Submission ID #: 68658
Scriptwriter Name: Poornima G
Project Page Link: https://review.jove.com/account/file-uploader?src=20940738

Title: Development, Characterization, and Evaluation of CAGE-Based Ionic Liquid Systems for Transdermal Delivery

Authors and Affiliations:
Saahil Sandeep Baghel, Maja Munck Nikolajsen, Stefanie Dietl, Ragna G. Diedrichsen, Martin Malmsten, Stine Rønholt

LEO Foundation Center for Cutaneous Drug Delivery, Department of Pharmacy, Faculty of Health and Medical Sciences, University of Copenhagen

Corresponding Authors:
Stine Rønholt stine.roenholt@sund.ku.dk

Email Addresses for All Authors:
Saahil Sandeep Baghel saahil.baghel@sund.ku.dk
Maja Munck Nikolajsen maja.nikolajsen@sund.ku.dk
Stefanie Dietl stefanie.dietl@sund.ku.dk
Ragna G. Diedrichsen ragna.diedrichsen@sund.ku.dk
Martin Malmsten martin.malmsten@sund.ku.dk
Stine Rønholt stine.roenholt@sund.ku.dk

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length
Number of Steps: 26
Number of Shots: 55 (7 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: Sound for the interviews was recorded separately

1.1. **Stine Rønholt:** Our research aims to develop safe, durable, and patient-friendly atopic dermatitis therapies using ionic liquids, enabling local delivery of poorly soluble drugs while elucidating their structural interactions with the skin barrier.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What are the most recent developments in your field of research?

1.2. **Saahil Baghel:** Ionic liquids enable local delivery of hydrophilic and hydrophobic compounds to lower skin layers, minimizing systemic exposure, while stabilizing labile molecules, including proteins and siRNA, for extended periods under ambient conditions.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What are the current experimental challenges?

1.3. **Stefanie Dietl:** A current challenge lies in accurately detecting and quantifying individual ionic liquid components within skin layers to understand and improve the selection of specific cations or anions for each application.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

What research questions will your laboratory focus on in the future?

1.4. **Stine Rønholt:** We would further translate our findings on Ionic liquids with Tacrolimus and Bariticinib from porcine to human skin and investigate its immunomodulatory effects to develop localized and clinically relevant treatments.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.3.1*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the Danish Veterinary and Food Administration

Protocol

Videographer's NOTE:
The video files can be found in the following path: 1. Video footage -> PRIVATE-> M4ROOT -> CLIP.
If there is a double digit number in the last slate number for a shot, fx. 2.11.12, it means that there is an extra take of 2.11.1.

2. Recrystallization of Geranic Acid

Demonstrator: Saahil Baghel

2.1. To begin, place a 500-milliliter round-bottom flask on a balance and record its weight **[1]**. Weigh 40 grams of geranic acid directly into the same flask **[2]**.

2.1.1. WIDE: Talent placing a 500 milliliter round-bottom flask on a digital balance and recording the weight.

2.1.2. Talent carefully adding geranic acid into the flask using a transfer pipette..

2.2. Using a transfer pipette, add 18.42 milliliters of acetone to the round-bottom flask to achieve a 70 to 30 weight ratio of geranic acid to acetone **[1]**.

2.2.1. Talent pipetting and adding 18.42 milliliters of acetone into the flask containing geranic acid.

2.3. Then, take a 5-liter beaker and prepare approximately 500 milliliters of dry ice and ethanol mixture **[1]**. Place the round-bottom flask into this cold bath for external cooling **[2]**. When everything except for approximately 18.42 milliliters of liquid has solidified, and no additional liquid solidifies, decant the acetone and impurities from the solidified geranic acid to improve purity **[3-TXT]**.

