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General Catalog

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Description — Page Numbers

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Diffusion Cell Basics

This document was written to help researchers, new to diffusion studies, choose a diffusion cell system that is best suited to their work.

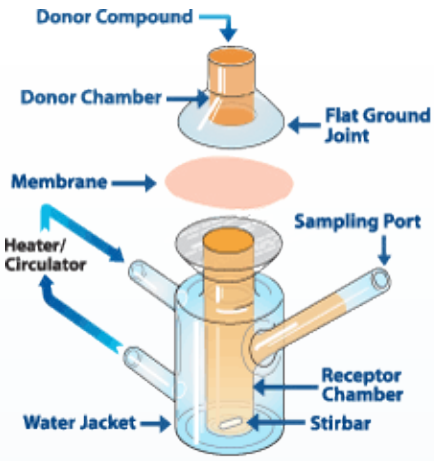
Terms

Diffusion - the concentration gradient driven, and random movement of particles

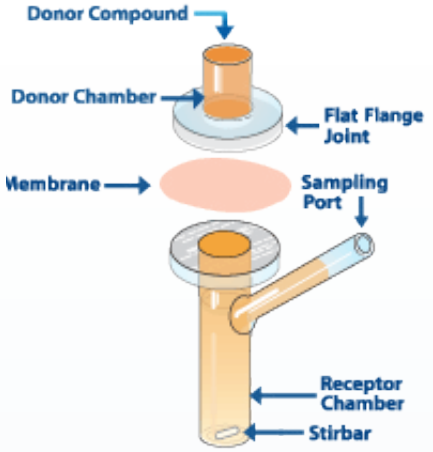
Membrane - any biological or manmade layer of material

Diffusion Cell - a donor chamber, receptor chamber, membrane, and cell clamp to hold the assembly together

Donor Chamber - the part of a diffusion cell, where the compound of interest, is placed at the beginning of a study



Jacketed Franz Cell With Flat Ground Joint

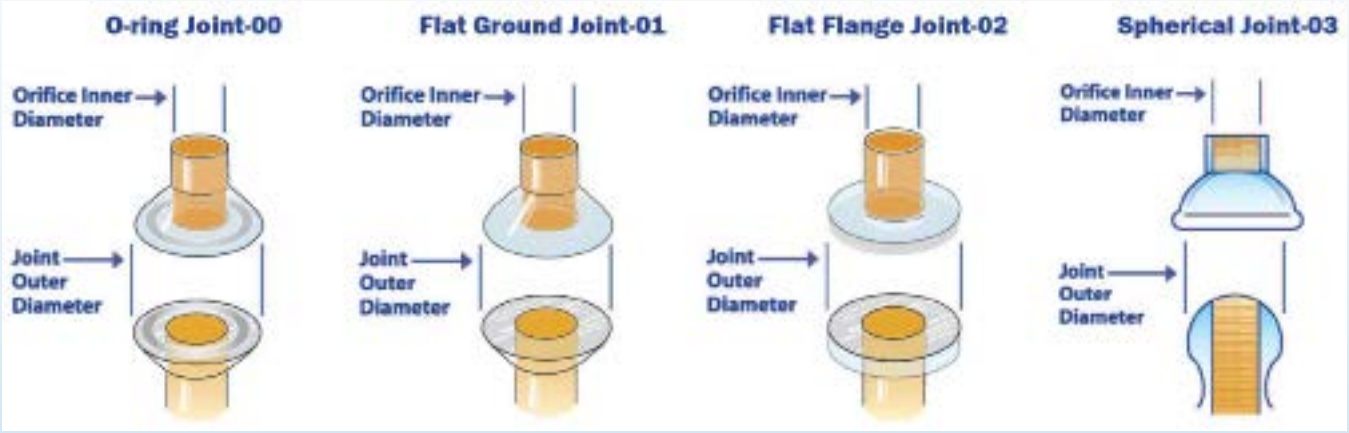


Unjacketed Franz Cell With Flat Flange Joint

Receptor Chamber - the part of a diffusion cell, into which particles of the compound of interest may migrate

Joint - the interface between the donor chamber and receptor chamber, or the interface surface of each chamber

Orifice - the hole in the joint surface of either the donor or receptor chamber, usually the receptor chamber, the same hole covered by the membrane



Orifice Diameter - the diameter, in metric units and usually millimeters, of the orifice - the orifice diameter is how diffusion cell size is specified. A 9mm Franz Cell has a 9mm orifice diameter. A 15mm Side-Bi-Side Cell has a 15mm orifice diameter.

Orifice Area - the area of the orifice in centimeters, which is important to know for diffusion calculations

Receptor Volume - the volume of the receptor chamber in milliliters, which is important to know for diffusion calculations

Sampling Arm - a port through which a device is inserted, to withdraw receptor fluid from the receptor chamber. Some unjacketed Franz Cells have large diameter sampling arms for pouring out all the receptor fluid for analysis, instead of just removing a sample. These are called dump cells.

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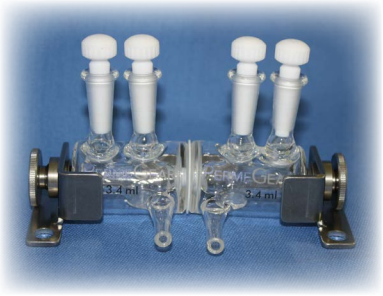
Diffusion Cell Basics



Side-Bi-Side Cell

Franz Cell - a versatile blown glass diffusion cell which locates the membrane horizontally and is usually used in the development of transdermal products having a donor chamber open to the air and a stirred receptor chamber

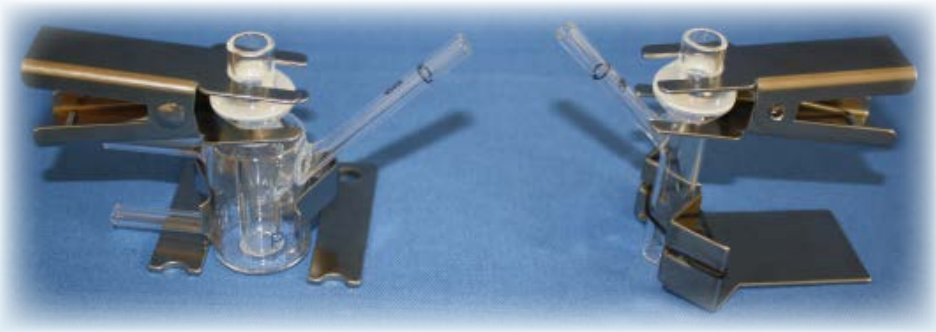
Side-Bi-Side Cell - a blown glass diffusion cell which locates the membrane vertically between the donor chamber and receptor chamber, both of which are stirred and closed to the air

Franz Cell

In-Line Cell - a polychlorotrifluoroethylene (CTFE) flow type diffusion cell which locates the membrane horizontally and has a donor chamber open to the air, a very small receptor chamber, and is almost always used in an automated sampling system

Flow Porting - additional ports added to the receptor chamber of a Franz Cell or Side-Bi-Side Cell which allow liquids to be pumped through the chamber to draw off the compound of interest as it permeates through the membrane. Flow porting may also be added to donor chambers to draw off volatiles or recirculate other fluids or gasses.

Heating Jacket - a glass chamber around the receptor chamber of a Franz Cell, or each chamber of a Side-Bi-Side Cell, that allows circulated liquid, usually water, to maintain other than ambient temperature in the diffusion cell



Jacketed Franz Cell *Unjacketed Franz Cell*

Jacketed Cell - a diffusion cell with a heating jacket

Unjacketed Cell - a diffusion cell without a heating jacket that may be placed in a temperature controlled environment to achieve the desired temperature, when other than ambient temperature is required.

Important Notes *- PermeGear makes three varieties of diffusion cells, Franz Cells, Side-Bi-Side Cells, and In-Line Cells. "Franz Cell" is sometimes used erroneously when "diffusion cell" is what was intended. A Franz Cell is a type of diffusion cell. All Franz Cells are diffusion cells but not all diffusion cells are Franz Cells. "Franz" is correctly pronounced when it rhymes with lands, not fronds.*

Diffusion Cell Selection

It is possible to successfully use a diffusion cell for a purpose other than what it was primarily designed for. Clever researchers are always conjuring up novel adaptations. If you feel that your study requires something other than the norm, don't be afraid to ask us for help. PermeGear makes over 800 different models of Franz and Side-Bi-Side Cells.

Generally, Franz Cells are used in the development of transdermal applications. Side-Bi-Side Cells are used when permeation through membranes inside the body are studied. In-Line Cells are primarily used in transdermal research. Any of these cells may be used for other applications if careful consideration is given to the study design.

Franz Cells

As noted in the Terms section above, a Franz Cell is referred to by its orifice diameter. A 25mm Franz Cell has an orifice of 25mm. A standard 25mm Franz Cell also has a receptor volume of 20ml but the cell should not be referred to as a "20ml Franz Cell"; it is a 25mm Franz Cell with a 20ml receptor volume. The outer diameter of the joint on a 25mm Franz Cell is about 42mm, but the cell is properly referred to as a 25mm Franz Cell. Any membrane with an outer diameter that is the same size as the orifice of a Franz Cell cannot be used with that Franz Cell, as the membrane will fall into the receptor chamber.

PermeGear manufactures Franz Cells with 5mm, 7mm, 9mm, 11.28mm, 15mm, 20mm, 25mm, 30mm, and larger orifice diameters. Experience has shown that Franz Cells with orifice diameters smaller than 5mm, do not expose enough of the membrane to the receptor fluid and detectability of the subject compound may not be possible.

When mounting a membrane on a Franz Cell receptor chamber, it is critical that the membrane completely cover the receptor chamber orifice. The larger the orifice, the larger the margin or distance between the edge of the orifice and the outer edge of the membrane, should be. When working with Franz Cells up to 9mm in orifice diameter, it is recommended that there be at least a 3mm margin. When using a Franz Cell with an orifice diameter of 11.28mm and 15mm, there should be at least a 5mm margin. 20mm and 25mm Franz Cells should have at least a 7mm margin. When using a biological membrane which tends to be slippery, particular care must be taken so the tissue remains in place over the orifice and does not slide out of position and expose the donor chamber directly to the receptor chamber. Improper placement of the tissue will also result in leakage of the compound from the donor chamber out through the periphery of the joint.

5mm Franz Cells are often used for studying permeation into and through mucosal tissues. Bigger cells may be used if tissue specimens large enough to be properly used in those cells are available.

9mm is the orifice diameter most used when working with human skin. Other sizes may be used if desired. Important considerations in cell selection when using human tissues are; the availability and cost of the tissues, regulatory requirements, and biohazardous material concerns.

15mm Franz Cells are commonly used with polymer membranes and artificial membranes. One reason for this is that many membranes are supplied 25mm in diameter so they cover the entire orifice with an appropriate margin. Also, the outer diameter of a 15mm flat ground joint is about 28mm, so the edge of the membrane is neatly inside the joint.

When using a Franz Cell there may be evaporation of fluid from either chamber out through the side of the joint. It is recommended to wrap the joint of an assembled Franz Cell with parafilm to help prevent evaporation. The end of the sampling arm should also be covered using three layers of parafilm.

