Permeability studies using PermeaPad® Barrier in a Franz-cell set-up

AIM

Investigate the permeation of caffeine across the artificial, biomimetic barrier, PermeaPad® Barrier.

MATERIALS AND CHEMICALS

Chemicals:

- Caffeine (CAS nr. 58-08-2)
- Purified water
- PBS

Materials:

- Ultrasonic bath
- Magnetic stirrer and stir bar
- Franz-cell permeation set-up with e.g. 5-7 mL chamber volume including temperature control.
- PermeaPad® Barrier, 3 pieces
- Vacuum pipette
- Volumetric flasks (e.g. 10 ml)
- Beaker
- Micropipette (1-5 mL)
- Syringe + Cannula
- Container for samples (depending on quantification method)

Explanation Video:

How to prepare a Franz Cell – using PermeaPad® Barrier - YouTube

➔ https://youtu.be/BDoHqrnSFPg

METHOD

Day 1 (Preparation of donor solutions and stock for calibration):

For the donor solution, prepare a 5 mM caffeine solution in purified water. For this, weigh in 8-10 mg caffeine in a 10 mL volumetric flask. Add 90% of the total volume of purified water and sonicate for approximately 30 min. Make up to final volume (i.e. 10 mL). Add a magnetic stir bar and stir to ensure complete dissolution. If particles still are visible, sonicate the donor solution for an additional 30 min before the permeation experiment. Repeat this procedure to prepare a stock of caffeine in purified water for preparation of a calibration curve. For the standard stock use at least 1 mg more than for the donor solution.

Day 1 (Permeation experiment):

To conduct the permeation experiment, fill the acceptor compartment with PBS (~5 mL; depending on cell size the volume may vary) and slightly overfill it to avoid air bubble formation after you put on the membrane. Adjust the volume to the calibration mark and put in the magnetic stir bar via the sampling neck. Place the PermeaPad® Barrier on top of the Franz cell with the vacuum pipette. After that place the flat flange joint on top of the barrier and then place the donor chamber on top of it. Mount the cell clamp and connect the cell to the temperature control. Set the water bath to 25 °C (the correct temperature is very important). Assemble two more Franz-cells to get 3 replicates.

To start the experiment, add 5mM caffeine solution to the designated donor compartments (depending on cell size the volume may vary) and start the stirring (500 rpm). To facilitate the sampling procedure and to ensure the replicates are following the same time profile, fill the donor compartments with 1 min between each cell.

Take max. 500µL (volume may depend on quantification method) samples every 30 min from all cells for at least 3.5h. Refill the withdrawn solution with the same volume of fresh PBS after each sampling. At the end of the experiment also take a sample from the donor solution. Also take a sample from the 'left over' donor solution.

Quantification (Day 1/2):

Depending on the sensitivity of the instrument, the samples can be analyzed be UV spectroscopy, HPLC-UV, LC-MS/MS, etc.. For quantification of caffeine, prepare standards for a calibration curve by dilution from the standard stock. The concentration range should be approximately 0.2-100 µg/ml.

Conduct the quantification of both acceptor and donor samples according to a suitable method.

ANALYSIS

To determine the apparent permeability (P_{app}) of caffeine, plot the cumulative amount of caffeine (Q; in µg) permeated across the PermeaPad® Barrier per area (A, in cm^2) against time (t; in sec). The linear part of this graph corresponds to steady state flux (*J*; in $\mu g/cm^2 \times s$):

$$J = \frac{dQ}{A \cdot dt}$$

To calculate the P_{app} (in cm/s), the steady state flux is normalized by the donor start concentration (C_0; in $\mu g/cm^3$; 1 cm^3 = 1 ml):

$$P_{app} = \frac{J}{C_0}$$

CONCLUSION

The PermeaPad® Barrier is regarded to have the correct permeation properties when the P_{app} is 2×10^{-5} cm/s (± 5%). However, when an alternative permeation set-up was used (i.e. different cell volumes and/or a side by side cell set-up) the P_{app} may eventually vary to a higher degree due to the different stirring conditions, geometry and/or local temperature differences.