2.3.1. Talent preparing a cold bath by adding dry ice and ethanol into a 5 liter beaker.

2.3.2. Talent placing the round-bottom flask gently into the cold bath.

2.3.3. Talent decanting the liquid layer and repeating the process for several rounds. **TXT: Decant 5 - 6 times**

2.4. Now, use a rotary evaporator set to 40 revolutions per minute under vacuum to remove the remaining acetone for a minimum of 20 minutes **[1]** or until the round-bottom flask no longer feels cold **[2]**.

2.4.1. Talent placing the flask in the rotary evaporator and operating it under vacuum at 40 revolutions per minute.

2.4.2. Talent touching the flask to feel if it is still cold.

2.5. Weigh the flask containing the recrystallized geranic acid and record the total mass **[1]**. Calculate the yield based on the previously recorded empty flask weight **[2]**.

2.5.1. Talent placing the flask on the balance. **Videographer's NOTE:** 2.5.1 and 2.5.2 in one shot

2.5.2. Talent writing in a notebook.

3. Salt Metathesis Reaction

Demonstrators: Maja Nikolajsen and Saahil Baghel

3.1. For the salt metathesis reaction, weigh choline bicarbonate corresponding to half the number of moles of geranic acid used **[1]**. Now, slowly add it to the geranic acid flask dropwise **[2-TXT]** and calculate the target weight of the tube after the correct amount of choline has been removed **[3]**.

3.1.1. Talent adding choline bicarbonate to a tube and weighing it on a digital balance.

3.1.2. Talent slowly adding the choline bicarbonate dropwise into the geranic acid flask. **TXT: CO_2 is released while mixing**

3.1.3. Talent placing the choline containing tube on weighing balance.

3.2. Stir the reaction mixture at 300 revolutions per minute at room temperature for 18 to 21 hours, or until carbon dioxide release stops **[1]**.

3.2.1. Talent setting the stirrer to 300 revolutions per minute and placing the flask to stir.

3.3. Then, use a rotary evaporator at 60 degrees Celsius and 30 mbar pressure to dry the sample for 40 minutes **[1]**. Begin by increasing the temperature and then slowly lowering the pressure from around 200 bar to prevent foaming **[2]**.

3.3.1. Talent loading the flask into the rotary evaporator.

3.3.2. Talent adjusting the settings in the control panel of the rotary evaporator. **Videographer's NOTE:** 3.3.2 should use the 3.3.23 take (C0020) clips C0021 and C0022 can be used as close up of the screen for the same action

3.4. Dispense the CAGE *(cage)* product into glass vials in appropriate volumes, such as 2 milliliters into 4 milliliter vials **[1-TXT]** and place them under vacuum at 60 degrees Celsius for 48 hours to complete drying **[2]**.

3.4.1. Talent aliquoting 2 milliliters of CAGE into labeled 4 milliliter glass vials. **TXT: CAGE: Choline and Geranic Acid**

3.4.2. Talent placing the vials in a vacuum drying chamber.

4. Characterization of CAGE

Demonstrators: Stefanie Dietl and Maja Nikolajsen

4.1. Dissolve the CAGE sample in deuterated DMSO to a final concentration of 25 milligrams per milliliter, making sure the total volume is at least 500 microliters **[1]**.

4.1.1. Talent pipetting CAGE into a vial and adding deuterated DMSO to dissolve it to the required concentration. **Videographer's NOTE:** 4.1.1 the take 4.1.12 (C0061) should be used

4.2. Using a pipette, transfer 500 microliters of the prepared solution into a clean, dry nuclear magnetic resonance or NMR tube of 5-millimeter diameter **[1]** and cap the tube tightly to prevent evaporation or contamination **[2]**.

4.2.1. Talent pipetting 500 microliters of the prepared solution into a nuclear magnetic resonance tube. **Videographer's NOTE:** 4.2.1. And 4.2.2 one shot

4.2.2. Talent sealing the tube with a plastic cap.

4.3. Place the tube into a 400-megahertz nuclear magnetic resonance spectrometer **[1]**. Set the scan parameters to 8 to 16 scans for proton NMR and 1,000 to 5,000 scans for carbon-13 NMR, depending on signal strength **[2]**.

