Chapter 5
In-Vitro & Ex-Vivo Studies

5.1 Introduction

In vitro diffusion of formulations is a valuable tool to predict the behavior of a particular formulation with respect to drug transport across the skin. In this regard, two different cases have to be distinguished: local delivery to selected skin layers (e.g., antimycotics) and systemic delivery (e.g., hormones). In the context of bioavailability assessment, knowledge on the absorption behavior of the active compound is essential. For ethical reasons, fundamental skin absorption data can normally not be obtained by conducting *in vivo* studies. Therefore, other techniques must be used to obtain the desired information. One option to obtain these data is the use of *in vitro* penetration and permeation models. Some basic information on these techniques is provided in a number of documents, such as the Organization for Economic Cooperation and Development (OECD) guideline 428 in combination with OECD guidance 28, the Scientific Committee on Cosmetic and Non-Food Products Intended for Consumers (SCCNFP) guideline, a European Commission (EC) guide, and a United States Food and Drug Administration (FDA) guidance.

Generally, the stratum corneum is considered to be the rate limiting layer of the skin with regard to transdermal drug absorption. However, for the invasion of very lipophilic compounds, the bottleneck moves from the stratum corneum down to the viable, very hydrophilic layer of the epidermis, due to substances' reduced solubility in this rather aqueous layer.

A simple, reliable, reproducible, relevant, and generally acceptable in vitro method to assess drug release from a semisolid dosage form would be highly valuable for the same reasons that such methodology has proved valuable in the development, manufacture, and batch-to-batch quality control of solid oral dosage forms.

When drugs are applied topically, a pharmacologically active agent must be released from its carrier (vehicle) before it can contact the epidermal surface and be available for penetration in the stratum corneum and lower layers of the skin. A topical formulation thus is a complex drug delivery system, and the dynamics of drug release from a vehicle have been a subject of debate and investigation for many years.

In vitro release is one of several methods used to characterize performance characteristics of a finished topical dosage form. Important changes in the characteristics of a drug product or in the thermodynamic properties of the drug substance in the dosage form should be manifested as a difference in drug release. Both static and flow-through diffusion cells are approved by the authorities and data are available on their relevance for predicting the *in vivo* situation. Basically, a donor and an acceptor compartment are separated by a membrane of either native skin or bioengineered materials. These materials can be of human, animal, or artificial origin.

Drug release is theoretically proportional to the square root of time ($\sqrt{t}$) when the drug release from the formulation is rate limiting. A plot of the amount of drug released per unit area (g/cm2) against the square root of time yields a straight line, the slope of which represents the release rate. This release rate measure is formulation specific and can be used to monitor product quality. As summarized in a FDA guidance document, recommended methodology for in vitro release studies is as follows:

Diffusion cell system: a static diffusion cell system with a standard open cap ground glass surface with 15-mm-diameter orifice and total diameter of 25 mm

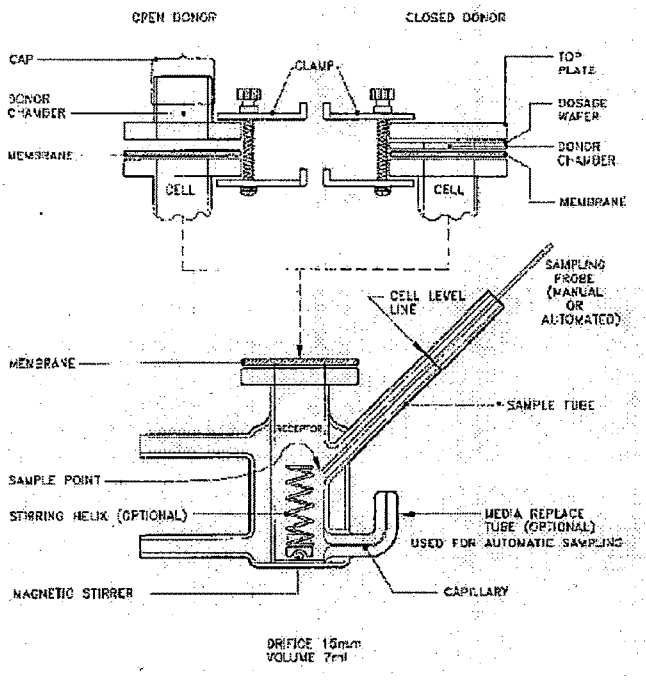

Fig. 5.1: A two compartment diffusion cell

Synthetic Membrane: appropriate inert, porous, and commercially available synthetic membranes such as polysulfone, cellulose acetate/nitrate mixed ester, or polytetrafluoroethylene 70 _m membrane of appropriate size to fit the diffusion cell diameter (e.g., 25 mm in the preceding case).

