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The challenges involved in *in-vitro* drug release testing for semi-solid formulations: advancements and rethinking on various diffusion systems

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ABSTRACT

Fast strides are being taken by the pharmaceutical industries and the academics the world over, in research related to recent advances in designing of static diffusion cells to assure batch-to-batch drug release equivalence for semisolid dosage forms and to facilitate an easy performance of quality control tests for semisolids dosage forms. Till today, there are no pharmacopoeial methods recommended to carry out *in vitro* release tests for semisolid dosage forms and about selection of diffusion cells. Majority of published transport studies, particularly for skin permeation, involves the use of FDC (Franz diffusion cell). Franz diffusion cell is the only existing device recommended both by FDA (Food and drug administration) and OECD (Organization for economic co-operation and development). Unfortunately this device suffers from several limitations such as formation of air bubbles, limited receptor compartment volume, laborious and large variation among experiments. To overcome the above limitations of Franz diffusion cell, several novel diffusional cells were invented like modified Franz diffusion cells, Keshary-Hein cell, Enhancer cell, United States of Pharmacopeia (USP) - 5, 6, 7 apparatus, automatic sampling Kelder cell, Insertion cell and Plexiglas cells etc., but each invented novel diffusion cell encountered other limitations. So until date, there is no widely accepted static diffusion cell recommended by any Pharmacopeias. The primary focus of this review makes an effort to compile some of the related recent findings and highlight some of the major issues related to various diffusion cells developed till today, their comparative assets and limitations.

Introduction

Semisolid dosage forms alleviate a pathological condition or offer protection against a harmful environment on their application to the affected skin or mucous membrane. From many years, skin has been shown to be the suitable delivery route for drugs formulated in transdermal delivery system. The commonly used semisolid dosage forms like creams, ointments, gels, etc. are generally applied on skin for systemic effect. The diffusion of drugs through skin is generally measured by both *in-vivo* and *in-vitro* techniques. In-vitro techniques are generally preferred, due to its simple and economical experimental conditions. The detailed skin permeation studies with its types and components are mentioned below in Figure 1.

A unique widely accepted novel diffusion cell should have the following characteristics:

- An optimal diffusion area/volume ratio that will allow sensitive permeate analysis in the receptor medium, especially at early sampling times when permeate concentrations are low. The proviso here is that the diffusion orifice, and the surrounding membrane-securing flange, should accommodate the smallest area of biological tissue usually obtained by the particular sampling technique employed. Large sheets of synthetic media are in ample supply and will not dictate the diffusion area of the cell.
- Homogeneous fluid mixing must be generated throughout the chambers

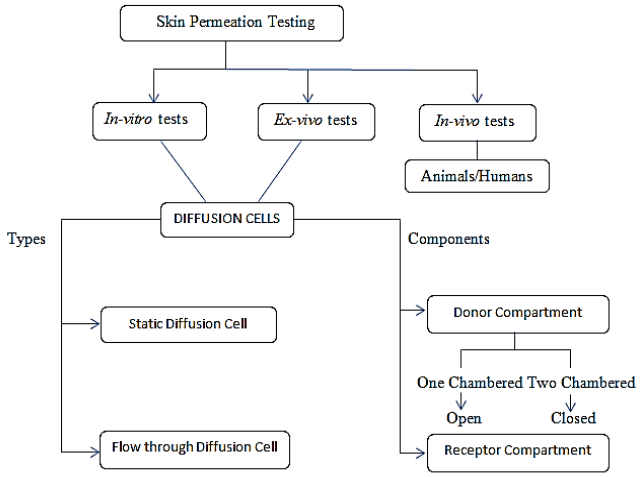

Figure 1: Conceptual diagrammatic design of skin permeation studies

- The cell design must enhance rapid temperature equilibration within the chambers so that gradients are not established between the bulk cell contents and the membrane interface.
- The conformation of the diffusion cell chambers should be as uniform as possible, with a minimum of appendages and no physical constrictions between the agitated bulk cell fluid and the membrane surface.
- The sampling port should form part of the cell body and should be attached in such a fashion as to ease adequate mixing

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of its contents with the bulk chamber fluid. The port should not contain a relatively large proportion of the chamber fluid and should have some stopper system to prevent evaporation of chamber contents.

