

Distance Learning Module IV: Separation of Anionic Compounds Using Micelle Electrokinetic Capillary Chromatography

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Abstract

This learning module expands the use of micelle electrokinetic capillary chromatography (MEKC) for the separation of structurally similar charged molecules. A new equation is derived to calculate retention factor to account for electrophoretic mobility of the charged analyte. Upon close examination of the equation, the learner will see there is a need to perform capillary electrophoresis and MEKC separations to calculate retention factor. The module culminates in the determination of retention factor for three anionic drug compounds (indoprofen, sulindac, indomethacin).

*Note regarding improvements in this module:

This learning module is a derivative work of the original learning module IV 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 MEKC for the separation of structurally similar charged molecules. This approach requires the learner to perform both capillary electrophoresis and MEKC runs to obtain the data necessary to calculate retention factor.

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Objective:

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

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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 charged compounds;
- (2) perform 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 three NSAIDs. Step 2 requires the user to determine retention factors experimentally for three NSAIDs using the protocol formulated in Step 1. The experiment outlined here is performed with recommended chemicals (n-decanophenone, dimethylformamide, indoprofen, sulindac, indomethacin). The experiment may be completed with other compounds. 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 uncharged analyte. The qualitative information provided by MEKC can assist in the identification of unknown compounds. The determination of retention factor for charged compounds requires the user to account for electrophoretic mobility of the analyte in order to estimate the association between analyte and micelle.

Application of MEKC to charged analyte. MEKC may also be applied to separate charged analyte of similar charge-to-size ratio. When charged analyte associates with micelle it assumes the micelle velocity and when charged analyte is not associated with micelle it has a velocity comprised of both bulk electroosmotic flow and electrophoretic mobility. Retention factor (equation 4.1) is also determined from measurable parameters for charged compounds subject to MEKC. However, the calculation includes contributions from electrophoretic mobility. In the case of MEKC separation of negatively charged analyte, the apparent velocity of the compound in aqueous component of the running buffer is the difference of the bulk electroosmotic flow toward the detection window, and

the electrophoretic velocity away from the detection window (towards the injection end of the capillary). The velocity components for an anionic compound separated using MEKC is described by equation 4.2. Using a derivation similar to that for neutral analytes the equation for capacity factor is expressed by equation 4.20. In this case, mobility, μ , is related to velocity by electric field, where electric field is applied voltage divided by total capillary length (see equations 4.17a-c).

$$k' = \frac{\text{moles}_{\text{micelle}}}{\text{moles}_{\text{aqueous}}} \quad (\text{equation 4.1})$$

$$v_{\text{apparent}} = v_{\text{eof}} \frac{\eta_{\text{aqueous}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} + v_{\text{eph-analyte}} \frac{\eta_{\text{aqueous}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} + v_{\text{micelle}} \frac{\eta_{\text{micelle}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} \quad (\text{equation 4.2})$$

[Click here to see the derivation of k' for anions in Appendix B](#)

$$k' = \frac{t_{\text{anion_MEKC}} \left(1 + \frac{\mu_{\text{eph-anion}}}{\mu_{\text{eof_MEKC}}} \right) - t_{\text{eof_MEKC}}}{t_{\text{eof_MEKC}} \left(1 - \frac{t_{\text{anion_MEKC}}}{t_{\text{n-dec_MEKC}}} \right)} \quad (\text{equation 4.20})$$

There are a variety of other conditions that may be used for MEKC to facilitate the separation of anions, neutral compounds, or cations. Micelles may have a net negative charge, as in the case of SDS. They may also have a net neutral charge, or net positive charge. In the later case, the user needs to consider the interaction of cationic surfactant with anionic capillary surface, since the introduction of cationic surfactant often reverses electroosmotic flow. Other, more sophisticated micelles have been used in MEKC, including, mixed micelles, polymeric micelles, polyelectrolyte micelle complexes and bicelles. Depending on the transport mechanisms for analyte and micelle, the derivation for retention factor may be different from what is outlined in Appendix B. The discussion of these other systems in further detail is beyond the scope of this Learning Module. Instead, the reader is referred to other sources that provide an in depth discussion of the derivation of expressions for retention factor [1-3].