Membranes of Animal Skin Origin: The limited availability of human skin, together with an increasing need to test the bioavailability of dermal products in pharmaceutics and cosmetics, as well as risk assessment of chemicals, has promoted the search for alternative models. A switch to animal skin, therefore, seems obvious. Species currently in use are mouse, hairless rat, hamster (cheek pouch), snake (shed skin), pig (ear, flank, abdomen, or back), and cow (udder). However, differences in stratum corneum thickness, number of corneocyte layers, hair density, water content, lipid profile, and morphology cause animal skin to be more permeable than human skin.

After excision, the skin should quickly be freed from subcutaneous fat and stored deep frozen at -20°C to -30°C in tightly sealed plastic bags (Shah et al, 2001).

Viability of Skin: Viable skin more closely simulates *in vivo* conditions. Cadaver skin is also acceptable for use in a skin absorption study.

Receptor Medium: appropriate receptor medium such as aqueous buffer for water soluble drugs or a hydro-alcoholic medium for sparingly water soluble drugs or another medium with proper justification. A physiological buffer such as a balanced salt solution or tissue culture medium is needed to maintain viability of the skin for at least 24 h. Bovine serum albumin is sometimes added to increase the solubility of lipophilic compounds. It is preferable to use a physiological buffer even when metabolism is not measured to simulate *in vivo* conditions. Some protocols use solubilizing agents in the receptor fluid so that skin absorption can be more easily determined by simply sampling the receptor fluid. Selection of an appropriate receptor medium is important to maintain sink conditions during *in vitro* release studies for products containing water insoluble drugs.

Temperature: may be controlled by using a water jacket around each permeation cell, an external water bath, or warm air in a drying oven. Usually, experiments are carried out at 32°C, that is, the temperature of the skin surface, or else a temperature gradient may be applied: of 32.C at the skin surface to 37°C in the acceptor compartment. Constant stirring of the acceptor phase ensures that diffusion is unhampered by the buildup of high local concentrations and provides sink conditions throughout the experiment.

Sample Applications: About 300 mg of the semisolid preparation is placed uniformly on the membrane and kept occluded to prevent solvent evaporation and compositional changes. This corresponds to an infinite dose condition. Dosing is possible in infinite (typically >10 µl/cm2 or 10 mg/cm2) or finite manner (<10 µl/cm2 or 10 mg/cm2). The donor chamber may either be left open or be occluded.

Sampling Time: Multiple sampling times (at least 5 times) over an appropriate time period are required to generate an adequate release profile and to determine the drug release rate.

***In vitro* release rate**: A plot of the amount of drug released per unit membrane area (g/cm2) versus square root of time should yield a straight line. The slope of the line (regression) represents the release rate of the product. An X intercept typically corresponding to a small fraction of an hour is a normal characteristic of such plots (Bronaugh et al, 2001)

Fick's laws of diffusion

Considering that the skin is such a heterogeneous membrane, it is surprising that simple diffusion laws can be used to describe the percutaneous absorption process. Since transdermal delivery involves the application of a device over a long period of time, it is generally assumed that steady-state conditions have been reached and that the most relevant law of diffusion is therefore Fick's first law.

The most quoted form of Fick's first law of diffusion describes steady-state diffusion through a membrane:

$J = KD/h \times (Co - Ci)$ (1)

Where J is the flux per unit area, K is the stratum corneum-formulation partition coefficient of the drug, and D is its diffusion coefficient in the

stratum corneum of path length h; Co is the concentration of drug applied to the skin surface, and Ci is the concentration inside the skin. In most practical situations, Co >>> Ci, and Eq. (1) simplifies to

$J = KpCi$ (2)

Where kp (DK/h) is the permeability coefficient, which has units of velocity, i.e., it is a heterogeneous rate constant and encodes both partition and diffusional characteristics (Barry, 1999 and Knepp et al, 1987).