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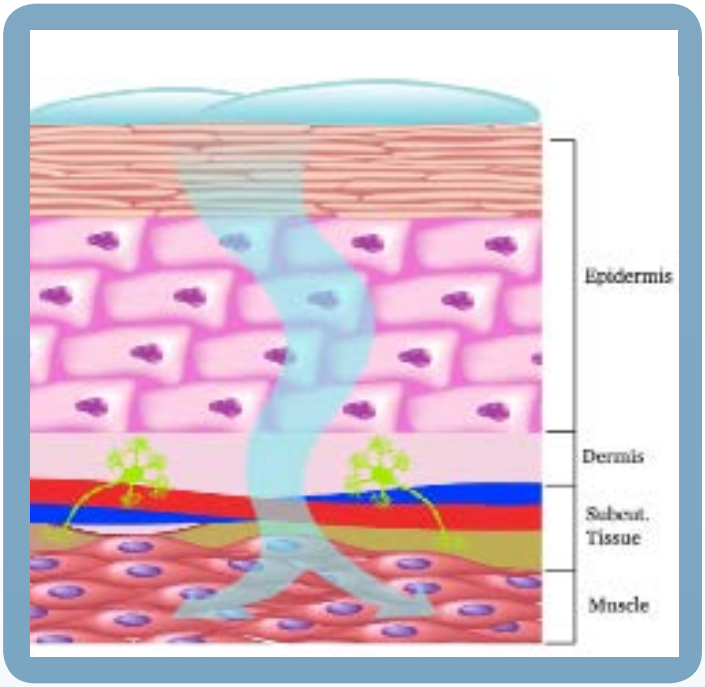
Diffusion Testing Fundamentals

Introduction

When deciding which type of diffusion cell system you need, or before running an experiment with your new diffusion cell or system, it is important to not only be familiar with the equipment but have a well planned experimental protocol with clear objectives. What do you want to know about a particular compound or membrane? Does it matter which type of membrane you use? What membranes are available that will fit your needs? How will you interpret and present the results? There are many parameters to consider, and working out the details beforehand will reduce the number of failed attempts and produce consistency between experiments that is crucial when comparing results. This paper will address basic concepts of diffusion, describe various materials from which to choose for your diffusion study, and describe the components of diffusion cells and diffusion systems.



Background Technology

Diffusion is, by definition, the random movement of molecules through a domain driven by a concentration gradient, from high concentration to low concentration. In vitro diffusion is generally passive diffusion of a permeant from a vehicle in the donor chamber, through an artificial or biological membrane into a receptor fluid in a receptor chamber, disregarding delivery systems such as iontophoresis and microneedles. The permeant is the molecular species moving through or into the tissue/membrane. Permeation is the movement of the permeant through the membrane that encompasses first partitioning the membrane and then diffusion through the membrane. Penetration can occur into the membrane without necessarily diffusing, or passing through, the membrane.

Flux is the amount of permeant crossing a membrane per unit area into the circulating system per unit time, and for in vitro permeation this “system” is the receptor chamber, expressed in units of mass/area/time. Similarly, accumulation is the amount of permeant crossing a membrane within a certain time, expressed in units of mass/area. Diffusivity is a property of the permeant and is a measure of how easily it penetrates a specific membrane expressed in units of area/time. The permeability coefficient (Kp) describes the rate of permeant penetration per unit concentration expressed in distance/time.

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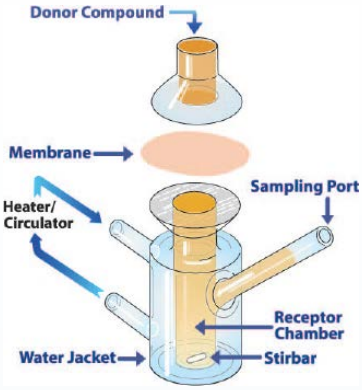
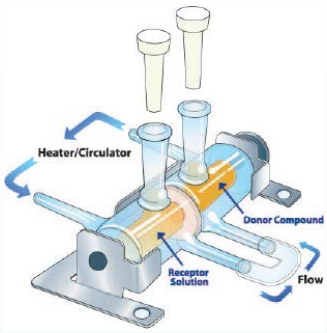
Diffusion Testing Fundamentals

Static cell (vertical or Side-Bi-Side):

Description: Franz type cell or Side-Bi-Side Cell, fixed volume receptor chamber, controlled temperature, port to sample receptor fluid, stirred receptor fluid (Side-Bi-Side Cells allow stirring of both the donor and receptor chambers.)





Uses: Evaluating compound uptake into a membrane, finite dose permeation, steady state flux of compounds (either alone or in formulations.)

Permeability of the compound

&

Permeability of the tissue

⇓

Determines rate across membrane (flux)

⇓

Determines concentration in receptor chamber

Considerations:

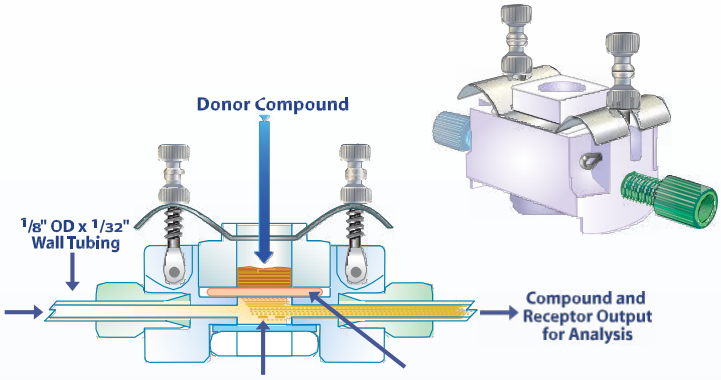
1. If you are using a highly permeable compound with a large volume receptor chamber, the large volume reduces the gradient, so build up of the compound in the receptor part is not a problem (sink conditions are maintained).

2. If you are using a highly permeable compound with a small receptor chamber, the buildup of compound reduces the concentration gradient and therefore slows the flux of the compound (non-sink conditions).

3. If you are using a low permeability compound, then detection of the compound in a large volume receptor chamber can be a problem.

Continuous flow cell or flow-thru cell:

Description: Franz type cell or In-Line Cell, fixed volume receptor chamber, controlled temperature - Franz type cells are stirred, In-Line Cells have continuous flow which causes turbulence in the receptor chamber and simulates stirring, flow rate is adjustable, permits automated sampling.



Uses: Mimics in-vivo (flow equates blood flow), evaluating compound uptake into membrane, finite or infinite dose permeation, steady state flux and Kp of compounds (either alone or in formulations.)

Permeability of the compound

&

Permeability of the tissue

⇓

Determines rate across membrane (flux)

⇓

Determines concentration in receptor chamber

⇓

Determines rate of clearance (flow rate)

Considerations:

1. If you are using a highly permeable compound, the high flow rate necessary to clear the receptor chamber to maintain gradient can result in a large volume of permeant to analyze.

2. If you are using a low permeability compound, the flow rate necessary to clear the receptor chamber can result in a large volume of permeant for which detection can be a problem.

3. A smaller receptor chamber requires less permeant to clear the receptor chamber which results in less permeant volumes and better detection.

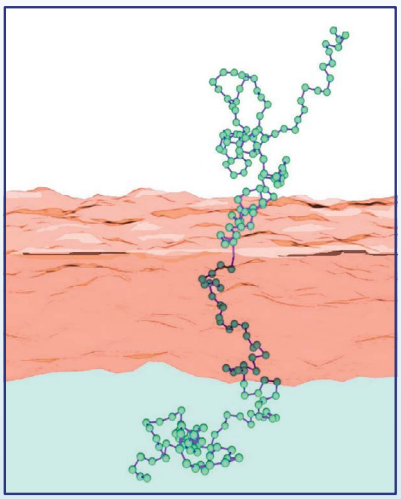
II. Membrane Types

1. Human tissue ex vivo: It may be difficult to obtain sufficient quantities of normal, healthy human tissue to perform permeability experiments with large enough sample sizes for statistical analysis. Many laboratories use human cadaver skin obtained from accredited U.S. human tissue banks. There might be ethical and legal considerations in obtaining human tissue biopsies and surgical specimens.

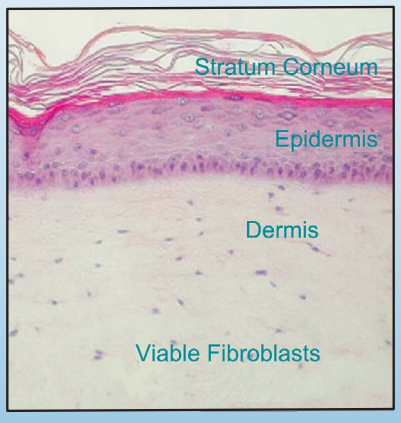
2. Small animals: Models such as rats, mice, and rabbits have traditionally been used in research for human tissue permeability as they are usually inexpensive to purchase and maintain. Drawbacks are the tissue areas are usually thinner than human skin and have a different morphology resulting in higher compound permeabilities.

3. Large animals: Monkeys, dogs, pigs, and other large animals have also been used extensively but may be expensive to purchase, especially in the cases of monkeys and dogs. Pig and monkey soft tissue is very similar to human soft tissue in terms of morphology and function, therefore is widely used as a surrogate for human skin.

4. Polymeric membranes: These kinds of membranes are usually used for in vitro release testing (IVRT). 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-polymeric membranes with a pore size of 0.45 µm are used. Once set during the IVRT method developing phase, the same membrane should be used for the entire duration of the project.



5. Human skin equivalents (HSEs): These are tissues engineered 3D skin constructs (such as MatTek products) that utilize a combination of cultured human skin cells and extracellular matrix components under controlled culture conditions. The advantage of these tissues is that viability can be maintained. The main disadvantage is that the permeability of the simulated tissue is generally higher than that of in vivo.



TISSUE PREPARATION / STORAGE

Once you have selected the membrane you will use for your study, it is imperative that you know the specifics for that membrane such as the source, age, integrity, and shelf life. If it is human or animal tissue, you will need to know the health and age of the animal, time of excision, race and gender of the human, site of excision (for skin) and storage conditions before utilization. Most skin can be frozen and used at a later date without affecting permeation. Certain tissues degrade quickly and must be used immediately or kept moist and refrigerated until use. It is also important to note if any chemicals were applied to the tissue such as an alcohol wipe that could alter the lipid permeability barrier or if there was possible damage from instruments or mechanical damage. Any damage to tissue or alteration of the permeability barrier can bias your results. Consistency in tissue retrieval, handling and storage can minimize erroneous data, and precise information about the tissue can help to determine if the results are valid. You can find information pertinent to the tissue type you plan to use and appropriate tissue handling technique by referencing published literature.

III. Donor Formulations

The compound of interest can be prepared in a variety of formulations - liquid solutions, suspensions, creams, gels, ointments, lotions, pastes, powders, or adhesive patches. The formulation chosen should mimic the real world application as much as possible. Varying the formulation, concentration, and adding permeability enhancers can also help you gain valuable information about the permeant characteristics and behavior.