- The cell design should be easily constructed with basic laboratory materials. Glass is the ideal construction material because it is inexpensive, it is easily worked into any idealized conformation, it is fairly inert to the normal laboratory chemicals, and it supports rapid thermal conduction. A totally inert material is not yet available, hence, the need for appropriate performance validation. Careful design and planning may incorporate other basic laboratory equipment into the diffusion cell system, such as thermostatic water baths, magnetic stirrers, thereby further simplifying construction and reducing cost without sacrificing sensitivity in the monitoring of permeation.
- Optimal design would also produce a cell that is versatile in performance: one that may be used for both steady-state and in vivo-mimic, finite- and infinite-dose diffusion experiments.
- A rate-limiting, discriminating membrane appears essential for demonstrating subtle differences between the drug release characteristics of similar topical formulations and for estimating drug absorption rates that may be expected in vivo. Equally, the receptor phase should compose a relatively innocuous solvent, but one in which the permeate is sufficiently soluble to facilitate partitioning from the membrane. This may require surfactant or lipophilic addition to aqueous media to enhance its biochemical similarity to the physiological environment of the skin.

Although these features may represent the ideal diffusion cell design, the incorporation of all these facets into a single system may be impractical; however, as many as possible should be included into any cell system proposed for study.

Diffusion cells

Diffusion studies are one of the vital evaluation parameters which decide the efficiency of transdermal dosage forms. Within this context, different *in-vitro* static and flow through diffusion cells to assess skin permeability is been discussed. The types of diffusion cells and their differences are given below in Table 1.

Table 1: Differences between static and flow through diffusion cells

Based on fluid refreshment in receptor compartment	
STATIC / NON-FLOWING	FLOW THROUGH
• The receptor medium is stirred continuously in the compartment	The receptor medium flows continuously through the receptor compartment
• The receptor medium is not refreshed	The medium is refreshed continuously.
• Receptor compartment is variable	• Receptor compartment volume is relatively large > 10ml

Static diffusion cells

Franz diffusion cell

In mid1970s [1, 2] Franz designed the Franz diffusion cell. The FDC is the standard criterion that has been commercially marketed and has been widely used cell recommended only by both FDA and OECD. Fully assembled Franz Diffusion cell showing the various parts and components are described in Figure 2. This system comprises of a donor compartment and a receptor compartment with a membrane placed between the compartments. It also comprises a side arm in connection with the receptor compartment for sample collection. The donor compartment is small and is the upper portion of the diffusion cell that is open to the atmosphere while the receptor compartment containing the magnetic stirrer bar, the lower portion of diffusion cell is either jacketed or non-jacketed. The jacketed portion is to maintain the temperature. All these components of the system are connected with a cell clamp.

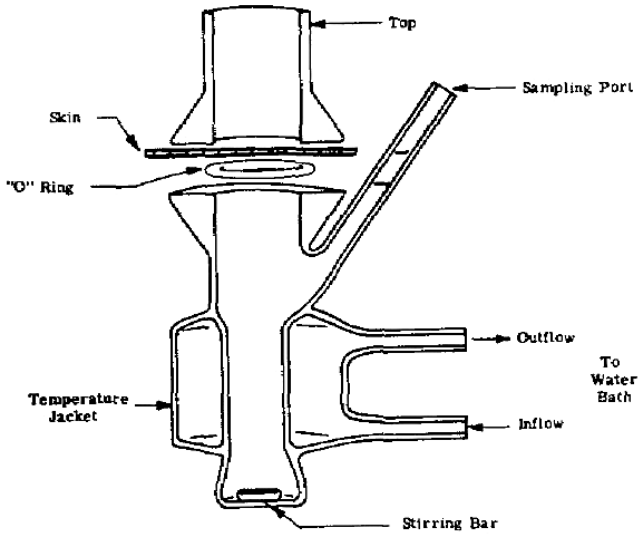

Figure 2: Schematic representation of Franz Diffusion cell (FDC) showing the various parts (1)

Advantages of FDC:

- Membranes can be replaced according to the view of researchers
- It can be well used for low soluble drugs, e.g.: BCS-II and IV (Biopharmaceutical classification system) since the receptor volume in FDC is low.
- Since its ideal construction material is glass,
- it is affordable
- reusable diffusion cell
- fairly inert to the normal laboratory chemicals and
- it supports rapid thermal conduction

Although the FDC has been used extensively for skin permeation studies, it has several obvious non-ideal features as listed below.