Mathematical analysis of the release of drug from different ointment bases can be carried out using the Higuchi equation for solution type of ointments (Higuchi, 1962). When the amount of drug released was plotted against the square root of time, a straight line was obtained. The diffusion coefficient of drug (D) was calculated using the following equation (Giirol et al, 1996):

$$Q = 2\,Co\,\sqrt{DT/\pi}$$

Q: Cumulative amount of drug released per unit area (mg/cm^2);

C: Initial concentration of drug in the ointment (mg/cm^3);

D: Diffusion coefficient (cm^2/h)

T: time (h)

5.2 Methods

5.2.1 Drug Diffusion Studies across Artificial Membrane

Semipermeable membrane

Dialysis membrane (LA-401, molecular weight cut off: 12000 Dalton; Himedia, India), 150μm in thickness was used as an artificial membrane for preliminary *In vitro* studies because of simplicity, homogeneity and uniformity. The membrane was activated by washing it in running tap water for 3-4 h, followed by treatment with 0.3%w/v of sodium sulphide solution at 80 C for 1 min. then it is washed with hot water at 60° C for 2 min followed by acidification with 0.2% sulphuric acid for 2-3 min. Finally it is rinsed with hot water at 60° C for 2-3 min.

Design of diffusion cell

For the present study a vertical type of membrane diffusion system was developed. The system consists of a hollow glass tube open at both ends with inner diameter of 18 mm and 6 cm length. The membrane was tied to one end of the tube with a nylon string and this tube acts as a donor compartment. The tube was dipped flush on the surface of a 100 ml beaker containing diffusion medium that is the receptor compartment. The receptor solution was stirred at 100 rpm using a Teflon coated magnetic needle (length = 2.5 cm, d = 0.5 cm) and the surrounding water bath by the aid of a magnetic stirrer (Remi, India). The temperature of the bulk of the solution was maintained at 32 + 1.0° C.

Validation of diffusion cell

The hydrodynamic characteristics of the diffusion cell were established using the benzoic acid disc method (Mojaverian et al, 1997; USP 24).

Selection of diffusion medium

The diffusion medium was selected based on the solubility of drugs so as to maintain sink conditions during the course of studies. Water: methanol (70:30 v/v) proved to be an effective diffusion medium for both the drugs.

Diffusion Protocol

In-vitro drug release studies were carried out in a two compartment apparatus. The donor contains the releasing system (formulation equivalent to 0.5 mg of HP/Tac); the drug reaches the receiving phase (25 ml, water: methanol 70:30 v/v) through a semi-permeable cellulose acetate membrane, previously activated. The apparatus was thermostated at 32°C and under constant slow speed stirring. At pre-selected time points aliquots were withdrawn from the receptor compartment for analysis of drug content and replaced by an equivalent receptor solution (Fini et al, 2008).

5.2.2 Drug Permeation and Skin Retention Studies in Rodent and Human Cadaver Skin

Franz diffusion cell

The *in vitro skin permeation* studies were carried out using franz diffusion cell. This cell consists of a hollow glass tube in the center having diameter of 10mm. The cell has two compartments viz. i) donor compartment and ii) receptor compartment. The donor compartment is used for holding the test formulation while the receptor compartment holds the respective diffusion media. The hydrodynamic characteristics of the franz diffusion cell was established using benzoic acid disc method (Chein and Valia 1984)

Permeation and Skin Retention Study Protocol

Male or female healthy Sprague dawley rats weighing between 250-350 g are selected for the study. The abdominal rat skin is cleaned using cotton swab and disinfectant solution. The animals were euthanized by ether anesthesia. The abdominal skin was surgically excised from the sacrificed animals and immediately placed in balanced salt solution or phosphate buffered saline pH 7.4. The excised skin is immediately freed from the subcutaneous and extra tissues surgically. The hair from the excised skin is shaved off to yield hairless skin or with negligible hair. The excised skin is kept in balanced balanced salt solution or phosphate buffered saline in deep freezer at -40°C till further use.

Human cadaver skin from the abdominal region was obtained from Baroda Medical College (Vadodara, India). The excised skin was made free from subcutaneous fat surgically. The skin was stored at -80° C and thawed at room temperature prior to use.

The two compartment jacketed Franz diffusion cells were filled with receptor medium and checked for any leakage and their effective area for diffusion is determined. The full thickness skin obtained from excision was then mounted on to these franz diffusion cells between the donor and receptor compartment and clamped. The mounted membranes were checked for any sort of leakage or irregularities.