1. Application - infinite dose/finite dose: The concentration of the permeant depends on the study aims.

a) An infinite dose, one in which the permeant will not be depleted from the donor formulation over the course of the experiment, will produce fundamental permeation behavior and is used when testing that behavior in the presence of permeability enhancers.

b) A finite dose mimics the amount of permeant that would be practical in actual use; for example, a specific amount of drug to be administered to a patient. The aim would be to determine what amount of the permeant needs to be incorporated in a specific vehicle that will deliver the required amount of drug to the patient in order to be effective.

c) An alternative application would be to mimic application of a compound that would in actuality temporarily remain on the tissue; for example, a salve applied to the skin or a drug to the inside of the mouth. The permeant would be applied to the tissue for a short period of time, it would then be removed, and samples would be collected for a length of time following permeation.

2. Vehicle formulation: Also named blank or control formulation, vehicle formulation is the application dose without the active pharmaceutical ingredient (API) or drug agent. The vehicle in which you choose to incorporate the permeant of interest, whether liquid, semi-solid or paste, will again depend on your study aims. There are several things to consider:

a) Aqueous solutions and phosphate buffers are good options for getting basic permeation data.

b) Lipophilic compounds have a low aqueous solubility and may require alternative vehicles such as water/alcohol mixtures or propylene glycol. It must be noted that these additives may interact with the structure and permeability barrier of the tissue and thus change the flux of the permeant.

c) Inert materials can affect the flux of the permeant through both artificial and animal membranes.

d) The nature of the vehicle can alter the potency of certain enhancers. That is, the same enhancer can affect the penetration of a specific permeant differently depending on the vehicle it is incorporated in.

e) Selection of buffer is important to control the degree of permeant ionization. Adjusting pH can also fully dissociate the permeant into charged ions.
f) The thermodynamic activity (the driving force for permeation) of the same concentration of permeant can change drastically by modifying the vehicle components.

3. Permeant detection: Consideration must be given to the method of permeant detection. This can be performed by chromatographic or spectroscopic methodologies, or the use of radio-labeled permeants. A discussion of advantages and problems associated with these methods can be found under Section V. SAMPLING.

4. Other considerations: For each formulation tested, a minimum of three parallel cells should be used for every run. Due to biological variability in skin, in many cases 5-6 replicates should be run per group. Evaporation of compounds, solvents, or formulations from donor chambers may occur, especially if the cells are maintained at high temperatures.

IV. Receptor Media

Selection of a receptor solution depends on the nature of the permeant and the type of diffusion cell used. An ideal receptor medium for an in vitro permeability experiment should mimic the in vivo situation. Flow-thru systems minimize accumulation in the receptor chamber, thus aqueous receptor fluid is usually adequate. This could be a concern for static cells where the permeant is not continually cleared.

Solubility of a compound should be taken into consideration so that the compound is at its desired form in the donor formulation as well as being delivered at a certain amount in the receptor medium; which would affect the diffusion gradient and could slow diffusion.

Aqueous receptors are common for hydrophilic and moderately lipophilic permeants. Phosphate buffer saline (PBS, pH 7.4) should be used if the permeant is ionizable. For un-ionizable permeants, a solubilizer added to the receptor medium should be sufficient. More lipophilic permeants or permeants with low aqueous solubility require additional solubilizing agents such as surfactants, protein (bovine serum albumin) or organic solvents (ethanol/water systems). The effect of these solubilizers on the penetration of your compound of interest needs to be considered as these solubilizers may cause damage to the barrier or changes in the tissue which can alter the permeability results. They should, therefore, be used at the minimum concentration necessary to solubilize the permeant.

V. Sampling

The best method of sampling in terms of time intervals, frequency, and volume, and the best method of reporting the permeation of a compound of interest will depend on your research question. Do you want to know how much compound crosses a membrane at short time intervals, the total amount of compound that crosses the membrane over a long sampling period, or the total amount of compound found within the membrane after a given exposure period? Do you want to calculate flux, accumulation or permeability constant (Kp)? The following addresses factors that will help you determine these parameters.

Flux and permeability can be determined using radio-labeled or unlabeled compounds. The calculation of each parameter can be found in the next section. The flux of a compound can be converted to an amount of compound (in ng or µg) per time interval. Regardless of how your analysis will be performed, you need to know the time points of the specific samples to report the results as either of two types of accumulation:
1 - The total quantity of the compound in the collected sample per time interval, regardless of volume, and
2 - As the concentration of the compound, i.e. the amount of compound per standard volume of sample.

Radio-labeled compounds can be detected in very small quantities and in a large volume of receptor medium. Analysis is usually quite rapid and accurate. The entire collected volume can be easily counted using a scintillation counter, and then the flux or Kp calculated from the counts per minute (cpm) obtained can be converted to the amount of compound in any given sample at any given time interval by knowing the specific activity of the labeled compound. Many compounds are readily available and inexpensive to purchase, but other compounds, such as pharmaceuticals, are very expensive to synthesize and may have handling and storage issues. For example, a radio-label could be unstable in the donor formulation.

Non-radio-labeled compounds can also be used in permeability experiments and detected with methods including high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and fluorescence. These methods are versatile; they can be adapted to many permeants, and you can often conduct multiple analyses. It is possible to determine if the same species of compound that permeated the membrane was that which was applied. The disadvantage to these detection methods is that very small quantities of compound can go undetected or under-detected because of the large volume in the receptor chambers of static cells or large quantities collected from flow-thru cells. As small samples are required for quantification by HPLC or ELISA, you need to take aliquots of the sample and then calculate the total compound in a given sample over a given interval. You can also decrease the volume of the sample by drying it down to a total quantity of sample that can then be analyzed.

V. Sampling (Cont'd.)

intervals for the samples from all the cells will be exactly the same. There may, however, be slight variations in the volume of receptor fluid pumped through each diffusion cell into the collection vials. This is due to slight variations of the tubing element diameters or clamping pressures in the pump cassettes but is not a concern if the total volume of sample is being analyzed. If aliquots are taken for analysis, then the volume of each collected sample must be measured so that the total amount of compound in the sample can be calculated precisely. For example, if a 100 µl aliquot of sample is determined to contain 2 pg of compound, then there would be 20 pg total in a 1 mL sample but 24 pg in a 1.2 mL sample.

It may be necessary to obtain a small volume of receptor fluid over a long period of time with your continuous flow system. This would occur when you want to reduce the amount of sample for analysis to avoid pipetting or to detect a compound that you know would only penetrate in very small quantities over a period of time. In essence, you are converting to a "static" system. In this instance you would turn the pump off for a period of time and then quickly purge the receptor chamber by running a specific volume through it to obtain your sample. This is a valid method if you report the time interval correctly. In this case, the time interval would be from the time the pump was turned off through the end of the purging of the receptor chamber. Keep in mind with this method that the driving force for diffusion is the concentration gradient. If the membrane is very permeable, the compound could accumulate quite rapidly in the receptor medium, consequently increasing the concentration in the receptor chamber and thus slowing diffusion. You must also be sure to adequately purge all the compound that has accumulated in the receptor chamber.

A buildup of compound in the tissue followed by a slow release into the receptor medium can occur. This has been demonstrated in experiments where a compound of interest formulated in a liquid, gel or paste is applied to a membrane and then removed after a short period of time but samples are collected after the removal. The compound may continue to diffuse for some time up to several hours after removal of the compound from the membrane surface. This can be attributed to a reservoir effect. The upper layers of the tissue absorb the compound fairly rapidly, and once saturated, the compound diffuses through the deeper layers of the tissue and into the receptor medium at a fairly constant rate. Again, as long as the concentration gradient remains, the diffusion will continue.

The frequency of samples collected will be determined by the goals of your experiment. If you have no prior concept of the permeability of a membrane to a particular compound, it is initially prudent to take small samples over a long period of time to determine the onset of steady state flux and the overall permeability. If you know the membrane is highly permeable to the compound, you will want to take very short and frequent samples. If you only want to know the total amount of compound to cross the membrane in a given time period, you can take fewer samples with longer time intervals. It is best to do several replicates for a given compound and condition, and then average the amount of compound collected at each time interval for those replicates. You can then plot these results over the entire experimental period, which will provide you with of reliable data. For example, one scenario would be to collect for very short intervals in the beginning of an experiment, such as 15 minute intervals for one hour, 30 minute intervals for 2 hours and then 60 minute intervals for several hours. This will allow you to determine how quickly a drug permeates, when it reaches steady state flux, and finally the total quantity of compound permeating the tissue over the entire sampling period. Using this you can calculate flux or Kp for whatever time period is of interest for this compound. You can determine the amount of radio- labeled compound still in the tissue by autoradiography or by solubilizing the tissue and counting in a scintillation counter. You can also identify compound in the tissue by grinding and/or homogenizing it in liquid and centrifuging off the liquid to be analyzed with HPLC, ELISA, Luminex or pyrosequencing

VI. Calculating Flux and Permeability Coefficient (KP)

Flux (J) is the amount of permeant crossing the membrane per time. It is given in units of mass/area/ time or in units of radioactivity/area/time. Note: If the permeant was applied in a finite dose, then you can only calculate a flux by the formula: J = Q /(A•t) where Q is the quantity of compound traversing the membrane in time t, and A is the area of exposed membrane in cm2. Units of flux are quantity/cm2/min.

Examples: (µg/cm2/h) or (pg/cm2/min) (cpm/cm2/min)

Steady state flux (Jss) is the amount of permeant crossing the membrane at a constant rate; this occurs after the lag phase when the amount continues to increase. When the amounts measured at successive sampling intervals are not significantly different, this is considered steady state.

Jss = Q /(A•t) where Q is the quantity of compound transported through the membrane in time t, and A is the area of exposed membrane in cm2. The unit of steady state flux is quantity/(cm2 • hr).

If the amount of permeant applied to the membrane was an infinite dose, then the permeability constant (Kp) can be calculated from the relationship Kp = Q /[A•t•(Co-Ci)] where Q is the quantity of compound transported through the membrane in time t (min), Co and Ci are the concentrations of the compound on the outer side (donor side) and the inner side (receptor side) of the membrane respectively, and A is the area of exposed membrane in cm2. Usually Co can be simplified as the donor concentration and Ci as 0. The units of Kp are cm/min or cm/hr.

VII. Methodology

However you design your experiments, you should be consistent and precise in your methods. It is imperative to become familiar with your equipment, to set some baseline values for the membranes or tissues you plan to use, and to run several compounds if possible to determine if your detection method will be adequate. Initially run some simple experiments with as few variables as possible. Once you have set baseline values, it will be easier to ascertain that your experiment is running smoothly or if there is a problem with the methodology.

Be consistent with preparation of the tissue and membranes. Mount the tissue carefully to avoid overstretching or damaging it by perforation. Specimens must be large enough that there is an edge of tissue which can be securely clamped in the diffusion cell around the orifice. Care must be taken to ensure that tissue does not protrude into the receptor chamber which could block the flow of receptor fluid.

Use tissue as soon as possible after excision or freeze it at -80°C until you do use it. Keep notes on the time from harvest to use when excising tissue from live animals.

Be precise in compound preparation, receptor medium preparation, pipetting samples, and sampling intervals. Changing the pH of or adding solubilizers to your solutions can affect the penetration of some compounds. You should be aware of this and be able to justify your methods.

Temperature can affect the results of your experiment so you must be consistent in using room temperature, skin temperature, or body temperature when comparing compounds. Report temperature in your methodologies. Always attempt to reproduce the in vivo situation as closely as possible.

