Permeability studies using PermeaPad® Barrier in a side-by-side set-up

AIM

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

MATERIALS AND CHEMICALS

Chemicals:

- Micronized hydrocortisone (CAS nr. 50-23-7)
- Purified water
- PBS

Materials:

- Ultrasonic bath
- Magnetic stirrer and stir bar
- Side-by-side permeation set-up with e.g. 5-7 mL chamber volume (see Figure 1) including temperature control.
- PermeaPad® Barrier, 3 pieces
- Vacuum pipette
- Volumetric flasks (e.g. 100 ml)
- Beaker
- Micropipette (1-5 mL)
- Syringe + Cannula
- Container for samples (depending on quantification method)

Explanation Video:

How to prepare a side-by-side diffusion cell – using PermeaPad® Barrier - YouTube

➔ https://youtu.be/VyvdH2fr7js

METHOD

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

For the donor solution, prepare a 0.1 mM hydrocortisone solution in purified water. For this, weigh in 3-5 mg micronized hydrocortisone in a 100 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. 100 mL). Add a magnetic stir bar and stir overnight to ensure complete dissolution. If particles still are visible the next day, sonicate the donor solution for an additional 30 min before the permeation experiment. Repeat this procedure to prepare a stock of hydrocortisone 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 2 (Permeation experiment):

To conduct the permeation experiment, assemble the Side-by-side cells (3 replicates) according to the manufacturer's instructions or video from PHABIOC using the PermeaPad® Barrier as permeation barrier and connect the cells to the temperature control. Set the water bath to 25 °C (the correct temperature is very important). Remember to add magnetic stir bars to the cells.

When the cells are assembled and connected to the water bath, add 5 mL PBS to the designated acceptor compartments of all cells (depending on cell size the volume may vary). To start the experiment, add 7 ml 0.1 mM hydrocortisone solution in purified water 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 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 2/3):

Depending on the sensitivity of the instrument, the samples can be analyzed be UV spectroscopy, HPLC-UV, LC-MS/MS, etc.. For quantification of hydrocortisone, 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 hydrocortisone, plot the cumulative amount of hydrocortisone (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 $15 \pm 1.5 \times 10^{-6}$ cm/s. However, when an alternative permeation set-up was used (i.e. different cell volumes and/or a Franz 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.