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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, sodium dodecyl sulfate, sodium hydroxide, indoprofen, sulindac, and indomethacin.
- (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. Outline the experiments you must do to determine the retention factor (capacity factor) for 3 NSAIDs.
2. Determine the retention factor (capacity factor) for indoprofen, sulindac, indomethacin. These values should be reported as mean values determined from replicate runs. For the MEKC runs, the running buffer is 25 mM CAPs, 100 mM SDS buffered to pH 10. For the capillary electrophoresis runs you will perform, the running buffer is 25 mM CAPs, buffered to pH 10. You should use a ~25 micron inner diameter fused silica capillary ~42 cm in total length, ~32 cm to the window, 20,000 V.

Table 4.1. Free Zone Data				
$L_w = 31.2$ cm, $L_t = 41.2$ cm, $V_{sep} = 20,000$ V, pH 10 CAPS				
	TRIAL			
	1	2	3	
Indoprofen migration time (s)				
μ_{app} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
DMF migration time (s)				
μ_{eof} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
Sulindac migration time (s)				
μ_{app} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
DMF migration time (s)				
μ_{eof} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
Indomethacin migration time (s)				
μ_{app} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
DMF migration time (s)				
μ_{eof} ($\text{cm}^2\text{V}^{-1}\text{s}^{-1} \times 10^{-4}$)				
μ_{eph} $\text{cm}^2\text{V}^{-1}\text{s}^{-1} (\times 10^{-4})$	1	2	3	AVE \pm SD
Indoprofen				
Sulindac				
Indomethacin				

Table 4.2. MEKC DATA			
$L_w = 31.2$ cm, $L_t = 41.2$ cm, $V_{sep} = 20,000$ V, pH 10 CAPS 100 mM SDS			
	Migration Time		
	Trial		
Marker	1	2	3
DMF			
n-dec			
MEKC μ eof $\text{cm}^2\text{V}^{-1}\text{s}^{-1}$ ($\times 10^{-4}$)			
	Migration Time		
	Trial		
Analyte	1	2	3
Indoprofen			
Sulindac			
Indomethacin			
	k'		
Analyte	1	2	3
Indoprofen			
Sulindac			
Indomethacin			
			AVE \pm SD

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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 2, 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.

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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 [4]. You have completed several self-guided exercises designed to expand your skill at performing 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 isotachopheresis, and capillary electrochromatography.

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References

- [1] Nielson, K.R.; Foley, J.P. 1998. Micellar electrokinetic capillary chromatography. 135-82. *In* P. Camilleri, (eds.), *Capillary electrophoresis: Theory and practice*, CRC Press, Boca Raton.
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- [3] Khaledi, M.G. 1994. Micellar Electrokinetic Capillary Chromatography. 43-93. *In* J.P. Landers, (ed.), *Handbook of Capillary Electrophoresis*, CRC Press, Inc., Boca Raton.
- [4] Pappas, T.J.; Gayton-Ely, M.; Holland, L.A. *Electrophoresis*. 2005. 26, 719-34.

Appendix B: Derivation of k' for Anionic Compounds

$$k' = \frac{\text{moles}_{\text{micelle}}}{\text{moles}_{\text{aqueous}}} \quad (\text{equation 4.1})$$

$$v_{\text{apparent}} = v_{\text{eof}} \frac{\eta_{\text{aqueous}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} + v_{\text{eph_analyte}} \frac{\eta_{\text{aqueous}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} + v_{\text{micelle}} \frac{\eta_{\text{micelle}}}{\eta_{\text{micelle}} + \eta_{\text{aqueous}}} \quad (\text{equation 4.2})$$

substitute equation 4.1

$$v_{\text{apparent}} = v_{\text{eof}} \frac{1}{1+k'} + v_{\text{eph_analyte}} \frac{1}{1+k'} + v_{\text{micelle}} \frac{k'}{1+k'} \quad (\text{equation 4.3})$$

Given that velocity is the arrival time at the capillary window (l = length to detection window)

$$v_{\text{apparent}} = \frac{l}{t_R} \quad (\text{equation 4.4a})$$

$$v_{\text{eof}} = \frac{l}{t_{\text{eof}}} \quad (\text{equation 4.4b})$$

$$V_{micelle} = \frac{l}{t_{micelle}} \quad (\text{equation 4.4c})$$

$$V_{eph_analyte} = \frac{l}{t_{eph_analyte}} \quad (\text{equation 4.4d})$$

substitute equations 4.4a-d

$$\frac{l}{t_R} = \frac{l}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{l}{t_{eph_analyte}} \left(\frac{1}{1+k'} \right) + \frac{l}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \quad (\text{equation 4.5})$$