Limitations of FDC:

- Air bubbles formation
- Limited receptor compartment volume for high soluble drugs
- Inadequate mixing efficiency
- Equilibration times of temperature requires 30 minutes
- Evaporation of the receptor solvent
- Fragile whilst its ideal construction material is glass
- Laborious and large variation among experiments

To overcome the limitations of FDC, several modifications were made to the cell and brought into light as described below

Keshary Chien cell

Several of modifications were suggested by Keshary and Chien 1984 to the introductory design of Franz to greatly ameliorate the hydrodynamics of the cell. The modifications proposed are mentioned in Table 2. The performance of the modified design (Keshary Chien cell see Figure 3.) was compared with that of the standard criterion of Franz cell for a number of physical variables. Equilibrium temperature maintenance in the bulk phase and at the membrane interface was more easily attainable, with less variation than in the Franz design, [3, 4, and 5]

Table 2: Modified Franz diffusion cell

Modifications to FDC proposed by Keshary	
Receptor compartment	Widened by a diameter of 20 mm
Height of diffusion cell	Reduced to 50 mm
Receptor volume	Approximately to 15.7 ml
Heated water jacket	Dimensions increased
Type of magnetic bead	Star-head magnet in place of the simple bar magnet
To minimize evaporation of the receptor solvent	A glass stopper was introduced into the sampling port.

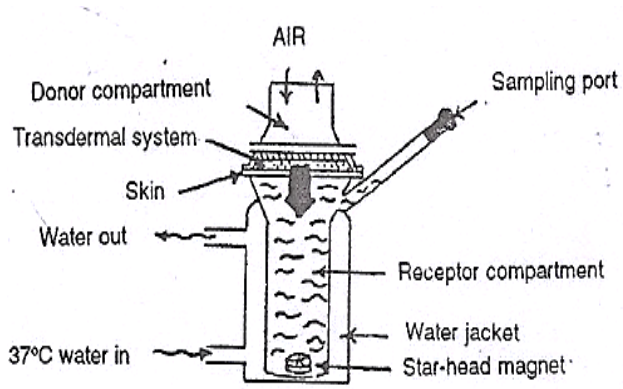

Figure 3: Schematic representation of Keshary Chien cell (K-C) showing the various parts (6)

Apart from the advantages of FDC, the advantages of Keshary Chien cell include:

- More efficient fluid mixing pattern
- Minimization of evaporation of the receptor solvent
- Temperature maintenance was more easily attainable

Moving forward, several modifications were introduced in Keshary chien cell, it still had to overcome the below listed features.

Limitations of Keshary Chien cell:

- Air bubbles formation,
- Limited receptor compartment volume for high soluble drugs,
- Fragile whilst its ideal construction material is glass,
- Laborious and large variation among experiments.

Other modifications done on Franz diffusion cell and patented by other researchers:

The proviso of the diffusion cell depicted by William Hanson in his patent is for the removal of the sample aliquots by means of a sample tube which is to be movable into and out of the receptor chamber. A refilling tube and a stirring device (in the form of helical coil) are positioned within the receptor chamber for addition of receptor fluid and for homogenous mixing of receptor fluid respectively. This change can be brought into automatic or manual devices [7]. In the next patent filled by Hanson, to the same above invention, the addition of the incorporation of filter screen within the coil was done to prevent the particulate matter entering the sample tube and clogging such [8]

Royal Hanson et al. described in the invention the usage of a quick release clamping apparatus which secures the donor housing that contains the donor chamber tightly to the main housing of the diffusion cell. The usage of the capillary port and the refilling tube for the removal of test aliquots and the filling of the receptor fluid respectively were a part of the invention [9]

Ongoing forward, USP 5(paddle over disk), USP 6 (Rotating cylinder), USP 7 (Reciprocating holder) developed by slight modifications done in USP 2 (Paddle), and were used for testing the drug release from transdermal drug delivery systems. These approaches were found to be good for transdermal patches rather than semisolid dosage forms.