The receptor compartment is filled with physiologically simulating receptor solution (25 ml capacity) and the skin is allowed to equilibrate and stabilize for 30 minutes at 32-35°C. After 30 minutes of stabilization of the skin, the receptor medium is replaced with a fresh medium. Weighed quantities (around 1 g) of creams are applied in donor compartments onto the skin ensuring proper spreading of the applied cream. Around 0.5 ml of

physiological simulating solution is also added to donor compartment to maintain normal skin conditions. The receptor or acceptor compartment is maintained at temperature between 32-35°C and stirred with a magnetic bead and stirrer at around 150 rpm. Care is taken to ensure avoidance of frothing or air bubble deposition in the receptor compartment and ensuring complete and uniform contact of the skin with underlying medium.

At predefined time intervals (4h, 6h, 8h, 10h, 12h and 24 h) 2 ml aliquot of the receptor medium is withdrawn and replaced with fresh medium. The removed aliquots are filtered through 0.2 μ nylon filter and then analyzed by HPLC with suitable dilution if required. At the end of experiments, after 24 h, the clamps are opened and the two compartments separated. The skin is also removed from the diffusion apparatus and adhering creams are removed. The skin is washed with 5 ml aliquots of methanol thrice for 2-3 secs each. The skin is then weighed to determine the weight of tissue and minced into very small pieces using homogenizer. The minced skin is kept for digestion with methanol overnight. The methanol + skin are then sonicated for 15 min followed by filtration through 0.2 μ nylon filter. The methanol extract so obtained is then analyzed by HPLC after suitable dilution, if required.

Quantity applied: 1g

Surface area for diffusion: ~ 3.75 sq.cm

Receptor media: Phosphate buffered saline pH 7.4 + propylene glycol (60:40)

Volume of receptor media: 25 ml

Sampling volume: 2ml

Sampling intervals: 4, 6, 8, 10, 12 and 24h

Volume of extracting solution for skin: 5ml of methanol.

Temperature: 32-35 °C

5.3 Results

5.3.1 Halobetasol Propionate

The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % drug release were shown in Table 5.1. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, Higuchi's equation and first order equations and recorded in Table 5.2. The formulations were compared for skin permeation and retention through rat skin and human cadaver skin for 24h. The results are recorded in table 5.3.

Table 5.1: *In vitro* diffusion study of HP formulations

Time (h)	% Drug Diffused (w/v) ± SD*					
	HP Solution	HPME	HPMEC (0.035%)	HPMEC (0.05%)	HP cream (0.05%)	HP ointment (0.05%)
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	24.00 ± 8.54	18.33 ± 5.03	5.00 ± 1.0	2.33 ± 1.53	1.67 ± 0.58	0.67 ± 0.57
0.50	41.66 ± 12.1	37.66 ± 4.16	10.33 ± 4.93	11.00 ± 4.58	5.67 ± 1.53	3.00 ± 1.0
1.00	72.66 ± 14.2	65.00 ± 9.0	14.00 ± 2.65	15.33 ± 3.06	12.67 ± 3.51	5.33 ± 1.53
2.00	88.00 ± 3.61	83.00 ± 4.0	24.67 ± 5.13	27.00 ± 4.58	23.67 ± 7.37	11.33 ± 2.08
4.00	89.66 ± 2.08	89.66 ± 3.1	38.67 ± 3.51	37.67 ± 6.66	33.33 ± 4.51	18.33 ± 2.52
6.00	-	-	68.66 ± 8.08	61.67 ± 7.02	56.00 ± 6.24	36.00 ± 7.81
8.00	-	-	82.00 ± 6.24	77.33 ± 3.51	67.34 ± 7.57	45.67 ± 8.02
24.00	-	-	88.34 ± 1.15	87.66 ± 4.51	85.00 ± 9.17	63.67 ± 8.50

*n=3

Table 5.2 Diffusion coefficient and regression coefficients of HP formulations

Formulation	Diffusion coefficient (cm^2/h)	Rate constant	Zero order	First order	Higuchi kinetics
			Regression Coefficient (R^2)		
HP Solution	0.293 ± 0.096	71.417	0.8818	0.7881	0.9506
HPME	0.218 ± 0.089	47.097	0.7323	0.5958	0.8665
HPMEC (0.035%)	0.031 ± 0.016	21.573	0.6655	0.5046	0.8548
HPMEC (0.05%)	0.028 ± 0.018	20.968	0.7087	0.4349	0.8913
HP cream (0.05%)	0.021 ± 0.015	20.492	0.763	0.4512	0.9281
HP ointment (0.05%)	0.008 ± 0.006	15.556	0.8337	0.494	0.9504