4.3.1. Talent inserting the sealed nuclear magnetic resonance tube into the instrument.

4.3.2. SCREEN: 4.3.2.mp4 00:10-00:20 and 00:34-00:35

4.4. Process the acquired data using the spectrometer's software **[1]**. Adjust phase and baseline to optimize peak shapes **[2]**. Use the residual solvent peak of deuterated DMSO as a reference **[3-TXT]**. Integrate the resolved peaks and assign them to their corresponding protons in the molecular structure **[4]**.

4.4.1. SCREEN: 4.4.1-(1).mp4 00:27-00:35.

4.4.2. SCREEN: 4.4.2-(1).mp4 00:10-00:15.

4.4.3. SCREEN: 4.4.3.mp4 00:06-00:16. **TXT: Reference: ~ 2.50 ppm for ^{1}H; ~39.52 ppm for ^{13}C NMR**

4.4.4. SCREEN: 4.4.4-(1).mp4 01:10-01:20.

4.5. To determine the water content, dissolve 300 milligrams of CAGE in 300 microliters of methanol **[1]** and load the solution into a 1 milliliter syringe **[2-TXT]**.

4.5.1. Talent preparing the CAGE-methanol solution in a small vial. **Videographer's NOTE**: 4.5.1 changed to 300ml prepared and on camera adding methanol use take 4.5.12 (C0056)

4.5.2. Talent loading the solution into a syringe. **TXT: Weigh the syringe before and after injection**

4.6. Inject the prepared sample into the coulometric Karl Fischer titrator through the septum **[1]**. Input the weight of the added sample and press **Enter** to display the water content in percent weight by weight **[2]**. Adjust for the water content of methanol to determine the actual water content in CAGE, which is typically 1 to 2 percent. **[3]**.

4.6.1. Talent injecting the sample into the titrator via septum using a syringe.

4.6.2. Talent inputting sample weight and display of water content result on screen.

4.6.3. SCREEN: 4.6.4-(1).mp4 00:02-00:16.

5. Dermatome-Processing for Ex Vivo Assessment of Skin Barrier Interaction

Demonstrator: Maja Nikolajsen

5.1. Cut expanded polystyrene to fit the dermatome blade and wrap it with grafting tape **[1]**.

5.1.1. Talent cutting expanded polystyrene to the required shape and wrapping it in grafting tape.

5.2. Using blunt dissection tools, remove all visible muscle and fat tissue from the underside of the skin **[1]**. Then, trim the skin using a hair trimmer to eliminate as much hair as possible **[2]**.

5.2.1. Talent scraping off fat and muscle tissue from the skin surface.

5.2.2. Talent trimming the skin surface with an electric hair trimmer. **Videographer's NOTE:** 5.2.2, the beginning is the end of the previous shot

5.3. Now, rinse the cleaned skin with PBS at pH 7.4 to remove any remaining blood and loose hair **[1]**. Using a knifecut the skin into 5 by 15-centimeter sections to fit the expanded polystyrene blocks **[2]** and fix the cut pieces onto the blocks using needles **[3]**.

5.3.1. Talent pouring PBS on the skin in a stainless steel tray.

5.3.2. Talent measuring and cutting skin into rectangular strips.

5.3.3. Talent pinning the cut skin pieces onto the polystyrene using surgical needles.

5.4. Then, set the dermatome to a thickness of 0.5 millimeters and section the skin across its surface **[1]**. Place the dermatomed skin between two layers of grafting tape and store it at minus 70 degrees Celsius **[2]**.

5.4.1. Talent operating the dermatome to section skin at 0.5 millimeter thickness.

5.4.2. Talent stacking and wrapping the dermatomed skin between grafting tape layers.

6. Trans-Epidermal Water Loss (TEWL) Assessment

Demonstrators: Saahil Baghel and Maja Nikolajsen

6.1. Prepare PBS by dissolving one tablet in 200 milliliters of ultrapure water **[1]** and degas the solution in an ultrasonic bath for 20 minutes **[2]**.