Enhancer cell

VanKel Industries with Duquesne University, USA introduced the enhancer cell, [10] a device which studies the drug release profiles of topical formulations. The cell system was made of Teflon an inert and non-reactive material which is a poor conductor of heat. Enhancer Cell (PN-12-4000, VanKel Industries, NJ) (Figure 4.) consisted of a cap, a washer, membrane, a O-ring, and a drug reservoir, an adapter plate, a cover, smaller sized shafts and collets. The semisolid dosage form was placed in the reservoir of the enhancer cell and the top cap was screwed up. The enhancer cell setup was introduced into a USP Apparatus 2 assembly which was modified with 200 ml flasks whilst 900ml flasks. The adapter plate was used to position the flasks in the center and to prevent the evaporation of the receptor fluid, a cover was used. The assembly was completed (Figure 5.) and the mixing efficiency was provided internally by a paddle [11]

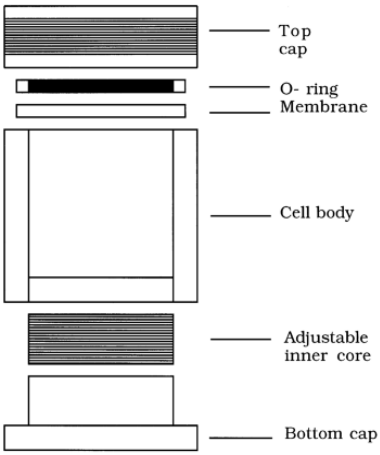

Figure 4: Schematic representation of the Enhancer cell (11)

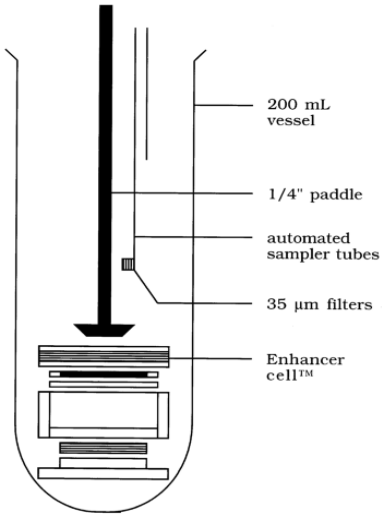

Figure 5: Fully assembled Enhancer cell (11)

Advantages of Enhancer cell:

- Receptor volume is larger when compared to other static diffusion cells.

- It requires less accessories and hence reduces the time and cost required for equipment setup.
- This method can be automated with relative ease whereby the sample can be collected and transferred to the HPLC (High performance liquid chromatography)
- Made of Teflon which is an inert material and thus has no problems of interaction of the formulation with the cell.
- The problem of breakage, common with most glass diffusion cells, is also avoided.

Although this modern type of cell implicates the modifications of the existing and easily available apparatus (USP-2) which is universal to most of the researchers, but keeping in mind the standard Franz diffusion cell, it has not still overcame the limitations of FDC.

Limitations of Enhancer cell:

- Formation of air bubbles
- Made of Teflon (a poor conductor of heat with a small heat transfer coefficient),
- The temperature equilibrium could take a finite time, requiring the cell and the formulation both are stored at the study temperature before use.
- Complicated and costly since using HPLC.

Vertical diffusion cell (VDC)

A VDC is commonly used to determine in vitro release testing of topical drug products such as creams, gels, ointments. The ideal material for construction is borosilicate glass. This system dwells in two chambers; a donor chamber and a receptor chamber, held together by a clamp (see Figure 6). The semisolid dosage form is smeared on the synthetic membrane which sits in the cavity of the dosage compartment that is covered with a glass disk. The overall setup is intern placed in the donor chamber. The diameters of the orifices of the donor chamber and the dosage compartment here defines the dosage delivery area, which should be sized within ± 5% of the specified diameter. The receptor chamber volume should be within ± 20% of specified volume and its receptor chamber orifice should be fancied to the same size of donor chamber [12]

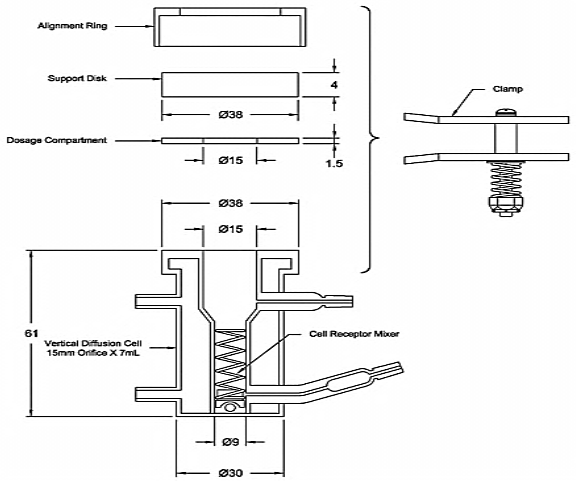

Figure 6: Vertical diffusion cell (All measurements are expressed in mm unless noted otherwise [12]