*n=3

Table 5.3: Skin retention data of HP formulation through rodent skin and human cadaver skin

Formulation	Rodent skin	Human cadaver skin
	Quantity (mcg) retained ± SD*	
HP Solution	35.67 ± 11.59	24.00 ± 9.16
HPME	32.67 ± 6.80	19.67 ± 9.86
HPMEC (0.035%)	20.33 ± 4.04	13.33 ± 4.16
HPMEC (0.05%)	25.33 ± 4.04	15.67 ± 3.78
HP cream (0.05%)	14.33 ± 4.51	10.33 ± 4.50
HP ointment (0.05%)	20.67 ± 3.05	15.00 ± 3.60

*n=3

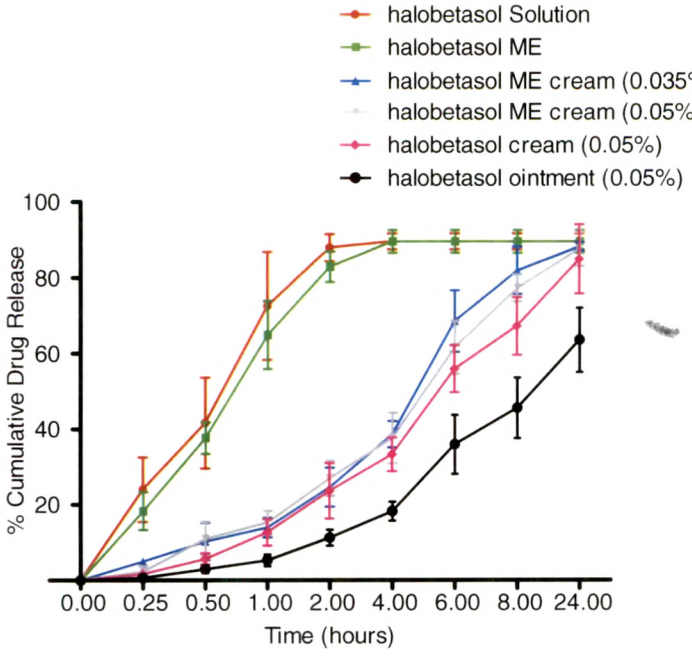

Fig. 5.2: Comparative cumulative HP release through semi permeable cellulose acetate membrane. Values are mean ± SD for n=3 experiments. Release profiles were statistically compared using Two Way ANOVA, Bonferroni post test with $p<0.05$ considered significant.

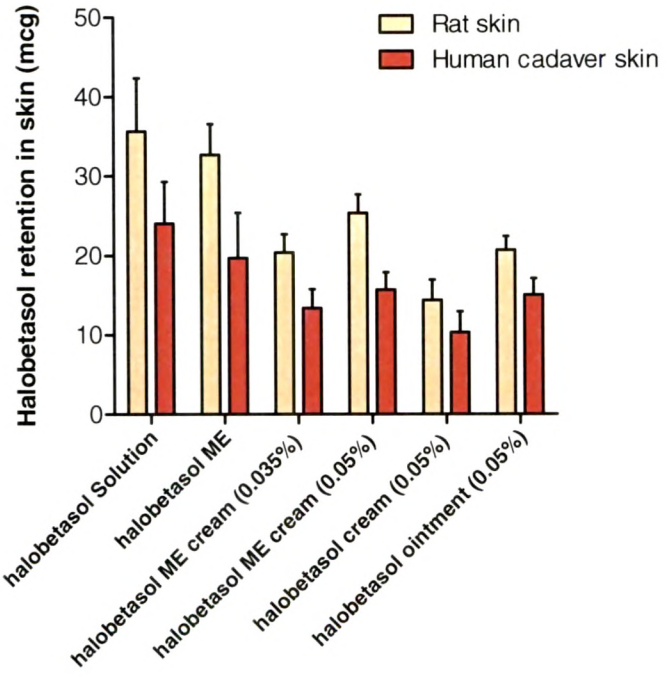

Fig. 5.3: Comparative HP retention in rat skin and human cadaver skin following 24 h application. The columns and the error bars represent means ± SEM for n=3. Results were statistically compared using Two Way ANOVA, bonferroni's post test $p<0.05$ considered as significant.