VIII. Suggested Reading

A. General references addressing permeability and diffusion.

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Choosing a Membrane for IVRT Studies Using PermeGear Cells

Diffusion cells are valuable aids in formulating topical and transdermal pharmaceuticals. Product development often requires measuring diffusion parameters either between immiscible phases, through continuous or heterogeneous phases, into and/or through barrier membranes, or some combination of each of these phenomena. The results of these preliminary studies facilitate product development and optimization of the final product. These experiments usually fall into one of two categories, permeation testing (IVPT) or release testing (IVRT). Permeation testing is where the membrane is the rate limiting barrier for API diffusion whereas release testing determines the availability of the API for diffusion. In both cases, API is diffusing out of one medium--a donor solution or formulation, also called a vehicle--into a “perfect” sink which is generally a medium where the solubility of the API in the receptor medium does not hinder the transfer of the API out of its vehicle or the membrane. The membrane separates the donor and the receptor medium. In both cases the membrane and the formulation determine the rate an API will diffuse from its vehicle into the receptor chamber.

The first consideration, whether for IVPT or IVRT studies, is choosing which diffusion cell is most appropriate for your study. For excised skin IVPT studies and topical formulation IVRT studies, the most common diffusion cell is the classic Franz Cell. For mucosal, stomach, intestinal or other biological membranes, the Side-Bi-Side cell may be the most appropriate. If you need automation, there are cells that have reservoir flow-through designs. The second consideration is choosing a membrane based on the objective of the experiment or studies to be carried out. Characterizing the membrane itself may be the goal for evaluating and selecting prospective delivery rate controlling components for medical devices (e.g. implants, transdermal patches, etc.). In these cases, membranes are usually solid and homogeneous, but could also be porous, heterogeneous, and/or layered to achieve a desired rate control for optimizing the release profile of an active pharmaceutical ingredient (API). To characterize components of medical and pharmaceutical devices, diffusion coefficients of the component materials can be calculated by determining permeation rates through various materials. It has been proposed that some polymer membranes may be useful as surrogates for viable tissues in certain situations. A bridging study between comparing the viable tissue to the permeability of polymer membranes is required when artificial membranes are proposed as substitutes for viable tissue.

Membranes developed for filtering liquids (membrane filters) are most popular for studying the release of an API from its formulation. In vitro release testing (IVRT) is required for batch to batch quality control of topical pharmaceutical products (Ref). Commercially available membrane filters differ in diameter, pore size, thickness, chemical composition, and type of porosity. Table II lists most common membrane filters with their corresponding membrane characteristics and where they can be purchased.

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The membrane of choice for permeation testing is the biological tissue for which the device or dosage form is to be applied. Thus for transdermal patches and most topical pharmaceuticals, excised human skin is recommended. Intestinal membranes and stomach lining are harvested for studying absorption of oral drugs. There are cells specially designed for studying permeation through corneal tissue. Excised human skin can be procured from burn banks, tissue supply houses, and hospitals following autopsies or surgical procedures.

Animal models such as hairless mouse, hairless rat, guinea pig, and pig skin are frequently used as substitutes for human skin. Synthetic polymers are used in place of human skin for permeation testing as well. When using substitutes instead of the target viable tissue, correlating the results with those obtained from preliminary or concurrent experiments on the harvested tissue is recommended. Strat-M is one commercially available synthetic membrane that has been developed to emulate the two-layer characteristics of human epidermis. A short and descriptive video and correlation data[1] are available. Table I contains a partial list of polymer films which may be useful for characterizing device components or as substitutes for viable tissues.

Most membrane filters are circular and commonly available in 13, 25, and 47mm diameters. Some filters are available as sheets. Dialysis membranes can also be used for IVRT experiments and are available as sheets or tubes. Although membranes are easily trimmed to suit one's cell dimensions, a 25mm diameter filter is most appropriate to fit 9mm and 15mm Franz cells and 47mm diameter filters are well-suited for 25mm cells. In-Line cells have square chambers and 25mm filters must be trimmed on each side to fit.

Membrane filters used for IVRT should provide minimal resistance to the API diffusing out of its formulation while completely retaining all other components of the formulation. Higuchi mathematically characterized the perfect release of an API from a topical formulation by defining its diffusion through the formulation matrix (Ref), the practical application of using porous or semi-porous membranes may require additional considerations, such as porosity, thickness, chemical make-up, and hydrophobicity.

For example, Hatanaka, et al[2]. derived an equation for predicting steady-state diffusion through a membrane with multiple pathways, which embodies most of the factors involving permeation through membranes. The steady-state rate of diffusion, or flux, is given by J = DKCp/th where D represents a coefficient of molecular diffusion through the liquid in the pores of the membrane, K is a partition coefficient between the membrane and the vehicle, and C is the concentration of the diffusant in its vehicle. Porosity, tortuosity, and thickness are represented by p, t, and h, respectively.

A membrane's pore size should be large enough that an API will easily diffuse through, but not other formulation components. With dialysis membranes, the largest molecules that can pass through the membrane determine the pore size. The corresponding molecular weight cut-off (MWCO) can be used as a rule of thumb in estimating the likelihood of any hindrance to diffusion through a dialysis membrane. Some common dialysis membranes[3], with their properties and suppliers, are shown in Table III. See Appendix A for more information on pore size and other membrane characterization measurements such as porosity and tortuosity.

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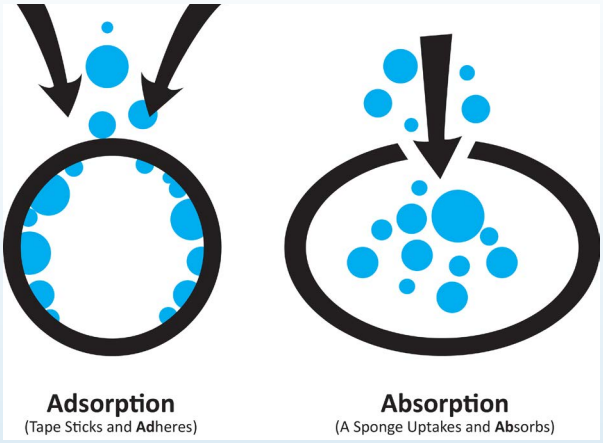
In general, if the membrane has a rate limiting influence on the diffusion of the API through the membrane matrix, the thicker a membrane is, the slower an API will diffuse through since permeability is inversely proportional to membrane thickness. This is useful for optimizing a rate-limiting delivery device or developing a polymer film as a model for a bio-membrane substitute. Ideally, IVRT membranes should be as thin as possible. Because thinner membranes can be more delicate or too flexible, some composite filters, such as Fluoropore[4], have sturdier support layers. Non-woven supports (as opposed to nets or screens) are bonded to the filter material by spraying melted polymer beads through nozzle guns to produce a random pattern, presumably adding physical strength without compromising any filtration properties.

Inertness of the membrane is an important factor since the chemical composition of membranes may affect the degree to which other molecules may be bound to or absorbed by them. Appendix B contains supplemental information on absorption, adsorption, and protein binding. Some polymer membranes contain ionizable groups which may provide functional value for filtration of charged molecules, but are potentially undesirable for IVRT where the membrane should be as inert as possible.

Adsorption to, or absorption into, the synthetic membrane matrix is an important consideration when choosing a membrane for IVRT experiments. Functional groups on membrane polymers affect the degree to which adsorption to the membrane can occur. Hydrophilicity and lipophilicity are rough indications of the solubility of APIs in water or oil media, respectively. Similarly, an API that is hydrophilic will have affinity to a hydrophilic polymer and a lipophilic API will have affinity for a lipophilic polymer. However, the terminology describing the hydrophobicity or hydrophilicity of membrane filters can be misleading. Hydrophilic filters are designed for fast wetting so that filtering aqueous solutions results in faster flow rates[5]. But this has little to do with the degree to which a lipophilic molecule may adhere to the membrane or be absorbed by it. When there is any concern over the possibility that an API will adsorb onto or be absorbed by a membrane, compatibility testing is recommended. At a minimum compatibility data should be consulted, if available. For example, Cole-Parmer's compatibility tool[6] rates polypropylene compatibility with naphthalene as good, whereas silicone is not recommended for use with naphthalene. Several compatibility resources are listed in Appendix C.



To test compatibility, expose the API, its formulation components, and the receptor fluid to a membrane and observe any adverse visual effects, like excessive shrinking or swelling. To judge adsorption and absorption, membranes should be immersed in low enough concentrations that disappearance of the API can be quantitated. Theoretically, the membrane itself may cause interference with sample analysis due to extractables produced during manufacture.

Membrane extractables[7]--artifacts present in synthetic membranes introduced during the manufacturing or packaging process—may interfere with chromatographic analysis by co-eluting with an API or introducing extraneous peaks. An artifact may be a component of the membrane or a particle trapped in the pore structure. Components of a drug formulation or a receptor fluid may trigger the release of extractables during a diffusion experiment. Chemical compatibility affects how significant the resulting sample contamination can be. Membranes should be exposed to formulations and receptor fluids preliminarily to assess the possibility of extractables appearing in diffusion samples. More information on extractables can be found on pages 18-19 of a technical paper on HPLC filtration[8].

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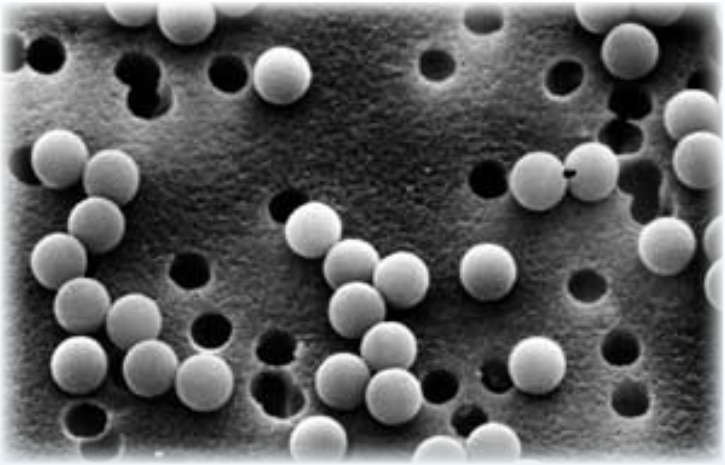
Appendix A

Membrane pore sizes are given in micrometers (µm) and represent an approximate diameter of channels or pores through which a molecule or particle might diffuse through. Thus moieties larger than the pore size will be retained and those smaller than the pore size will be able to pass through. Most commercial membrane filters are fibrous and pore size is somewhat nominal. Some membranes have a very narrow pore size distribution and thus a sharp molecular mass cut-off. Others have a wider pore size distribution and therefore a more diffuse cut-off. More uniform pores exist in membranes made with ion beams from accelerators[9], such as the track-etched membranes. These have a top to bottom hole through the membrane.

Pore size can be determined by scanning electron microscopy, capillary flow porometry, or filtering particles of defined size to determine the minimum size retained. With dialysis and ultrafiltration, filter pore size is too small to be meaningful. These membranes are rated according to their MWCO or nominal molecular weight limit (NMWL). This is not a sharp cutoff. For example, a membrane rated at 30,000 will exclude a test protein with a molecular weight of 30,000 Daltons. Ninety percent of that test protein will be retained on the upstream side and 10% will pass through into the filtrate, resulting in concentration of the protein[10].