Apart from FDC, advantages of vertical diffusion cell:

- Uniform mixing of receptor fluid
- Minimization of evaporation of the receptor solvent

Limitations of VDC:

- The design of VDC should facilitate proper alignment of dosage compartment within donor compartment to align on receptor compartment.
- Formation of air bubbles
- Exuberant care during sampling and replenishment of the receptor fluid
- Laborious and large variation among experiments.

Flow through diffusion cell

The flow through cells have several advantages over static: cells can include automated or manual sampling, sink conditions can be maintained throughout the course of the experiment, which is crucial for monitoring the permeation profile of substances with low solubility in receptor fluid. Thus, the flows through cells mimic the blood flow through skin closer than the static types.

Novel diffusion cell

Mahajan et al., invented three new designs of diffusion cells that were developed for *in-vitro* transdermal permeation. All the cells consisted of an inlet compartment (A), a donor compartment (B) and a receptor compartment (C). The diffusional are is 0.51cm^2 and the receptor volume is approx. 84µl. The depth of an inlet compartment was increased in only one of the three designs. The constructive material of cell was acrylic sheet. The membrane (D) was positioned between the donor and the receptor compartments using O- ring (E). The flow of the receptor fluid first entered the cell via the inlet compartment then flowed through inlet channel (F) to the receptor compartment and left the cell via the outlet channel (G) to the sample collector (J). In the two designs (Figure 7. and 8.) entrapment of air bubbles was a problem but in (Figure 9.) it was avoided by increasing the depth of the inlet compartment. The performance of the cells were examined for 8 hours diffusion experiment using Nimesulide as the model drug and the results were found to be similar to the Franz diffusion cell [13]

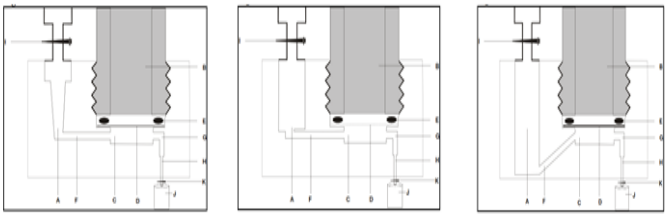

Fig.7. First design of newer cell **Fig. 8.**Second design of newer cell **Fig. 9.** Third design of newer cell

A = inlet compartment; B = donor compartment; C = receptor compartment; D = membrane; E = O-ring; F = inlet channel; G= outlet channel; H = outlet tube; J = sample collector (13)

Advantages of novel diffusion cell:

- **Entrapment of air bubbles was avoided** (The only cell where the major problem is avoided)
- The problem of cell breakage is avoided
- Constant attention is not required during sampling since sample collector is fixed to cell

Limitations of novel diffusion cell:

- The model of the cell as merely focused on the design and not on temperature control
- The volume of the receptor compartment was very less (84µl)
- Mixing of the receptor fluid was not performed

Plexiglas flow through cell

Plexiglas flow through diffusion cell was designed by Chattaraj and Kanfer to monitor drug release from semisolid dosage forms (see Figure 10.). This cell is peculiarly useful for measuring the effect of variables such as membrane type, flow rate of a receptor fluid, and temperature on release rates. The cell system consists of two reservoirs, a receptor fluid reservoir and sample reservoir with a base plate supporting it. The semipermeable membrane is positioned between the two reservoirs. The receptor-fluid reservoir is divided into two equal parts, one carrying the inlet and the other carrying the outlet for the receptor fluid. A solid Plexiglas block seals the top of the receptor-fluid reservoir that acts as a support for clamping the cell in position. The entire cell is immersed in a constant temperature water bath. The system is automated and computer controlled by connecting it to a pump for the receptor fluid, a medium splitter, and a fraction collector [14]