5.3.2 Tacrolimus

The formulations were studied in triplicate for diffusion studies and the mean cumulative values for % drug release were shown in Table 5.4. The release kinetics of diffusion was studied by calculating the regression coefficient for zero order, Higuchi's equation and first order equations and recorded in Table 5.5. The formulations were compared for skin permeation and retention through rat skin and human cadaver skin for 24h. The results are recorded in table 5.6.

Table 5.4 *In vitro* diffusion study of Tac formulations

Time (h)	**% Drug Diffused (w/v) ± SD***			
	Tac Solution	**Tac ME**	**Tac MEC 0.1%**	**Tac ointment 0.1%**
0.00	0.00	0.00	0.00	0.00
0.25	33.00 ± 8.54	23.66 ± 5.03	3.67 ± 1.53	0.33 ± 0.57
0.50	51.66 ± 10.26	44.33 ± 6.11	8.00 ± 2.00	0.67 ± 1.15
1.00	82.66 ± 8.50	75.00 ± 9.00	15.00 ± 2.00	3.00 ± 1.00
2.00	89.00 ± 3.00	89.66 ± 3.05	24.66 ± 4.04	9.66 ± 1.53
4.00	88.00 ± 4.60	87.66 ± 5.2	40.66 ± 5.13	14.33 ± 2.08
6.00	-	-	68.66 ± 12.85	21.67 ± 3.05
8.00	-	-	82.00 ± 6.24	34.00 ± 3.0
24.00	-	-	86.66 ± 2.51	64.66 ± 8.50

Table 5.5 Diffusion coefficient and regression coefficients of Tac formulations

Formulation	Diffusion coefficient (cm^2/h)	Rate constant	Zero order	First order	Higuchi Kinetics
			Regression Coefficient (R^2)		
Tac Solution	0.101 ± 0.025	62.563	0.7904	0.7313	0.8845
Tac ME	0.078 ± 0.027	72.85	0.8657	0.7609	0.9417
Tac MEC 0.1%	0.008 ± 0.004	21.882	0.6733	0.4761	0.8867
Tac ointment 0.1%	0.0012 ± 0.001	15.056	0.959	0.5205	0.9802

Table 5.6: Skin retention data of Tac formulation through rodent skin and human cadaver skin

Formulation	Rodent skin	Human cadaver skin
	Quantity (mcg) retained ± SD	
Tac Solution	15.86 ± 3.94	13.03 ± 2.61
Tac ME	13.63 ± 5.20	10.70 ± 2.43
Tac MEC 0.1%	13.30 ± 3.51	8.90 ± 2.74
Tac ointment 0.1%	4.26 ± 1.19	3.20 ± 1.30

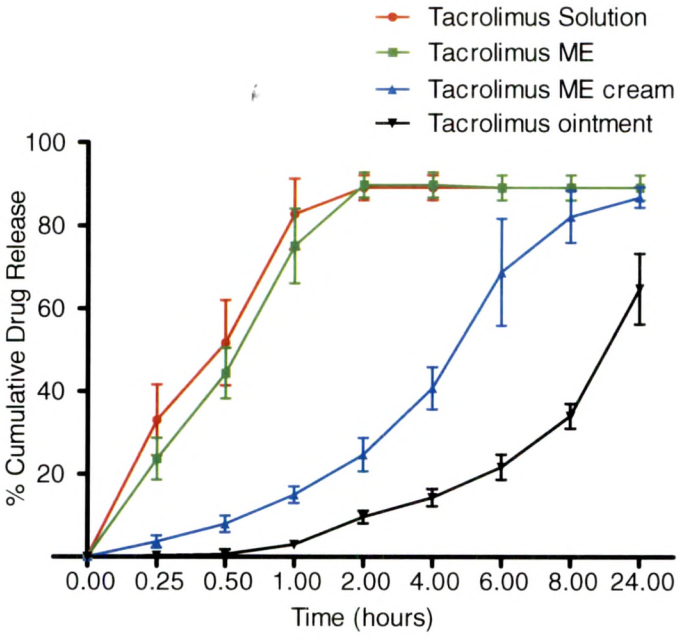

Fig. 5.4: Comparative cumulative Tac release through semi permeable cellulose acetate membrane. Values are mean ± SD for n=3 experiments. Release profiles were statistically compared using Two Way ANOVA, Bonferroni post test with $p<0.05$ considered as significant.