$$\frac{1}{t_R} = \frac{1}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{eph_analyte}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \quad (\text{equation 4.5a})$$

$$\frac{1+k'}{k't_R} = \left(\frac{1+k'}{k'} \right) \left[\frac{1}{t_{eof}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{eph_analyte}} \left(\frac{1}{1+k'} \right) + \frac{1}{t_{micelle}} \left(\frac{k'}{1+k'} \right) \right] \quad (\text{equation 4.6})$$

$$\frac{1+k'}{k't_R} = \frac{1}{k't_{eof}} + \frac{1}{k't_{eph_analyte}} + \frac{1}{t_{micelle}} \quad (\text{equation 4.7})$$

$$\frac{1+k'}{k't_R} - \frac{1}{k't_{eof}} - \frac{1}{k't_{eph_analyte}} = \frac{1}{t_{micelle}} \quad (\text{equation 4.8})$$

$$\frac{1+k'}{k't_R} - \frac{\left(\frac{t_R}{t_{eof}} \right)}{k't_R} - \frac{\left(\frac{t_R}{t_{eph_analyte}} \right)}{k't_R} = \frac{1}{t_{micelle}} \quad (\text{equation 4.9})$$

$$\frac{1}{k't_R} \left(1+k - \frac{t_R}{t_{eof}} - \frac{t_R}{t_{eph_analyte}} \right) = \frac{1}{t_{micelle}} \quad (\text{equation 4.10})$$

$$\left(1+k' - \frac{t_R}{t_{eof}} - \frac{t_R}{t_{eph_analyte}} \right) = \frac{k't_R}{t_{micelle}} \quad (\text{equation 4.11})$$

$$k' - \frac{k't_R}{t_{micelle}} = \frac{t_R}{t_{eof}} + \frac{t_R}{t_{eph_analyte}} - 1 \quad (\text{equation 4.12})$$

$$k' \left(1 - \frac{t_R}{t_{micelle}} \right) = \frac{1}{t_{eof}} \left(t_R + \frac{t_R t_{eof}}{t_{eph_analyte}} - t_{eof} \right) \quad (\text{equation 4.13})$$

$$k' = \frac{t_R + \frac{t_R t_{eof}}{t_{eph_analyte}} - t_{eof}}{t_{eof} \left(1 - \frac{t_R}{t_{micelle}}\right)} \quad (\text{equation 4.14})$$

$$k' = \frac{t_R \left(1 + \frac{t_{eof}}{t_{eph_analyte}}\right) - t_{eof}}{t_{eof} \left(1 - \frac{t_R}{t_{micelle}}\right)} \quad (\text{equation 4.15})$$

The term $t_{eph_analyte}$ is difficult to measure. In free zone capillary electrophoresis the apparent velocity of anionic analyte is the sum of two components: the velocity of the electroosmotic flow and the electrophoretic velocity of the anionic analyte.

$$v_R = v_{eof} + v_{eph_analyte} \quad (\text{equation 4.16a})$$

$$v_{eph_analyte} = v_R - v_{eof} \quad (\text{equation 4.16b})$$

To simplify the representation of the final equation we will substitute mobility, μ , with velocity, v (L =capillary length).

$$\mu_R = \frac{v_R L}{V} = \frac{lL}{t_R V} \quad (\text{equation 4.17a})$$

$$\mu_{eof} = \frac{v_{eof} L}{V} = \frac{lL}{t_{eof} V} \quad (\text{equation 4.17b})$$

$$\mu_{eph_analyte} = \frac{v_{eph_analyte} L}{V} = \frac{lL}{t_{eph_analyte} V} \quad (\text{equation 4.17c})$$

substitute equations 4.17 a,b,c into equation 4.16b

$$\frac{V \mu_{eph_analyte}}{L} = \frac{V \mu_R}{L} - \frac{V \mu_{eof}}{L} \quad (\text{equation 4.18})$$

$$\frac{t_{eof}}{t_{eph_analyte}} = \frac{\mu_{eph_analyte}}{\mu_{eof}} \quad (\text{equation 4.19})$$

$$k' = \frac{t_R \left(1 + \frac{\mu_{eph-analyte}}{\mu_{eof}}\right) - t_{eof}}{t_{eof} \left(1 - \frac{t_R}{t_{micelle}}\right)} \quad (\text{equation 4.20})$$

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