the retention time of the hydrophobic/neutral analyte, t_{R} , and the time of an analyte that serves as a micelle marker, t_{micelle} .

$$k' = \frac{\text{moles}_{\text{micelle}}}{\text{moles}_{\text{aqueous}}} \quad (\text{equation 3.1})$$

$$v_{\text{apparent}} = v_{\text{eof}} \frac{\eta_{\text{aqueous}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} + v_{\text{micelle}} \frac{\eta_{\text{micelle}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} \quad (\text{equation 3.2})$$

[Click here to see the derivation of k' for neutral compounds in Appendix A](#)

$$k' = \frac{t_R - t_{eof}}{t_{eof} \left(1 - \frac{t_R}{t_{micelle}}\right)} \quad (\text{equation 3.14})$$

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Materials

In order to complete Learning Module III 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, dimethylformamide, n-decanophenone, sodium dodecyl sulfate, sodium hydroxide, *trans*-1,2,4,-trimethoxy-5-(1-propenyl)benzene (α -asarone), and *cis*-1,2,4,-trimethoxy-5-(1-propenyl)benzene (β -asarone),
- (3) Standard laboratory equipment: electronic balance, pH meter, volumetric pipets, sonicator (for degassing running buffer).

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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

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. How are the analytes separated by MEKC? Describe fundamental principles that govern the method.
2. Define retention factor (or capacity factor) as it pertains to MEKC. Write the equation you would use to calculate the retention factor of α -asarone in 25 mM CAPs, 50 mM SDS buffered to pH 10. Describe what experiments are necessary to obtain retention factor.
3. Determine the migration time for dimethylformamide, α -asarone, β -asarone and n-decanophenone by MEKC. Be sure to perform replicate runs. Sample should be dissolved in background electrolyte (BGE). The sample should contain DMF, α -asarone, β -asarone, and n-decanophenone. The run buffer for the MEKC separations is 25 mM CAPs, 50 mM SDS buffered to pH 10. You should use a ~25 micron inner diameter fused silica capillary record the length to the capillary window, total capillary length and applied voltage. You may consider performing the separations after flushing the capillary adequately before you begin. For these separations it is likely you will not need to perform flushes in between each run. This instrumentation does not allow you to control the run temperature. You may use the tables below to organize your data.

TABLE 1: MEKC DATA				
	$L_w = \text{ cm}, L_t = \text{ cm}, V_{\text{sep}} = \text{ V}, \text{ BGE} =$			
	Migration Time			
	Trial 1	Trial 2	Trial 3	
DMF				
n-decanophenone				
β -asarone				
α -asarone				
	k'			
	Trial 1	Trial 2	Trial 3	AVE \pm SD
β -asarone				
α -asarone				

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Follow-up Activities

Upon completing Learning Module III, you have documented procedures for implementing MEKC 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 charged analytes. Take a look at the answer key we have provided for Learning Module III. 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.

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Conclusions and Future Direction

If you have mastered the learning outcomes for Learning Module III, congratulations! MEKC is a flexible separation technique. Learning Module IV is a self-guided exercise designed to expand your skill in MEKC by analyzing a multi-component sample and performing qualitative and quantitative analysis of an unknown. This will assist you in devising separation strategies for future MEKC analyses. The procedures you have mastered in Learning Module III will be required to further refine your laboratory skills in the fourth Module.

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References

[1] Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchiya, A.; Ando, T. *Anal. Chem.* 1984. 56, 111-13.

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Appendix A: Derivation of k' for Neutral Compounds

$$k' = \frac{\text{moles}_{micelle}}{\text{moles}_{aqueous}} \quad (\text{equation 3.1})$$

$$v_{apparent} = v_{eof} \frac{\eta_{aqueous}}{\eta_{micelle} + \eta_{aqueous}} + v_{micelle} \frac{\eta_{micelle}}{\eta_{micelle} + \eta_{aqueous}} \quad (\text{equation 3.2})$$

substitute equation 3.1

$$v_{apparent} = v_{eof} \frac{1}{1+k'} + v_{micelle} \frac{k'}{1+k'} \quad (\text{equation 3.3})$$

Given that velocity is the arrival time at the capillary window

$$v_{apparent} = \frac{l}{t_R} \quad (\text{equation 3.4a})$$

$$v_{eof} = \frac{l}{t_{eof}} \quad (\text{equation 3.4b})$$

$$v_{micelle} = \frac{l}{t_{micelle}} \quad (\text{equation 3.4c})$$

substitute equations 3.4a-c

$$\frac{l}{t_R} = \frac{l}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{l}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \quad (\text{equation 3.5})$$

$$\frac{1}{t_R} = \frac{1}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \quad (\text{equation 3.5a})$$

$$\frac{1+k'}{k' t_R} = \left(\frac{1+k'}{k'} \right) \left[\frac{1}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \right] \quad (\text{equation 3.6})$$

$$\frac{1+k'}{k' t_R} = \frac{1}{k' t_{eof}} + \frac{1}{t_{micelle}} \quad (\text{equation 3.7})$$

$$\frac{1+k'}{k't_R} - \frac{1}{k't_{eof}} = \frac{1}{t_{micelle}} \quad (\text{equation 3.8})$$

$$\frac{1+k'}{k't_R} - \frac{\left(\frac{t_R}{t_{eof}}\right)}{k't_R} = \frac{1}{t_{micelle}} \quad (\text{equation 3.9})$$

$$\frac{1}{k't_R} \left(1+k' - \frac{t_R}{t_{eof}}\right) = \frac{1}{t_{micelle}} \quad (\text{equation 3.10})$$

$$\left(1+k' - \frac{t_R}{t_{eof}}\right) = \frac{k't_R}{t_{micelle}} \quad (\text{equation 3.11})$$

$$k' - \frac{k't_R}{t_{micelle}} = \frac{t_R}{t_{eof}} - 1 \quad (\text{equation 3.12})$$

$$k' \left(1 - \frac{t_R}{t_{micelle}}\right) = \frac{1}{t_{eof}} (t_R - t_{eof}) \quad (\text{equation 3.13})$$

$$k' = \frac{(t_R - t_{eof})}{t_{eof} \left(1 - \frac{t_R}{t_{micelle}}\right)} \quad (\text{equation 3.14})$$

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