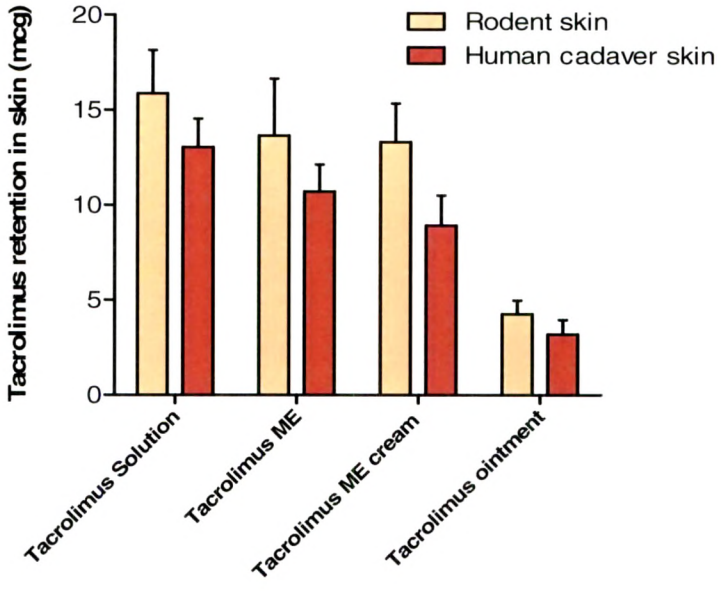

Fig. 5.5: Comparative Tac retention in rat skin and human cadaver skin following 24 h application. The columns and the error bars represent means ± SEM for n=3 experiments. Results were statistically compared using Two Way ANOVA, bonferroni's post test with $p<0.05$ considered as significant.

5.4 Discussion

HP formulations [HP solution in propylene glycol, HPME, HPMEC 0.035% and 0.05%, HP cream and ointment (marketed preparations) were subjected to *in vitro* diffusion studies through semi-permeable cellulose acetate membrane. The percent cumulative drug diffused across the cellulose acetate memebrane was calculated and recorded in Table 5.1. The kinetic pattern of the diffusion was studied by fitting % drug diffused in given time in different order kinetics like zero order, first order and higuchi kinetics. Regression coefficients of all formulations in different orders were compared and found that the release pattern of HP from the formulation across the semi-permeable cellulose acetate followed higuchi kinetics rather than zero order and first order. This was concluded by higher regression coefficient value in curve fitting. Rate constant for all the formulations was calculated and recorded in Table 5.2. Among the formulations subjected to *in vitro* diffusion studies and it was observed that the drug release from solution and ME was faster than semisolid preparations and reached more than 75% in first hour. When drug loaded ME was dispersed in the cream base, the release was slow and prolonged for 8 h. The drug release from commercial HP cream was slightly slower in comparison to ME based cream. The drug release from the commercial ointment of HP was least and reached around 65% in 24 h. The results of in-vitro drug release study showed significant difference in the release profile of formulations (Fig. 5.2). Solution and drug loaded ME showed near complete release in the first hour as there is no physicochemical barrier to drug release. The ME based cream showed slightly faster drug release in comparison to the commercial cream. The ointment demonstrated a significantly slower and incomplete release in 24h which may be due to the lipophilic nature of the vehicle and consequently increased affinity of the drug for the vehicle.

Formulations were compared for drug permeation and skin retention of HP in an *ex-vivo* study with rat skin and human cadaver skin. The profiles were compared for solution, ME, 0.05% and 0.035% MEC, and marketed cream and ointment of HP (Fig. 5.3). Drug could not be detected in receptor solution even after 24 h of application. The drug retention in skin followed the rank order - HP Solution > HP ME > HP MEC (0.05%) > HP ointment ~ HP MEC (0.035%) > HP cream. The data is recorded in table 5.3. It can also be seen that the drug retention in both biological membranes follow similar trend. The values are lower for human cadaver skin in comparison to rat skin. Rodent skin is often used for *ex-vivo* drug permeation studies. It has been reported that the kinetics in rodents often mimic the human data. The drug permeation and skin retention studies in rat skin and human cadaver skin showed enhanced permeation with HPME and HPMEC (0.05%). Drugs being present in a supersaturated condition in MEs enhance dermal penetration due to high thermodynamic activity of the drug. MEs alter solvent properties of stratum corneum to favor drug partitioning into the skin. The lipophillic drug is retained in lipidic skin layers and thus, drug is not detectable in receptor solution. Although cream base being viscous retards drug release in comparison to ME, but it is probably compensated by permeation enhancement due to the partial occlusivity and hydration provided by cream base. Ointments are highly occlusive and increase hydration to open up channels for drug transport. HP Solution in propylene glycol exhibited highest retention in skin which may be due to solvent drag effect of propylene glycol and its transdermal permeation enhancement capabilities (Godin et al, 2007, Teichmann et al, 2007, Loth, 1991 and Kalbitz et al, 1996).