Although pore size ratings vary from manufacturer to manufacturer and from product to product, these differences are not likely to be significant for release testing. Unless there is a major difference between the API or CoI and all other formulation components, pore size will not be able to retain the components exclusively. The more important considerations are porosity, tortuosity, thickness, and absorption or adsorption. Tortuosity is another factor that can influence diffusion through a membrane, and the release rate of an API from its formulation. Tortuosity increases the path length through which a molecule must diffuse as it passes through the membrane. Therefore, with all other factors the same, release rates from the more tortuous membranes should be slower. Tortuosity should be least for track-etched membranes which have cylindrical straight-through pores. Greater tortuosity should favor the release of smaller molecules from a formulation relative to other components, because the diffusion rate will be faster for smaller molecules than for larger ones.

Although porosity and tortuosity values for membranes are not usually available, bulk air and solvent flow rates are commonly provided in the specifications for any membrane filter. Possibly the flow rate may be an indication of the combined effects of porosity and tortuosity. For membranes rated with equal pore sizes, the membrane with the faster flow rate will probably be the one with greater porosity and/or least tortuosity.

Appendix B

Adsorption is where molecules adhere to the surface of polymer matrix, whereas absorption involves molecular penetration into the polymer itself. Adsorption involves the affinity of permeant to functional groups on the polymer. Protein binding is generally all adsorption, especially if the protein is large and penetration into the bulk of the membrane is negligible. Membranes with high porosity (about 80%) provide high surface area and more likely to be susceptible to adsorption or binding[11]. Small molecules are more likely to be absorbed by the membrane. The degree of absorption depends not only on the solubility of a permeant in the polymer, but its tendency to remain in the formulation or receptor solution. Therefore a molecule with a small oil/water partition coefficient in an aqueous solution would be less likely to be absorbed by a polymer membrane filter than a more lipophilic molecule. Solubility and partition coefficient data may aid in the prediction of the compatibility of compounds with prospective polymer membranes, although a final membrane choice is best determined by experiment.

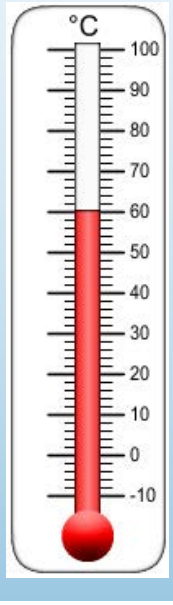
Small molecules may also adsorb to a membrane. Differentiating between adsorption and absorption can be accomplished with experiments by varying polymer dimensions and permeant concentrations.

Appendix C

The main manufacturers of membrane filters all have charts predicting the compatibility of their filters with various solvents. These compatibility references are of questionable value, because the compatibility test conditions may not apply to the conditions used in actual diffusion experiments. For example, a compound indicated as incompatible when exposed to the membrane in pure form may have little effect at the concentration present as a component of a formulation. On the other hand, a small concentration of a compound in a formulation may be absorbed by a membrane, cause it to swell, and subsequently affect the release rate of the API in the formulation.

EMD Millipore's compatibility guide[12] contains the following pertinent caveats:

1. **These recommendations assume pure solutions at room temperature and pressure without applied stresses. Time of exposure is not considered. These are critical assumptions as polymer properties are strongly affected by environmental conditions, time, the presence of external stress and the presence of additives.** It is not safe to assume that property changes are linearly related to changing temperature. A 10°C increase in temperature, for example, may place the test conditions closer to the glass transition of the polymer, thus allowing greater penetration of solvent molecules. This has a plasticizing effect, further lowering the glass transition and resulting in a modulus drop of up to three orders of magnitude. The glass transition of nylons, for example, has been shown to range from below -50 °C to +70 °C depending upon their moisture content.



2. **These recommendations assume that each polymer category has a uniform chemistry, molecular weight distribution and thermomechanical history. This assumption will never be true and, in some cases, variation has a distinct influence on compatibility.** For example, solvent compatibility of cellulose esters is strongly dependent upon their degree of substitution (acetylation/nitration). Crystalline morphology and degree of crystallinity influences compatibility of semi-crystalline polymers and can vary significantly. Polyethyleneterephthalate, for example, can be quenched to obtained samples with almost no crystallinity

or annealed to obtain samples with >50% crystallinity. The response time of these two polyesters, although chemically identical, will be quite different. The effect of molecular weight distribution and degree of branching on solvent compatibility can be seen by comparing the solvent compatibility of LDPE, LLDPE, HDPE and UPE. Such specific information concerning polymers evaluated does not accompany published compatibility tables.

3. The definition of solvent compatibility for EMD Millipore products differs from that used in determining the ratings given in published compatibility tables. **Such tables are generally concerned with chemical attack and significant losses in strength and/or dimensional changes.** A top designation, for example, might be designated for solvent-polymer combinations with <10% swelling, which is high. Other compatibility tables may make recommendations based upon dimensional change as a function of time. This is difficult to relate to a membrane that may respond almost immediately to immersion in solvent. In addition, solvent-membrane compatibility requires additional consideration of filtration-specific factors. **None of these published compatibility guides, for example, monitors the solvent's ability to wet a membrane or increase extractables.**

Pall's chemical compatibility guide[13] lists HPLC solvents and provides recommendations on the resistance to solvent flow or bubble point which usually means a change in pore size. In some cases, a limited resistance or not resistant rating applies to the housing containing the filter membrane, yet the membrane polymer might be perfectly compatible when used alone in a release experiment.

GE Healthcare Life Sciences has a similar table of solvents vs. their compatibility with all of their Whatman membrane filters[14]. Another compatibility chart has data for Nylon, PTFE, PVDF, and Regenerated Cellulose membranes[15].

Cole Parmer's compatibility tool is an interactive one where you can choose from a list of solvents or compounds and another list of materials, such as polymers, to see if they are compatible. The ratings go from excellent compatibility to not recommended for any use.

1. https://www.emdmillipore.com/US/en/life-science-research/drug-discovery-development/strat-m-transdermal-diffusion-membrane/yrOb.qB.rrIAAAE_5nsRHeiO,nav

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3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3986717/#B32-pharmaceutics-02-00209

4. https://www.emdmillipore.com/US/en/product/Fluoropore-Membrane-Filter,MM_NF-FSLW02500#overview

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9. https://s3.amazonaws.com/academia.edu.documents/51634821/s1350-4487_2801_2900228-120170204-6424-15df5in.pdf?AWSAccessKeyId=AKIAIWOWYYGZ2Y53UL3A&Expires=1505839897&Signature=lebGZJMGVC676o4EtHDbkFosSg4%3D&response-content-disposition=inline%3B%20filename%3DTrack_etching_technique_in_membrane_tech.pdf

10. https://www.emdmillipore.com/US/en/life-science-research/chromatography-sample-preparation/membrane-learning-center/Binding-Properties-of-Filters/596b.qB.Hj0AAAFM5FB88eJw,nav

11. https://www.emdmillipore.com/US/en/life-science-research/chromatography-sample-preparation/membrane-learning-center/Chemical-Compatibility/Glqb.qB.awMAAAFM9D588eJs,nav

12. https://laboratory.pall.com/content/dam/pall/laboratory/literature-library/non-gated/Chemical_Compatibilities-Media.pdf

13. http://www.gelifesciences.com/gehcls_images/GELS/Related%20Content/Files/1363086058160/lidoc29046171_20161015100012.pdf

14. http://www.lifescience.ca/data/catalogue/35~v~filtration_products_information.pdf

15. https://www.coleparmer.com/chemical-resistance

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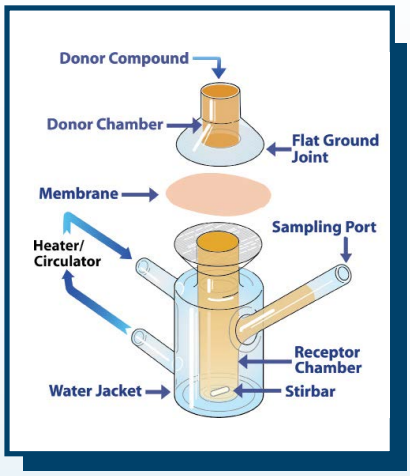
Franz Cells

PermeGear vertical glass diffusion cells are classic Franz Cells. Standard orifice diameters are 5mm, 7mm, 9mm, 11.28mm, 15mm, 20mm and 25mm, other diameters are custom. The word "orifice" as it refers to a Franz Cell is the area at the top of the receptor chamber that is exposed to the membrane or device through which transport or permeation is being studied. Corresponding volumes from 5ml to 20ml are standard. Clamping is achieved through o-ring joints, flat ground (ground o-ring) joints and flat flange joints. We manufacture our own clamps for the most popular Franz Cell models. Our clamps are all stainless steel and much easier to use than clamps from other suppliers.

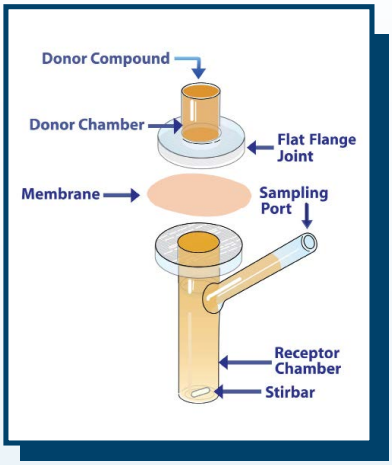
PermeGear offers ISO compliant Franz Cell calibration paperwork, as well as serial numbers on the cells and donor chambers. If you would like to perform an in-house calibration, we offer the appropriate ISO compliant paperwork. For more information, or to request either or both of these services, please e-mail us at: support@permegear.com

This diffusion cell is the most common of all Franz Cells. It is a jacketed Franz Cell with a 9mm orifice diameter, flat ground joint, and 5ml receptor volume. It is the de facto standard for working with human skin and may be used with any membrane 4mm or less in thickness.

We welcome requests for custom cells!



Jacketed Franz Cell



Unjacketed Franz Cell

Part numbers are generally made up of 2-character codes separated by hyphens.

1st code is cell type:
4G is for a jacketed cell, 6G is for an unjacketed cell

2nd code is joint type:
00 = o-ring joint
01 = flat ground (ground o-ring) joint
02 = flat flange joint
03 = spherical joint generally used for corneal work – please see additional information in the 4th code.

3rd code is glass type:
00 = clear glass
01 = for amberized glass

4th code is orifice diameter in millimeters, 05 through 25, if cells do not have a spherical (ball and socket) joint

Franz Cells with a spherical (ball and socket) joint are often used for corneal research and have 4 digit codes. The first two digits are for the orifice diameter, the last two digits are for the diameter of the spherical surface surrounding the orifice.

The following configurations are available:
0312 – 3mm orifice with a 12mm spherical diameter
0512 – 5mm orifice with a 12mm spherical diameter
0718 – 7mm orifice with a 18mm spherical diameter
0918 – 9mm orifice with a 18mm spherical diameter
1228 – 12mm orifice with a 28mm spherical diameter
1528 – 15mm orifice with a 28mm spherical diameter

5th code is receptor volume in ml

6th code is optional and indicates:
VD - volatile donor compartment with ground glass joint and Teflon stopper
VDFT - flow through volatile donor compartment with ground glass joint and Teflon stopper
VDOC - completely occluded volatile donor compartment
VDOF - flow thru volatile donor compartment without ground glass joint and Teflon stopper

7th code only follows the 6th VD or VDFT codes if a stoppered donor is required:
0725 = 7/25 ground glass joint with teflon stopper
1420 = 14/20 ground glass joint with teflon stopper
1922 = 19/22 ground glass joint with teflon stopper

Examples:

#4G-03-00-0918-10 describes a clear 9mm jacketed cell with a 18mm diameter spherical joint and 10ml receptor volume.