6.1.1. Talent dissolving a tablet into a beaker of ultrapure water and stirring. **Videographer's NOTE:** 6.1.1 use take 6.1.12

6.1.2. Talent placing the PBS solution into an ultrasonic bath for degassing.

6.2. Then, turn on the water bath connected to the Franz diffusion cells and add approximately 12 milliliters of degassed PBS and a magnetic bead to each cell **[1]**. Start magnetic stirring and adjust the temperature to 43 degrees Celsius **[2]**.

6.2.1. Talent pouring PBS into Franz cells and dropping in magnetic beads. **Videographer's NOTE:** 6.2.1 and 6.2.2 one shot

6.2.2. Talent turning on the magnetic stirrer.

6.3. Using a scalpel, cut circular sections of dermatomed skin approximately 3 centimeters in diameter **[1]**. Carefully mount each section onto the Franz diffusion cells using clamps and sealing rings with the stratum corneum facing upward **[2]**.

6.3.1. Talent cutting out skin discs using a scalpel and circular template.

6.3.2. Talent mounting the skin on the Franz cells and securing with clamps and rings.

6.4. Next, fill the receptor chambers completely with PBS using a glass Pasteur pipette, making sure they are filled up to the mark that indicates the known volume of receptor medium **[1]**. Invert the Franz cells to remove air bubbles and hydrate the mounted skin for 30 minutes **[2]**.

6.4.1. Talent carefully filling each receptor chamber with PBS using a Pasteur pipette. **Videographer's NOTE:** 6.4.1 and 6.4.2 one shot, use take 6.4.12

6.4.2. Talent inverting the cells to release trapped bubbles and letting them sit for hydration.

6.5. Turn on the trans-epidermal water loss device and allow it to equilibrate for 20 minutes or until stable **[1]**. Now, place the TEWL probe on the surface of each Franz cell **[2]** and press **Start** in the software. Hold the probe steady until a reading is displayed **[3]**.

6.5.1. Talent powering on the TEWL device. **Videographer's NOTE:** 6.5.1 and 6.5.2 combined (system was already on)

6.5.2. Talent positioning the TEWL probe onto the Franz cell.

6.5.3. SCREEN: 6.5.3.mp4 00:04-00:10.

6.6. Then, apply 300 microliters of the sample onto the surface of the mounted skin in triplicate **[1]**. Occlude the surface by placing grafting tape over the Franz cell and incubate a for 24 hours **[2]**.

6.6.1. Talent pipetting 300 microliters of sample onto each skin section in three replicates.

6.6.2. Talent sealing the Franz cells with grafting tape.

6.7. After incubation, remove the sample by wiping the skin with lint-free wipes **[1]** and let the skin rest for 30 minutes before measurement **[2]**. Finally, measure the trans-epidermal water loss for each cell using the TEWL device **[3]**.

6.7.1. Talent gently wiping residual sample from the skin surface with lint-free wipes. **Videographer's NOTE:** 6.7.1 and 6.7.2 one shot (the talent just put it up)

6.7.2. Talent setting the cells aside.

6.7.3. Talent performing TEWL measurements on all Franz cells post-incubation.

Results

7. Results

7.1. The proton NMR spectrum confirmed the 2 to 1 molar ratio of geranic acid to choline, validating the expected stoichiometry of CAGE **[1]**.

7.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the peak labeled "9"*

7.2. The carbon-13 NMR spectrum further confirmed successful CAGE synthesis with consistent chemical shifts for all expected carbon environments **[1]**.

7.2.1. LAB MEDIA: Figure 2.

7.3. Transepidermal water loss measurements showed no significant difference between samples treated with PBS, CAGE, or CAGE plus 50 millimolar Span 20, indicating preserved skin barrier integrity **[1]**.

