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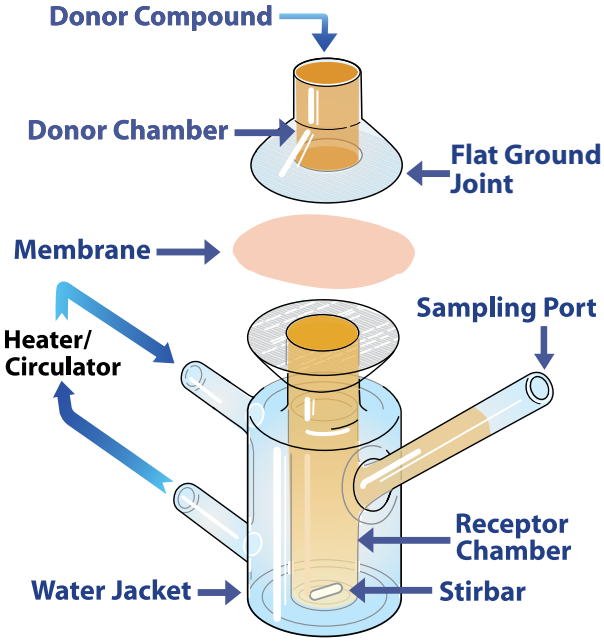
Franz Cell Operating Procedures

1 Preparing Franz Cells for Use

Prior to using the cells, clean the stirbars and donor chambers shown in Figure 3 by rinsing them with Methanol (MeOH) and deionized water several times. Let them dry. Also, fill the receptor chambers with MeOH and stir for 10 minutes. Use a disposable plastic pipette to suck and release MeOH from the receptor chambers several times to clean the receptor chambers, as well as the upper surfaces of the receptor chambers and the sampling ports. Replace MeOH with deionized water and stir for an additional 10 minutes. Finally, turn off the stirring and replace the used deionized water with fresh water. Keep the receptor chambers filled with water until testing. Repeat the cleaning procedures after use.



2 IVRT and IVPT Diffusion Testing

Before testing, the following should be determined based on the project: The fluid in the receptor, membrane (polymeric or skin), the temperature of the receptor fluid, sampling volume and sampling time points.

Prior to starting, set the temperature on the water bath/circulator to a predetermined value and place a stir bar into each cell. Fill the receptor fluid to a level somewhat higher than the receptor surface to avoid creating bubbles when the membrane is mounted. Then, carefully mount a membrane on the top of the receptor chamber, pushing the excessive fluid to the side until the membrane is flush with the joint surface. If there is still some air trapped beneath the membrane, remove the membrane and add more fluid to the receptor chamber to make it overflow slightly. Repeat previous mounting step until no air bubbles appear beneath the membrane. Also make sure the membrane completely covers the orifice of the receptor chamber.

Next, place the donor chamber on top of the membrane and secure it with a clamp. Apply an amount (usually a specific amount stated in the test protocol) of the subject product - such as a gel, lotion or suspension - to each membrane. Cover the donor chamber with triple layers of Parafilm®. Cover the sampling arm with double layers of Parafilm®.

At each predetermined sampling time, remove the Parafilm covering the sampling port. Withdraw a sample (or a volume of sample specified in the test protocol) by inserting a long needle-shaped pipette (e.g., Fisher brand, 9” Pasteur Pipets, #13-678-6B or Fisher pipette tips #02-681-419) far enough into the sampling port so that the sample will be removed from the middle of the chamber and not just from the sampling arm. The same amount of fresh fluid is refilled into the cell through the sampling port by a regular pipette and the sampling port is covered by the same Parafilm removed earlier.

To avoid bubbles, limit the sample volume to 0.5 ml or less. If bubbles are created during the sampling process, carefully lift the cell up out of the cell holder. Quickly reverse the cell to turn it upside down so that bubbles will be released out through the sampling port. Be cautious to prevent the test product leaking out of the donor chamber.

3 Cleaning Franz Cells After Use

After the testing is completed, turn off the V9-CA and the heater/circulator. Remove the donor chambers and membranes. Empty the receptor chambers. Fill the receptor chambers with MeOH. Stir the cells for 10 minutes. Use the MeOH in the receptor chambers to clean the joint surface of the receptor chambers and the sampling arm as described in Section 3. Replace MeOH with deionized water. Stir for another 10 minutes and repeat the previous cleaning step. Finally, replace the used deionized water with fresh water. Keep the receptor chambers filled with water until the next use. The donor chambers should be washed with MeOH as well, rinse first in tap water and then with deionized water.

4 Q&A

a - Can Franz cells be autoclaved? YES. They can be sterilized in an autoclave.

b - Can I use the mobile phase for the HPLC analysis as the receptor chamber's fluid? YES. In general, you can use any solvent for the receptor chamber's medium as long as the solvent provides "sink" conditions for the test compound. Generally, sink conditions are such that the concentration of the compound remains below 10% of the compound's solubility in the receptor chamber's medium. This ensures the permeation or release profile is not rate-limited by the compound's solubility in the receptor chamber fluid. The effect organic solvents have on the membrane should also be considered.

c - How do I select a membrane for *In Vitro* release testing? According to the "FDA Guidance for Industry: Nonsterile Semisolid Dosage Forms: Scale-Up and Postapproval Changes: Chemistry, Manufacturing, and Controls; In Vitro Release Testing and In Vivo Bioequivalence Documentation, May 1997, SUPAC-SS CMC 7", any "appropriate inert and commercially available synthetic membranes such as polysulfone, cellulose acetate/nitrate mixed ester, or polytetrafluoroethylene membrane can be used." Hydrophilic polymeric membranes with a pore size of .45µm are widely used. After determining a suitable membrane during the IVRT method development phase, the same membrane should be used for the duration of the project.

d - How do I decide how much sample volume to remove, i.e. the entire receptor volume or a couple of hundred microliters? If the study has only one time point, the entire receptor chamber's volume can be sampled out. Otherwise, if sampling more than one time point based on the FDA Guidance previously mentioned, a two or three hundred microliter aliquot is recommended for each sample. All of the receptor chamber's fluid may be removed from the receptor chambers, if necessary, but full replacement necessitates having to remove the cells from their holders to refill the receptor chambers without introducing bubbles. Sample volume considerations include the maximum volume that can be removed without introducing bubbles and the minimum volume required for sample analysis.

e - How can I remove the bubbles beneath the membrane? At the beginning of the test, before mounting the membrane, the fluid should be filled to a level somewhat higher than the receptor chamber's upper surface to avoid creating bubbles when membrane is mounted. Then slide the membrane on top of the receptor chamber, pushing the excessive fluid to the side until the membrane is centered around the orifice. If there is still some air trapped beneath the membrane, remove the membrane and add more fluid to the receptor chamber to make it overflow a little bit. Repeat previous mounting step until no air bubbles appear beneath the membrane.

During the testing, remove bubbles only after the samples are taken. Carefully lift the cell up out of the station and turn it upside down so that bubbles are released out through the sampling port. Be careful to prevent the formulation in the donor chambers from exiting the donor chambers.

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