#4G-01-00-11.28-08-VD-0725 describes a clear 11.28mm jacketed cell with a flat ground (ground o-ring) joint and 8ml receptor volume with a volatile donor chamber that has a 7/25 Teflon stopper.

#4G-01-01-20-15-VDFT-1420 describes an amber 20mm jacketed cell with a flat ground (ground o-ring) joint and a 15ml receptor volume with a volatile donor chamber that has flow porting and a 14/20 Teflon stopper.

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Dimensions of Standard Franz Cells

Standard 5mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	5mm	N/A	5mm
Joint O.D.	19mm	N/A	19mm
Receptor Volume	5ml	N/A	5ml
Orifice Area	.20cm^2	N/A	.20cm^2
Jacket Diameter	30mm	N/A	30mm

Standard 7mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	7mm	N/A	7mm
Joint O.D.	22mm	N/A	22mm
Receptor Volume	5ml	N/A	5ml
Orifice Area	.38cm^2	N/A	.38cm^2
Jacket Diameter	30mm	N/A	30mm

Standard 9mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	9mm	9mm	5mm
Joint O.D.	25mm	27mm	19mm
Receptor Volume	5ml	5ml	5ml
Orifice Area	.64cm^2	.64cm^2	.64cm^2
Jacket Diameter	30mm	30mm	30mm

Standard 11.28mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	11.28mm	11.28mm	11.28mm
Joint O.D.	28mm	30mm	28mm
Receptor Volume	8ml	8ml	8ml
Orifice Area	1.00cm^2	1.00cm^2	1.00cm^2
Jacket Diameter	30mm	30mm	30mm

Standard 15mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	15mm	15mm	15mm
Joint O.D.	30mm	35mm	30mm
Receptor Volume	7.5 or 12ml	7.5 or 12ml	7.5 or 12ml
Orifice Area	1.77cm^2	1.77cm^2	1.77cm^2
Jacket Diameter	30mm	30mm	30mm

Standard 20mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	20mm	20mm	20mm
Joint O.D.	37mm	43mm	36mm
Receptor Volume	15ml	15ml	15ml
Orifice Area	3.14cm^2	3.14cm^2	3.14cm^2
Jacket Diameter	30mm	30mm	30mm

Standard 25mm Jacketed or Unjacketed Franz Cell Specifications

	Flat Ground Joint	Flat Flange Joint	O-ring Joint
Joint I.D.	25mm	25mm	25mm
Joint O.D.	44mm	50mm	44mm
Receptor Volume	20ml	20ml	20ml
Orifice Area	4.91cm^2	4.91cm^2	4.91cm^2
Jacket Diameter	30mm	30mm	30mm

Standard Spherical Joint Jacketed or Unjacketed Franz Cell Specifications

	Two Sizes Available	
Orifice in Sphere	5mm	9mm
Sphere Diameter	12mm	18mm
Receptor Volume	5ml	5ml
Orifice Area	.20cm^2	.64cm^2
Jacket Diameter	30mm	30mm

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Franz Cell Certification, Installation & IQ/OQ Services

Calibration and Certification Documents optional with every Franz Cell.

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Franz Cell Certification

PermeGear Job #: _____
Franz Cell Serial Number: _____
Receptor Chamber Volume: _____
Actual Receptor Chamber Volume: _____
Receptor Chamber Orifice Diameter: _____
Actual Receptor Chamber Orifice Diameter: _____
Donor Chamber Orifice Diameter: _____
Actual Donor Chamber Orifice Diameter: _____

Test Procedure/Data: Testing performed in accordance with SOP # FCC-3-17-01

PermeGear certifies the above information to be true/accurate to the best of our ability, and the Franz Cell meets PermeGear's quality standards.

Testing performed by: _____ Date: _____
Jim Siverly, Ph.D. Principal Scientist

Review/Approval by: _____ Date: _____
Andrew Wilt, President

www.permegear.com 1815 Leithsville Road Hellertown, PA 18055 484-851-3688 Fax 484-851-3688 support@permegear.com

FC-CERT-3/17-01

Installation, Training & IQ/OQ Services

PermeGear manual Franz Cell systems are straightforward to set up so assistance from PermeGear personnel is usually not necessary. However, if you have no experience using Franz Cells and would like some help to begin your study, we will come to your facility, install the equipment, and help you set up your first run of samples. While doing so we can also help with formulation questions if needed. This can all be done in one day at your facility.

Our automated systems are more complicated and we recommend that we install your system. However, it is certainly possible for someone with good technical skills to do it. A single system can be installed in a morning and the afternoon can be spent guiding you through its use. And, as above, we can assist you with any questions you might have in starting your research.

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1. Cell Clamp - must be used to hold chamber, cell and membrane in place
2. Donor chamber
3. Franz Cell
4. Gaskets which may be used to seal the membranes and help prevent leaks, if needed. The gaskets are made of closed cell Teflon® foam. These are optional and can be placed on top of or below the membrane.
5. Stir Bars - One is to be placed in the donor chamber, an additional one is provided as a spare.

What's in the Box?

Proper Care of PermeGear Diffusion Cells

Diffusion Cell Cleaning Basics

It's generally easier to clean glassware if you do it right away. When detergent is used, it's usually one designed for lab glassware, such as Liquinox or Alconox. These detergents are preferable to any dishwashing detergent you might use on dishes at home. Much of the time, detergent and tap water are neither required nor desirable. You can rinse the glassware with the proper solvent, then finish up with a couple of rinses with distilled water, followed by final rinses with deionized water.

Autoclaving - PermeGear's diffusion cells are made of standard borosilicate glass and are autoclaveable. Autoclave for 15-20 minutes at 100-120ºC. Remove all closures, clamps, clasps, etc. when autoclaving. If it is not possible to remove them, at least loosen or unscrew them to avoid pressure buildup.

How to Wash Out Common Lab Chemicals

Water Soluble Solutions (e.g., sodium chloride or sucrose solutions) Rinse 3-4 times with deionized water then put glassware away.

Water Insoluble Solutions (e.g., solutions in hexane or chloroform) Rinse 2-3 times with ethanol or acetone, rinse 3-4 times with deionized water, then put the glassware away. In some situations, other solvents need to be used for the initial rinse.

Strong Acids (e.g., concentrated HCl or H_2SO_4) & **Strong Bases** (e.g., 6M NaOH or concentrated NH_4OH) Under the fume hood, carefully rinse the glassware with copious volumes of tap water. Rinse 3-4 times with deionized water then put the glassware away.

Weak Acids (e.g., acetic acid solutions or dilutions of strong acids such as 0.1M or 1M HCl or H_2SO_4) Rinse 3-4 times with deionized water before putting the glassware away.

Weak Bases (e.g., 0.1M and 1M NaOH and NH_4OH) Rinse thoroughly with tap water to remove the base, then rinse 3-4 times with deionized water before putting the glassware away.

Washing Glassware Used for Organic Chemistry - Rinse the glassware with the appropriate solvent. Use deionized water for water-soluble contents. Use ethanol for ethanol-soluble contents, followed by rinses in deionized water. Rinse with other solvents as needed, followed by ethanol and finally deionized water. If the glassware requires scrubbing, scrub with a brush using hot soapy water, rinse thoroughly with tap water, followed by rinses with deionized water.

Drying - If you are autoclaving, it is acceptable to allow the diffusion cells to dry in the autoclave. If you will be adding water to the glassware, it is fine to leave it wet (unless it will affect the concentration of the final solution). If the solvent will be ether, you can rinse the glassware with ethanol or acetone to remove the water, then rinse with the final solution to remove the alcohol or acetone. It is inadvisable to dry glassware with a paper towel or forced air since this can introduce fibers or impurities that can contaminate the solution. If water will affect the concentration of the final solution, triple rinse the glassware with the solution.

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Proper Care of PermeGear Diffusion Cells

Diffusion Cell Do's & Don'ts

Don't autoclave diffusion cells with the clamps on or stoppers in them.

Do use a soft bristled brush, never a wire brush to clean diffusion cells.

Don't use harsh acids to clean your diffusion cells as this could cause irreperable damage to the markings on the cells.

Do clean the diffusion cells after each use for best results.

Don't bump or clank the diffusion cells, remember they are glass and will break. Borosilicate glass is glass and requires the same care and handling procedures.

Do contact PermeGear if you **break a sampling arm or port, chances are we can repair it**. Send a photo to **support@permegear.com**

Don't send broken Franz Cells like this, (don't leave tubing on, tape pieces or cells).

Do send your broken Franz Cells just like this. Please send them **CLEAN!**

Additional Tips About Borosilicate Lab Glassware

Remove stoppers and stopcocks when they are not in use. Otherwise, they may 'freeze' in place.

You can degrease ground glass joints by wiping them with a lint-free towel soaked with ether or acetone. Wear gloves and avoid breathing the fumes.

The deionized water rinse should form a smooth sheet when poured through clean glassware. If this sheeting action is not seen, more aggressive cleaning methods may be needed.

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Side-Bi-Side Cells

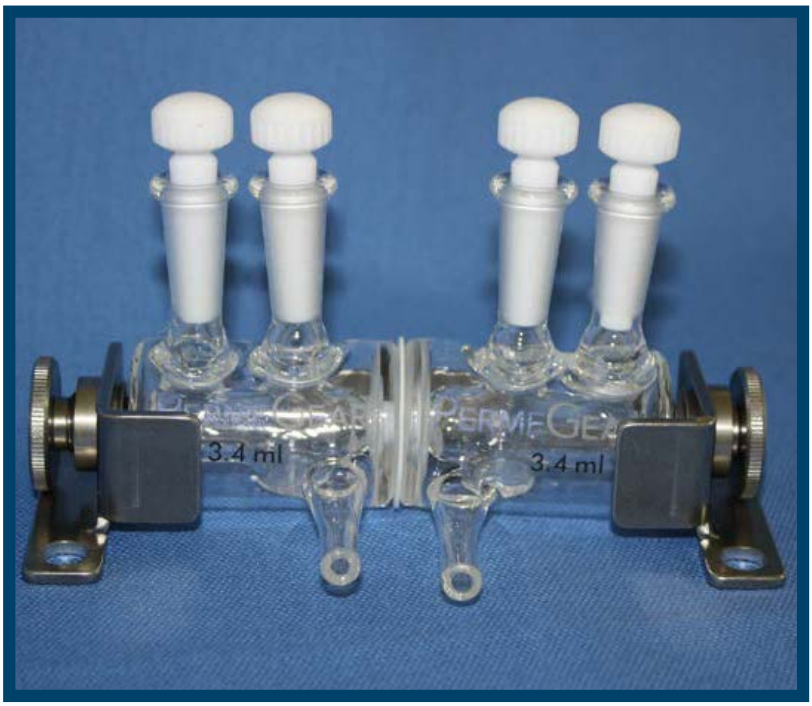
PermeGear makes three different classes of Side-Bi-Side Cells. The smallest class includes the most common Side-Bi-Side Cells having orifice diameters from 5mm to 15mm. The medium size class has orifice diameters up to 25mm. The largest class has orifice diameters between 15mm and 60mm. In all classes of Side-Bi-Side Cells available volumes are dependent on orifice diameter and other factors. Our Side-Bi-Side Cells come with stoppers and stirbars, they do not come with Cell Clamps.