Tac formulations [Tac solution in propylene glycol, Tac ME, Tac ME based cream 0.1% and Tac ointment (marketed preparation)] were subjected to *in vitro* diffusion studies through semi-permeable cellulose acetate membrane.

The % cumulative drug diffused across the cellulose acetate membrane was calculated and recorded in Table 5.4. The kinetic pattern of the diffusion was studied by fitting % drug diffused in given time in different order kinetics like zero order, first order and higuchi kinetics. Regression coefficients of all formulations in different orders were compared and found that the release pattern of HP from the formulation across the semi-permeable cellulose acetate followed higuchi kinetics rather than zero order and first order. This was concluded by higher regression coefficient value in curve fitting. Rate constant for all the formulations was calculated and recorded in Table 5.5. Among the formulations subjected to *in-vitro* diffusion studies and it was observed that when this ME was dispersed in a cream base, the drug release was slow and prolonged for 8 h. The drug release was slowest in the commercially available ointment of Tac as it showed around 70% drug release in 24 h. The results of in-vitro drug release study showed significant difference in the release profile of the drug (Fig. 5.4). Solution and drug loaded ME showed near complete release in the first hour indicating no physicochemical barrier to drug release. The increased viscosity of cream base sustained the drug release for a longer duration of time from ME based cream. At the same time it did not hinder drug release from the base system since more than 85% of drug release was seen in 8 h. The ointment demonstrated a significantly slower and incomplete release in 24h. This may be due to lipophillic nature of the vehicle and increased affinity of the drug for the vehicle.

Formulations were compared for drug permeation and skin retention of Tac during and at end of 24 h application in an *ex-vivo* study with rat skin and human cadaver skin. The profiles were compared for Tac solution, Tac ME, Tac MEC 0.1%, and commercially available ointment of Tac. The data is recorded in table 5.6. Even after 24 h of application drug concentration in the receptor solution was below detection levels for both rat skin and human

cadaver skin. The drug retention in skin followed rank order: solution > ME > MEC > ointment (Fig. 5.5) for both biological membranes. Comparable skin retention profiles for ME and MEC were observed. The drug retention in skin with ME and ME based cream was almost two fold as compared to the ointment. The drug solution too exhibited significant drug retention in skin as compared to the ointment. The drug retention through human cadaver skin followed similar pattern to rat skin except for retention being less with human cadaver skin. The drug retention studies in rat skin and human cadaver skin showed enhanced permeation with ME and ME based cream. MEs are known to enhance dermal penetration by increasing thermodynamic activity of the drug and providing steep concentration gradient as well as by altering the solvent properties of stratum corneum. The increased thermodynamic activity due to super-saturation favors its partitioning into the skin. The comparative hydrophilicity of the cream vehicle further enhances its partitioning into skin because the drug is highly lipophilic in nature (log P~3.2). The lipidic nature of the skin serves to retain the drug in its layer and thus, very low quantities were present in the receptor solution. Although cream base with its increased viscosity delays drug release in comparison to ME, but it is probably compensated by the enhancement due to the partial occlusivity and hydration provided by cream base. Ointment being lipophilic in nature probably did not favor drug partitioning into skin; however occlusivity afforded by ointments is highest amongst all. This increases hydration and opens up channels for drug transport. Solution too exhibited comparative retention in skin which may be attributed to the solvent drag effect of propylene glycol. The drug retention was less in human cadaver skin in comparison to rat skin but followed a similar pattern. Among rodents, rat skin is said to be structurally more similar to human and often skin permeation kinetic parameters through rat skin are comparable with human skin (Godin et al, 2007, Teichmann et al, 2007, Loth, 1991 and Kalbitz et al, 1996).

5.5 References

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