**Skin electroporation**

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Duration of the experiment: day 1: 60 min, day 2: 30 min  
Max. number of participants: 4  
Location: Laboratory for skin and planar lipid bilayers  
Level: Basic

**PREREQUISITES**
Participants should be familiar with Laboratory safety (S1). No other specific knowledge is required for this laboratory practice.

**THEORETICAL BACKGROUND**

Transdermal drug delivery offers several advantages over conventional routes of administration. It avoids liver metabolism and the gastrointestinal tract, it is a noninvasive mode of drug delivery with no trauma or risk of infection. In spite of these advantages, only a small number of drugs can be delivered transdermally due to the barrier properties of the skin: namely only small potent lipophilic drugs can be delivered at therapeutic rates by passive diffusion. Transport of most drugs across the skin is very slow and lag-times to reach steady state fluxes are measured in hours. Achieving a therapeutically effective drug level is therefore difficult without the use of some sort of skin permeation enhancement technique. Skin electroporation is one of the physical enhancement methods used to enhance transdermal delivery of the drugs. Although electroporation is normally used on unilamellar phospholipid bilayers of viable cell membranes, it has been demonstrated that it can also be used on multilamellar, intracellular lipid bilayers of the stratum corneum. Hence, electroporation has taken its place among the physical techniques of enhancement of transdermal drug delivery, like iontophoresis and ultrasound.

![Electroporation of the stratum corneum.](image)

**Figure 1:** Electroporation of the stratum corneum.

**The aim** of this laboratory practise is to see how different pulse parameters affect the transdermal molecular flux.
EXPERIMENT
We will measure the concentration of Calcein (fluorescent molecule) which will traverse the skin after different pulses of electroporation and compare it to passive diffusion (Table 1).

Protocol 1/2 (Preparation of the skin and Franz diffusion cells system for skin electroporation):
Cut previously prepared dermatomed skin into pieces that are suitable for use on Franz diffusion cells. Put the skin in between the donor and receiver compartment and fix it with a clamp. Fill the receptor chamber with buffer (PBS, pH = 7.4) to the mark. After putting the cell into the Franz cell holder, fill the donor compartment with 1 ml of calcein solution (concentration 0.1 mM). When you have prepared the whole system, deliver the pulses with electric pulse generator Cliniporator (Igea, Italy), through a pair of platinum electrodes. To assist transdermal delivery with electric current (calcein is negatively charged molecule), put the negative platinum electrode in donor calcein solution and the positive one in the acceptor solution. You leave the system running overnight.

Table 1: Pulses for skin electroporation.

<table>
<thead>
<tr>
<th>Number of the Franz cell</th>
<th>F1-F4</th>
<th>F5-F8</th>
<th>F9</th>
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</thead>
<tbody>
<tr>
<td>Electroporation pulses</td>
<td>Voltage: 1000 V</td>
<td>Voltage: 80 V</td>
<td>Passive diffusion</td>
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<tr>
<td></td>
<td>N. of pulses: 3</td>
<td>N. of pulses: 3</td>
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<tr>
<td></td>
<td>Pulse duration: 500 µs</td>
<td>Pulse duration: 300 ms</td>
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<td>Pause between pulses: 500 µs</td>
<td>Pause between pulses: 100 ms</td>
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Protocol 2/2 (Fluorescence measurements): Take 300 µl samples from the acceptor solution from all of the Franz diffusion cells and measure calcein concentration. Pipette 100 µl of the taken sample into a cuvette and put it into the spectrofluorimeter. Measure the fluorescence at the wavelength of 495/515 nm, which are the excitation and emission wavelengths of calcein.

FURTHER READING:
## NOTES & RESULTS

<table>
<thead>
<tr>
<th>Number of the Franz cell</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
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<th>F6</th>
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<tbody>
<tr>
<td>Measured Fluorescence</td>
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