Side-Bi-Side Cells are easily customized with different volumes, additional porting, and amberization. They are well suited to iontophoresis studies.

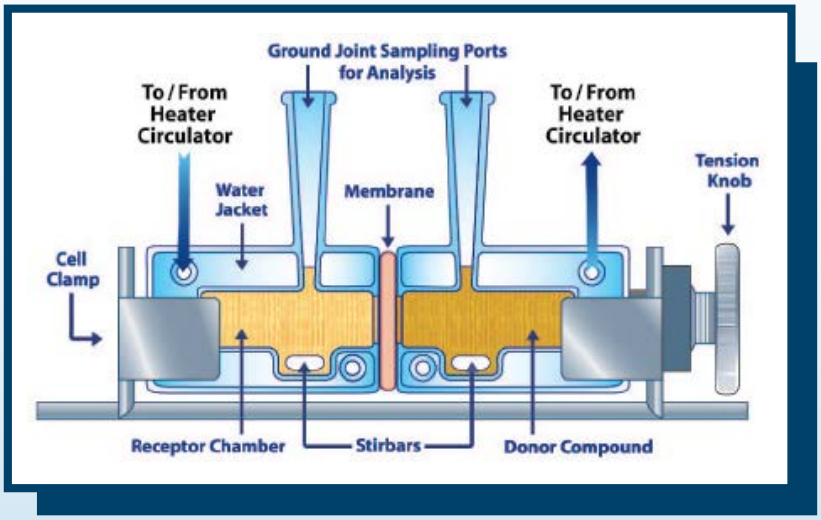
After a membrane is placed between the cell halves, the Cell Clamp is placed around them. The adjusting knobs are then gently tightened to hold the glass halves and membrane or device together. The assembly is then located over the stirring magnets of an H-Series Stirrer by placing the holes in the clamp over pins on the top of the stirrer.

There is another variety of Side-Bi-Side Cell known as a Valia-Chien Cell. It is generally the same as a Side-Bi-Side Cell except that the surfaces of the joint are not the ends of the heating jackets. The joint is like that of a Franz Cell with the joint's halves being fused to the heating jackets. An additional clamp holds the membrane in place. Valia-Chien are available with o-ring, flat ground o-ring, or flat flange joints.

Please note that the wrap around Cell Clamp for a Valia-Chien Cell is not the same as the Cell Clamp for a Side-Bi-Side Cell and that clamps for one variety of cell will not work properly with the other cells. There are also different stirrers for each kind of cell as the center-to-center distance for the stirbars in a Side-Bi-Side Cell is 29mm and the corresponding distance in Valia-Chien Cell is 41mm. For corneal work the Valia-Chien Cell must be used because there is no practical option for a Side-Bi-Side Cell to have a spherical surface.



This Side-Bi-Side Cell has the common 9mm orifice diameter with 3.4ml standard volumes.



Part numbers are made up of five 2-character codes separated by hyphens. Additional codes may be added by PermeGear if the cell is customized with extra porting or other modifications.

1st code is cell type: 5G is for a Side-Bi-Side Cell, 7G is for a Valia-Chien Cell
2nd code is joint type: 00 is a flat joint with ground surfaces, 03 is for a spherical joint (for corneal work)
3rd code is glass type: 00 is for clear glass, 01 is for amberized glass
4th code is orifice diameter in millimeters, 05 through 60 and is class dependent
5th code is the internal volume on either side of the joint

Examples 5G-00-00-15-05 is for a clear 15mm cell with 5ml volumes
5G-00-01-09-02 is for an amber 9mm cell with 2ml volumes
7G-03-00-0918-06 is for a clear Valia-Chien Cell with a 9mm orifice, 18mm spherical diameter, and 6ml volumes

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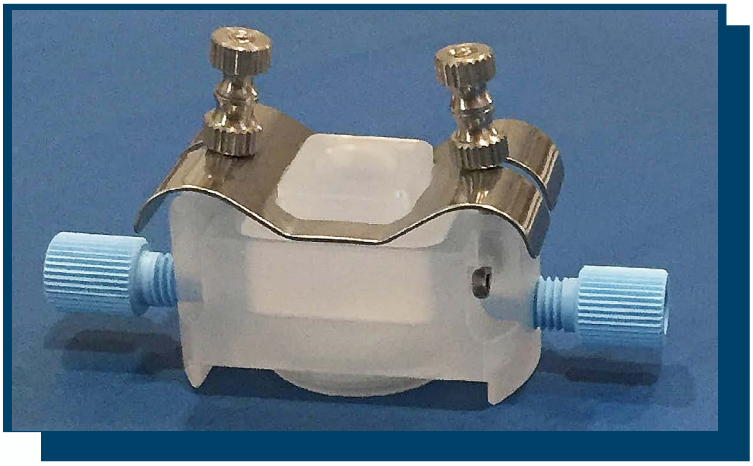
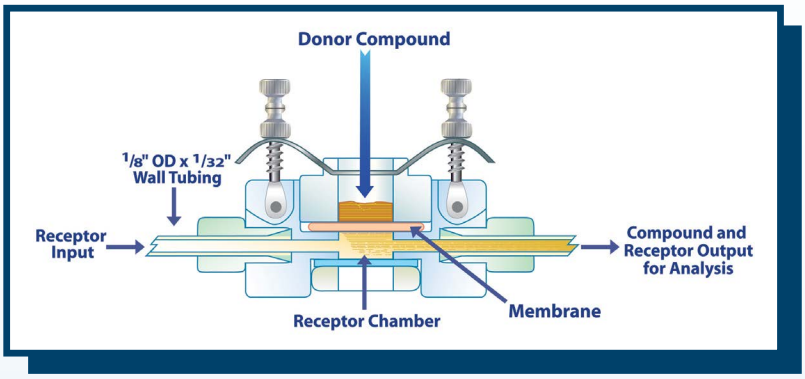
In-Line Cells

PermeGear In-Line Cells may be thought of as flow type Franz Cells with very small receptor volumes. In-Line Cells locate the membrane of interest in the horizontal plane and have donor chambers open to the air although occluded donor chambers are available.

In-Line Cells were designed to replace the Bronaugh Cell and are available in orifice diameters from 5mm to 15mm. They are made from Chlorotrifluoroethylene (CTFE). In-Line Cells have a unique clamping system which features user preset, repeatable, secure clamping of tissue or membranes. Clamping is achieved with a stainless steel spring that applies pressure preset by the user to the upper surface of the donor compartment for leakproof clamping. The cell design locates the orifice diameters within .1mm of each other. HPLC connectors are used to connect the cells to 1/16 ID tubing. Stirring effects within the cells are easily checked with our unique Twin-Flow Conversion System.



In-Line Cell



H1C Stirrer pictured above with 2 In-Line Cells

ILC07 Automated System with seven In-Line Cells

The most common sizes are 5mm, 9mm, and 11.28mm. Standard In-Line Cells are supplied with open donor chambers which are 14mm high. In-Line Cells are also available with volatile donor chambers that have septum caps. The volatile donor chamber height not including the septum or the cap is 26mm. The septum caps are the same on all In-Line Cells and the inner diameter at the top of all volatile donor chambers is 9mm. The orifice diameter at the clamping surface adjacent to the membrane is the same as the In-Line Cell body supplied with the volatile donor chamber.

In-Line Cells are usually used in our ILC07 Automated System, but they can be used with our Twin-Flow Conversion System, or individually, as either flow type or static diffusion cells. Our Twin-Flow unit allows two In-Line Cells to be used with a Side-Bi-Side Cell H-Series stirrer. The Twin-Flow unit maintains the desired temperature in the cells when connected to a source of flowing temperature controlled water.

In-Line Cells & Components – "xx" denotes orifice diameter

- 1K001-XX In-Line Cell 5mm-12mm
- 1K001-XX-VD In-Line Cell w volatile compound donor chamber
- 1KM01-XX In-Line Cell 5mm-12mm with interchangeable membrane support

Cell Extra Components – "xx" denotes orifice diameter

- 1K012-XX Membrane Support
- 1K015-XX Donor Chamber
- 1K016-XX Donor Chamber Cap
- 1KS02-XX Volatile Compound Donor Chamber

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Custom Cells

We Welcome Custom Requests!

Most researchers will find the products they require within our standard diffusion cell offerings. Occasionally an experiment requires non-standard features. At PermeGear, we understand the varying technical needs of researchers and are almost always able to customize our cells to your specifications.

Standard Franz Cells have a donor chamber open to the air, we can provide ground glass joints and matching stoppers of many varieties if you wish to occlude your Franz Cell donor chamber. We can provide flow porting to both the donor and receptor chambers. The donor chamber can be jacketed as well if needed.

Standard Side-Bi-Side Cells have one port on each side of the joint for filling and sampling the chambers. We can add ports for performing iontophoresis experiments, studies using ultrasound probes, threaded fittings for accessories, etc.. Standard Side-Bi-Side Cells are supplied with equal volumes on each side of the joint, the volumes may be different if needed. We welcome custom requests! Email **support@permegear.com** for more information.

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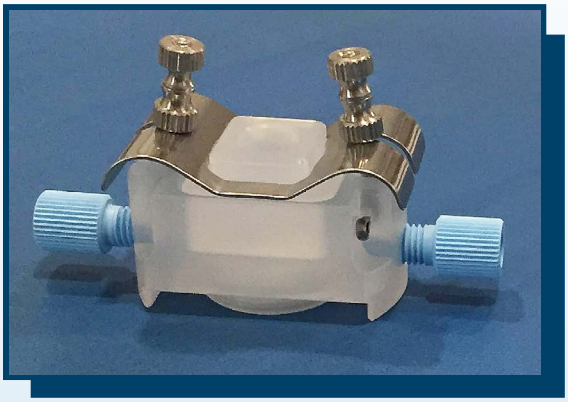
ILC07 Automated System

PermeGear's Automated Systems incorporate In-Line Cells, a peristaltic pump, a fraction collector, and a heater/circulator into a versatile system for analyzing flux over time. Collection vials are at the option of the user, racks for 20ml / 28mm diameter scintillation vials are provided. Adapters are available to allow the use of many different vials. Because of the varied requirements of individual users, vials ARE NOT supplied by PermeGear.