7.3.1. LAB MEDIA: Figure 3.

7.4. Differential scanning calorimetry demonstrated that both CAGE samples remained thermally stable from minus 70 degrees Celsius to 80 degrees Celsius, with no sharp phase transition peaks observed **[1]**.

7.4.1. LAB MEDIA: Figure 4.

1. Geranic acid

Pronunciation link:
https://www.merriam-webster.com/dictionary/geranic%20acid Merriam-Webster

IPA (American): /d͡ʒəˈræn.ɪk ˈæs.ɪd/ Wiktionary+1

Phonetic spelling: juh-RAN-ik AS-id

2. Deuterated DMSO (“DMSO-d6” / dimethyl sulfoxide-d6)

Pronunciation link:
_no direct Merriam-Webster entry found; the generic term “Deuterated DMSO” is explained at Wikipedia “Deuterated DMSO” and other chemistry resources. Wikipedia+1

IPA (American): /duːˈteɪreɪtɪd diːɛmˈɛsoʊ/

- Break-down:

- “Deuterated” /duːˈteɪreɪtɪd/
- “DMSO” spelled out /diː-ɛm-ɛs-oʊ/

Phonetic spelling: doo-TAY-ruh-tid DEE-EM-ESS-OH

1. **Stratum corneum**
   Pronunciation link: https://www.merriam-webster.com/dictionary/stratum%20corneum Merriam-Webster
   IPA (American): /ˌstreɪˈtʌm ˈkɔːrniəm/ Cambridge Dictionary+1
   Phonetic spelling: STRAY-tum COR-nee-um

---

2. **Karl Fischer**
   Pronunciation link: https://www.howtopronounce.com/karl-fischer How To Pronounce
   IPA (American): /ˈkɑrl ˈfɪʃər/
   Phonetic spelling: KARL FISH-er

---

3. **Titration** (in “Karl Fischer titration”)
   Pronunciation link: https://www.merriam-webster.com/dictionary/titration MilliporeSigma+1
   IPA (American): /ˌtaɪˈtreɪʃən/
   Phonetic spelling: TY-tray-shun

---

4. **Deuterated** (as in “deuterated DMSO”)
   Pronunciation link: *No confirmed link found for “deuterated” on Merriam-Webster with the exact term “deuterated” in context, but standard dictionaries and scientific usage guide pronunciation.*

IPA (American): /duːˈteɪreɪtɪd/
Phonetic spelling: DOO-tay-ray-tid

---

5. **Rotary evaporator**
Pronunciation link: *No single link for the full phrase; separate "rotary" and "evaporator" are in dictionaries.*
IPA (American): /ˈroʊtəri ɪˈvæpəˌreɪtər/
Phonetic spelling: ROH-tuh-ree ih-VAP-uh-ray-ter

---

6. **Acetone**
Pronunciation link: https://www.merriam-webster.com/dictionary/acetone
MilliporeSigma
IPA (American): /ˈæsəˌtoʊn/
Phonetic spelling: AS-uh-tohn

---

7. **Ultrapure** (as in "ultrapure water")
Pronunciation link: *No confirmed single link with "ultrapure" in technical dictionaries; general English dictionaries cover it as 'ultra-pure'.*
IPA (American): /ˈʌltrəˌpjʊr/
Phonetic spelling: UL-truh-pyoor

---

8. **Replicate** (when used as a noun or verb)
Pronunciation link: https://www.merriam-webster.com/dictionary/replicate
MilliporeSigma
IPA (American): /ˈrɛplɪˌkeɪt/
Phonetic spelling: REP-li-kate

---

9. **Microliter**
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
MilliporeSigma
IPA (American): /ˈmaɪkroʊˌlaɪtər/
Phonetic spelling: MY-kroh-lie-ter

---

10. **Vacuum** (as in “under vacuum”)
    Pronunciation link: https://www.merriam-webster.com/dictionary/vacuum
    MilliporeSigma
    IPA (American): /ˈvækˌjum/ or /ˈvækjuəm/
    Phonetic spelling: VAK-yoom