Permeability studies using PermeaPad® GIT Plate

AIM

Investigate the permeation of caffeine across the artificial, biomimetic barrier, PermeaPad® Barrier integrated in a 96-well plate.

MATERIALS AND CHEMICALS

Chemicals:

- Caffeine (CAS nr. 58-08-2)
  - 2 mM (about 0.4 mg/mL) of caffeine dissolved in PBS pH 7.4
- Purified water
- PBS (pH 7.4 (285 – 300 mOsm/kg)

Materials:

- Orbital shaker
- PermeaPad® GIT Plate, 1 set consisting of a bottom plate, insert-plate with integrated barriers and a cover
- Vacuum pipette
- Micropipette (1-5 mL)
- Container for samples (depending on quantification method)

Explanation Video:

https://youtu.be/u2zLglpmu-s

METHOD

Procedure 1 – shaker, Top-Bottom (T-B) orientation, sampling/moving top plate

1. Set orbital shaker-incubator to desired temperature (e.g. 25 °C)
2. Separate the top/insert (donor) and bottom (acceptor/receiver) plates of the PermeaPad® GIT Plate
3. Fill 200 – 400 µL PBS in each acceptor/receiver well
4. Set top/insert plate back into bottom plate
5. Fill the donor wells with up to 200 µL of drug solution
6. Seal the plate with transparent adhesive sealing foil
7. Place the plate on the orbital shaker plate and secure in place. Start rotation at desired speed (e.g. 200 rpm)
    a. use aluminum foil to protect samples from light, if necessary
8. Collect samples from the acceptor/receiver compartments at appropriate time intervals (e.g. every 30 minutes for a total of 5 hours) by either a, b or c:
    a. withdraw samples (100 – 200 µL) and replace withdrawn volume with fresh PBS every time. Samples are collected in separate multiwell plates for quantification
    b. use one well per time point. Samples are collected in separate multiwell plates for quantification
    c. move top/insert plate into a new bottom plate with fresh 200 – 400 µL PBS in each well
9. Drug concentration in the samples is determined using an appropriate quantification method (e.g. UV/Vis microplate spectrophotometer, HPLC-UV, etc.)

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® GIT Plate is regarded to have the correct permeation properties when the P_{app} is 1.31 × 10-5 cm/s (± 4%). However, when an alternative permeation set-up was used (i.e. different cell volumes and/or different buffers) the P_{app} may eventually vary to a higher degree due to the different stirring conditions, geometry and/or local temperature differences etc.