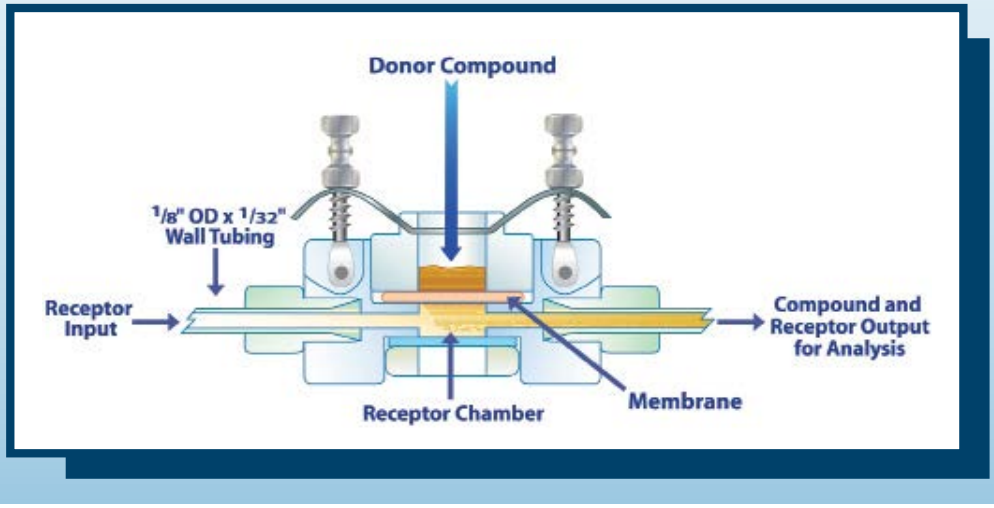
All PermeGear automated diffusion cell systems are based on the continuous flow principle. A multi-channel peristaltic pump draws receptor solution from a reservoir through a distribution manifold and sends it to the In-Line Cells. The cells are warmed to 35º-37ºC by a heater/circulator that pumps water through the Cell Warmer in which the In-Line Cells are located. After filling the receptor chambers of the cells and purging the system of bubbles, the subject compound is then placed in the donor chambers of the cells. From that time, the fluid is collected in the vials of the fraction collector at user set time intervals. The vials are taken for analysis manually after the sampling time has elapsed.

The components of the ILC07 are as follows:
- (7) In-Line Cells (many configurations)
- (1) #10035 Cell Warmer
- (1) #10027-14 1-liter reservoir & manifold
- (1) #10033 Fraction Collector
- (1) #10060 Heater/Circulator
- (1) #10090-08 Peristaltic Pump



The ILC07 Automated System is compact assembly of integrated components. Its footprint is 27" deep by 29" wide. Once the system is set up, no space on either side of it is required for operation.

PermeGear offers installation and training, as well as IQ/OQ services and documentation.



For more information, please visit:
http://permegear.com/automated-systems/

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H-Series Stirrers

PermeGear H-Series Stirrers are made for use with our Side-Bi-Side cells. All but the H1 Stirrers have aluminum supply manifolds mounted toward the rear, which connect to a heater/circulator. The long horizontal manifold tubes have hose barb fittings that allow the water jackets of the diffusion cells to be connected to them with latex tubing. Our stirrers have been around for 30 years and many are still operating today.

All H-Series Stirrers run at 120v/600rpm 230v/500rpm.

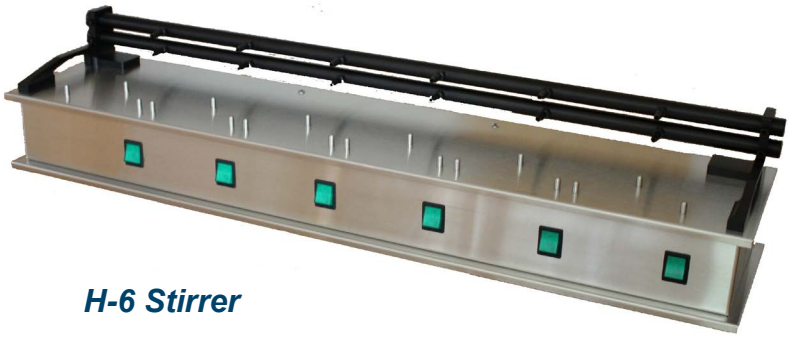
H-3 Stirrer

SIDE-BI-SIDE CELLS MUST BE ORDERED SEPARATELY!

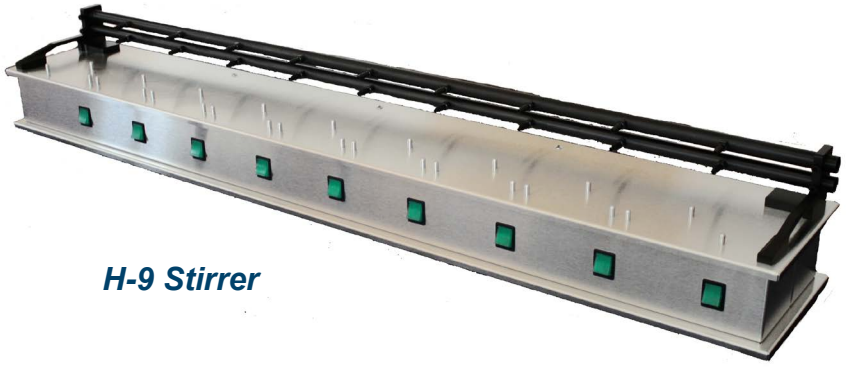
PermeGear's Side-Bi-Side Cells are currently in laboratories around the world and used for everything from measuring the migration of molecules into and/or through biological membranes to an instrument of measurement in the development of fuel cells. To best mimic in vivo conditions, permeation experiments are typically done at near normal body temperature. Therefore, the fluid inside each chamber of a Side-Bi-Side Cell is heated and stirred.

H-3 Stirrer shown above with Side-Bi-Side Cells

Each half cell is surrounded by a "jacket" through which heated water is circulated. Cell Clamps securely locate the Side-Bi-Side Cells directly above individual magnets. H-Series Stirrers provide easy access for connecting the jackets to a heater/circulator. PermeGear provides heater/circulators, but often these are already available in users' labs.



H-6 Stirrer



H-9 Stirrer

For more information, please visit:
http://permegear.com/side-bi-side-cells/

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V-Series Stirrers

PermeGear V-Series Stirrers are made for use with our jacketed Franz cells. They have aluminum supply manifolds mounted toward the rear that connect to a water bath and pump, or heater/recirculator.

All V-Series stirrers run at 120v/60hz/600rpm or 230v/50hz/500rpm.

V3A Stirrer

V3B Stirrer

FRANZ CELLS MUST BE ORDERED SEPARATELY!

V6-CB Stirrer

V6-CA Stirrer

PermeGear's V-Series Manual Diffusion Systems are an apparatus employing Franz Cells for carrying out in vitro release testing (IVRT) and in vitro permeation testing (IVPT). The systems enable a researcher to measure the release or permeation of a compound into or through membranes.

V9-CA Stirrer

V9-CB Stirrer

Franz Cells in the V-Series Manual Diffusion Systems are firmly held directly above individual stirrers and surrounded with a "jacket" through which heated water is circulated. The result is a safer and more secure apparatus with better ability to control experimental variables.

For more information, please visit:
http://permegear.com/v-series/

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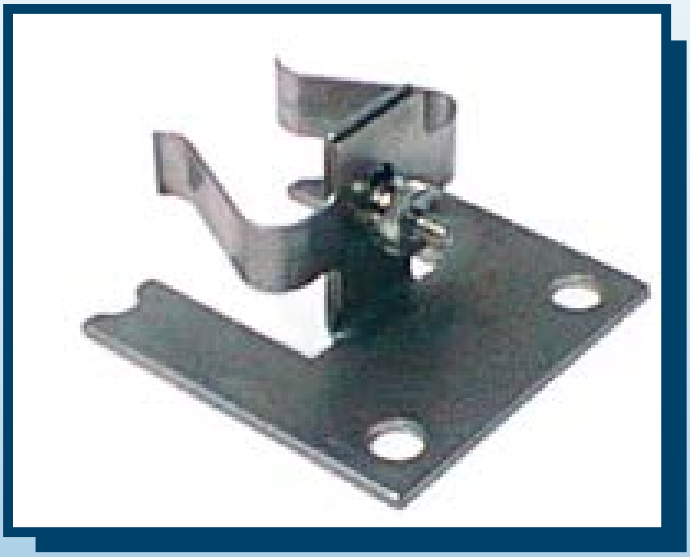
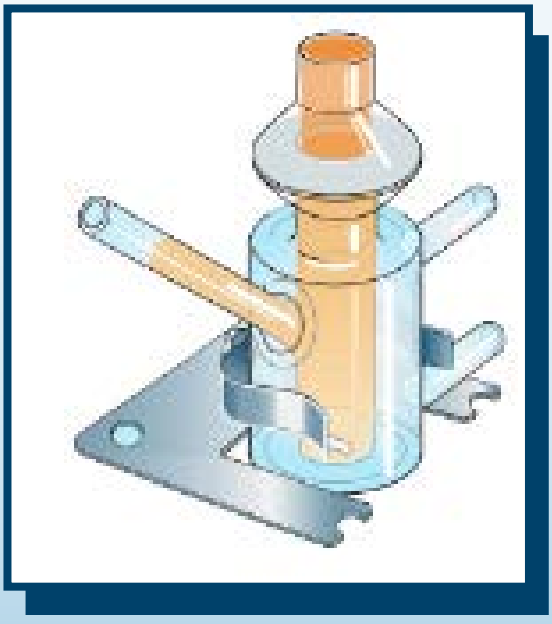
Cell Stands

PermeGear Cell Stands are stainless steel assemblies designed to locate Franz cells over the stirring magnets in laboratory stirplates, and Side-Bi-Side Cell Stirrers. These Cell Stands are convenient for holding any vertical cell with a 30mm heating jacket so it may be more easily handled with two free hands. The stainless springsteel component is vertically adjustable to allow for the handblown quality of the cells and the placement of the tubes for the heating jacket. If 9mm or 11.28mm Franz Cells are used with pinch clamps, two cells may be used over each station of any of our H-Series Side-Bi-Side Cell Stirrers.

Part number for a jacketed Franz Cell is CS-01.
Part number for an unjacketed Franz Cell is CS-02.

Typical Dimensions
Cell Stands for jacketed Franz Cells are about 2.5" or 6cm square. If you have an H-Series Side-Bi-Side Cell Stirrer, you can locate two 9mm, 11.28mm, or 15mm Franz Cells over the stirring magnets in each station.





For more information, please visit:
http://permegear.com/franz-cell-stands/

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Pipettes & Pipette Tip Assemblies

PermeGear is pleased to announce our new 100µl to 1000µl Pipettes (Part # PP-01) & Pipette Tip Assemblies. They are a three piece flexible assembly, the first designed specifically for insertion into the center of Franz Cells for more accurate sampling. The tip assemblies are available in packages of 100 (Part # PT-100) or 250 (Part# PT-250).

Because of this unique - never before introduced, slender design, you will need to dial in the pipette at a slightly higher volume to achieve your target aliquot. Once a PermeGear Pipette is dialed in, it will maintain ± 2% accuracy.

Please keep in mind many factors can affect pipetting results, i.e. temperature, humidity, angle, rate at which sample is pulled, operator techniques etc... Remember to sample slowly for micropipetting.

PermeGear Pipettes:

- Light weight
- Soft spring system for extremely low pipetting forces
- Controlled volume setting to prevent accidental volume changes
- Large volume display
- Contoured shape: fits either large or small hands
- Large pushbutton, rounded and freely rotating
- Finger hook: takes the weight, for a more relaxed grip
- 3-position tip ejector button for left & right handed users
- Easy on site calibration (calibration key included)
- A unique serial number
- UV resistance even prolonged exposure
- Fully autoclavable (120°C/0.1MPa/20 min)
- Calibration key included in the box

If you need additional information or have any questions, please e-mail us at: support@permegear.com