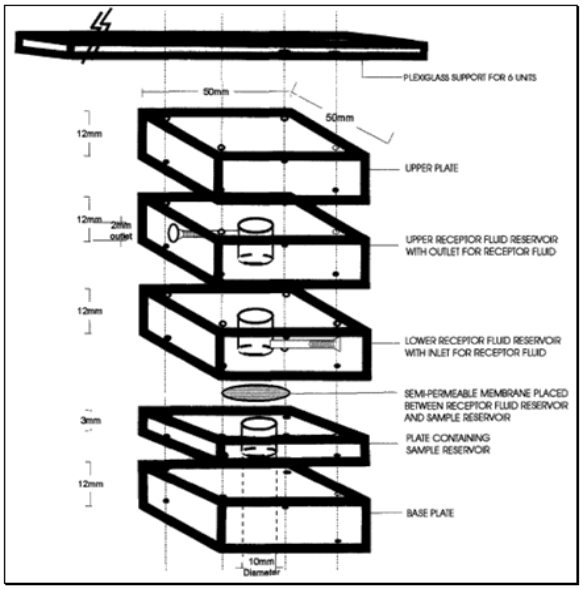

Figure 10: Plexiglas flow through cell [14]

An insertion cell

An insertion cell developed by Chattaraj and Kanfer with dimensions that permit the cell to be used with the compendial flow-through cell, has been contrived (see Figure 11.). The cell comprises of merely three components, the upper section, the middle section and the lower section. The upper section of the insertion cell consists of an oblong Plexiglas block with a 9-mm circle cut out of it. The middle section consists of a matching oblong Plexiglas block with a similar 9-mm circle cut out of it, which acts as the sample holder. The lower component is a solid Plexiglas block.

All three sections are screwed together. A membrane is placed between the upper section and the middle section. A stainless steel spring supports the insertion cell (for the turbulent-flow mode), and a layer of glass beads in the conical section of the flow-through cell supports the insertion cell (for the laminar flow mode). The insertion cell is positioned 10 mm from the conical section of the flow-through cell when used with spring support. The 'insertion cell' offers distinct advantages compared to the Franz cells in that it is easier to use and readily adaptable for use with the compendia flow-through apparatus and does not suffer from the problem of having to remove air bubbles at the membrane/liquid interface, which commonly occurs when using Franz cells [15]

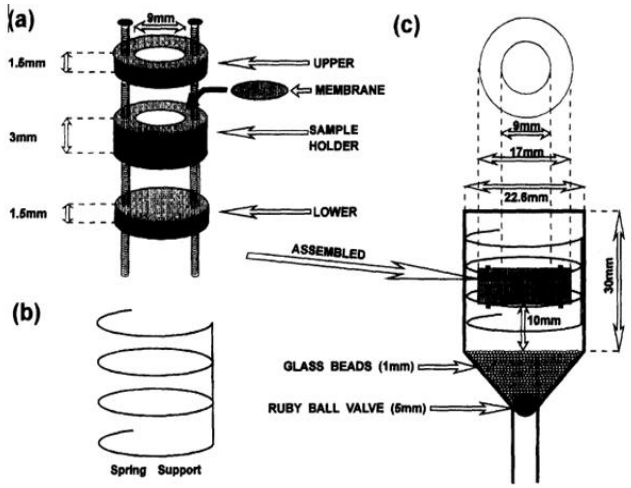

Figure 11: An insertion cell using compendial flow through diffusion cell [(a) insertion cell (b) Spring support used during turbulent flow studies (c) Insertion cell inside compendial flow-through cell apparatus.] (15)

Kelder cell with ASPEC (Automatic Sample Preparation with Extraction Columns)-system

Three designs of Kelder-cells were developed and all these consisted of an inlet compartment (A), a donor compartment (B) and a receptor compartment (C) (Figure 12.). The cells were made from Plexiglas and the main difference among the three designs was the size and the depth of the inlet compartment. The membrane (D) was positioned between the donor and receptor compartment using a Viton® O-ring (E).

The donor compartment was covered with parafilm to prevent evaporation of the solvent. The receptor solution entered the cell via the inlet compartment (A), flowed through the inlet channel (F) to the receptor compartment (C) and left the cell via the outlet channel (G). An outlet tube (H) made of stainless steel was fixed at the end of the outlet channel.

The inlet compartment of the cell is sealed with a polypropylene cap (J) to force the buffer to flow through the cell when fresh buffer is injected. The receptor solution with permeated drug was collected in polyethylene tubes, placed below the cells. The entire cell is very compact with the diffusional area of 0.51 cm and the receptor compartment volume of 77µl.

The newly developed Kelder-cells were made compatible with the ASPEC-system (Automatic Sample Preparation with Extraction Columns). As depicted in (Figure 12.1), the ASPEC-system consists of three sections: a Model 401 dilutor (A), a sample processor (B), and a set of racks and accessories to handle SPE-columns and solvents (C).

The dilutor allows to transfer a specified volume of solvent from a reservoir (D) through the needle (E) into a container and also to aspirate air or liquid from a container into the needle. The needle of the ASPEC-system is able to move blocks with SPE-columns to a programmed position. These three features of the ASPEC system were used to perform permeation experiments [16]

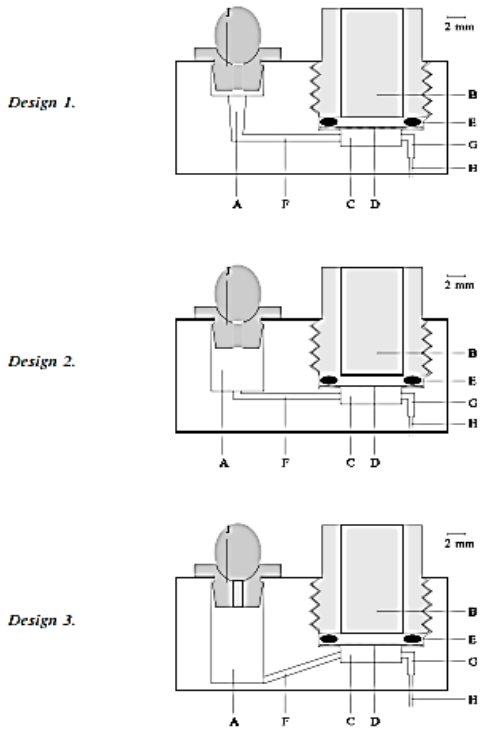

Figure 12: Cross-sections of the three Kelder-cell designs A = inlet compartment; B = donor compartment; C = receptor compartment; D = membrane; E = O-ring; F = inlet channel; G = outlet channel; H = outlet tube; J = polypropylene cap (16)

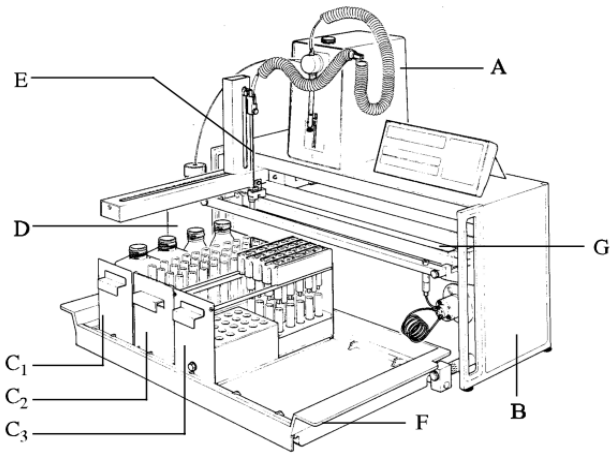

Figure 12. 1: The ASPEC-system [16] A = Model 401 dilutor; B = sample processor; C1 = solvent rack; C_2; sample rack; C_3 = SPE rack; D = reservoir; E = needle; F = polypropylene tray; G = rinsing station

Conclusion

Semisolid dosage forms constitute a significant proportion in the modern pharmaceutical formulations as they have extensive better patient compliance. This review was mainly focused on the advanced diffusion cells and their designs to test the batch-to-batch drug release equivalence for semisolid dosage forms which can facilitate an easy performance of quality control tests for semisolids dosage forms. Currently no official procedure exists for the evaluation of *in vitro* drug release testings of semisolid dosage forms. Till today, there are no pharmacopoeial methods recommended to carry out *in vitro* release tests for semisolid dosage forms and about selection of diffusion cells. Majority of the skin permeation studies involves the use of Franz-type diffusion cells but this system encounters the aforesaid problems. A unique widely accepted diffusion cell should be developed in such a manner, that a drug with low solubility or with high solubility permeation studies can be conducted in a single diffusion cell. This indicates there is a clear need and challenging task to the pharmaceutical scientists as well as industries to develop a simple, reliable and reproducible diffusion technique which acts as an *in vivo* surrogate marker for drug release testings of semisolid dosage forms.

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