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Original Article

Effect of Different Diffusion Membranes on the Diffusion Rate of Niacinamide and Diclofenac Sodium From Topical Formulations

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ABSTRACT

The aim of the study was to correlate diffusivity of Niacinamide from a cream formulation and Diclofenac sodium from a gel formulation. Evaluation is done by *in vitro* drug release through Franz diffusion cell and different diffusion membranes. The membranes used for the study were Polytetrafluoroethylene (PTFE), Polyvinylidene fluoride (PVDF) and Dialysis membranes. Phosphate buffer pH 5.5 was the receptor medium. Studies showed significant variations in the diffusion rate of niacinamide and Diclofenac sodium across different membranes. The percentage of Niacinamide diffused from niacinamide cream after 120 min was found to be PTFE (1.5%), PVDF (54.15%) and Dialysis membranes (21.6%). The percentage of diclofenac sodium diffused from diclofenac gel after 180 min was found to be PTFE (2.42%), PVDF (14.42%) and Dialysis membranes (9.8%). From the study it was concluded that membranes having different characteristic property shows different diffusivity. Therefore interpretation of *in vitro* diffusion data to correlate with *in vivo* dermal penetration need further in-depth study.

Key Words: Polytetrafluoroethylene (PTFE), Polyvinylidene fluoride (PVDF), Diclofenac sodium, Dialysis membrane, Niacinamide.

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INTRODUCTION

The skin is a physical barrier to most of the microorganisms, water, and most UV light. The acidic surface (pH 4.0–6.8) retards the growth of most of the pathogens. The skin protects the body from desiccation (dehydration) when on dry land and from water absorption when it was immersed in water. A normal body temperature of 37˚C (98.6˚F) is maintained by the antagonistic effect of shivering and sweating, as well as vasodilation and vasoconstriction of the blood vessels to the skin. Small amount of UV light is necessary for synthesis of vitamin D which is permitted through the skin. It is important to note that the certain toxins and pesticides also may enter the body through cutaneous absorption. The skin synthesizes the melanin (a protective pigment) and the keratin (a protective protein). Numerous sensory receptors are located in the skin, especially in parts of face, palms, and fingers of the hands, soles of the feet, and genitalia. The skin interacts with numerous body systems in accomplishing these various functions including the circulatory system, and nervous system.[1]

Structure of Human Skin

Human skin is an extraordinary organ that allows the terrestrial life by regulating water and heat loss from the body and at the same time preventing the entry of the harmful chemicals or microorganism.[2] The structure of skin is well known; however the deep description of this barrier highly in relation to skin kinetics is provided herein. The area of about 2 m^2 was covered by skin in an average human adult. It receives approximately 1/3 of blood circulating through the body. Skin with the thickness of few millimetres and separates the underlying blood circulation network from the outside environment. Anatomically, the skin contains three major tissue layers: the epidermis, dermis and hypodermis.[3] The outermost layer of the skin is called as epidermis and it comprises of stratified keratinised and squamous epithelium varies in thickness in different region of the body.[4] Epidermis consists of different layers such as stratum corneum, stratum lucidum, stratum granulosam, and stratum spinosam and stratum germinativum. Stratum corneum acts as rate limiting barrier which restricts or control the inside and outside movement of the chemical substances. It Consists of 15–20 layers of deeply flattened, metabolically inactive, polygonal cells having the dry weight density of 1.3–1.4 g/cm^3. Epidermis has no blood vessels or nerve endings, but its deeper layer are bathed in the interstitial fluid from dermis, which delivers oxygen and nutrients and is shattered away as lymph.[2]

Dermis is tough elastic, about 3-5 mm thick, which consists of a matrix of connective tissue woven from fibrous proteins.[4] Dermis essentially consists of 80% of protein.[3] Water binds collagen fibres and give the skin its tensile strength.[2] Branches from the arterial plexus supply blood to sweat glands, subcutaneous fat, hair follicles and dermis. Sweat glands are found all over the skin. The palms, soles of the feet, axillae and groins have the maximum sweat glands in number. Except soles of the feet, palms of the hand, red part of the skin, and selected portion of the sex organs hair follicles were found all over the skin surface.[3]

Routes of drug permeation through skin

Majorly three routes are there by which skin absorption may occur. Primarily, the chemical moieties are transported through the keratin-packed corneocytes via partitioning into and out of the cell membrane (trans cellular). Secondly, the molecule is passed around the corneocytes in the lipid rich extracellular region (intercellular). Thirdly, the shunt transport supported by the sweat glands, sebaceous glands and hair follicles (transappendageal).[5]

The permeation potential is through intercellular or trans cellular route and is highly dependent on their relative ability and partitioning in each phase. Thus, hydrophilic and lipophilic molecules follow separate pathway to transport across the skin layers.[6,7] Further, the non-ionic and the lipophilic compounds are easily permeated. Alternatively, skin appendages such as sebaceous gland, hair follicles and sweat glands acts as a diffusional shunt through rate-limiting barriers, facilitating the absorption of the topically applied molecules. Transappendageal absorption may be dominant pathway of dermal permeation, in case of slowly diffusing molecules.[6,8]

Transport mechanisms

Absorption of the drug molecules into the skin majorly done by passive diffusion. The transport rate of drug across the skin layers obeys Fick's Law of Diffusion. When the concentration gradient reaches zero, diffusion process stops.[9] The following equation defines the drug flux following passive diffusion

$$J = \frac{DPA\Delta C}{h}$$

Where, J is the steady state flux through the stratum corneum, D is the diffusion coefficient / diffusivity of drug molecule (cm^2/sec), ΔC is the concentration gradient of drug across the stratum corneum (g/cm^3), P is the partition coefficient of the drug between skin and formulation, h is the thickness of the stratum corneum (cm), A is the surface area of stratum corneum (cm^2). According to this equation, the rate of drug passage depends on the aqueous solubility, and directly proportionate to its partition coefficient (oil/water), concentration of drug in formulation and exposed surface area of the skin; and inversely proportionate to the thickness of skin. In reality, due to continuous blood stream there is low concentration on the receiver side. Thus, the donor side have relatively high drug concentration; equation becomes[10]

$$J = P_M AC$$

Where, P_M is the permeability constant, C is the concentration of the drug at the absorption site. However, the P_M can be determined by

$$P_M = \frac{DP}{h}$$

In Vitro method to predict skin diffusion[11]

Most common methods employed for evaluation of *in vitro* skin penetration is by using diffusion cells. The most commonly used solutions to diffusion equations which are applied to *in vitro* situation make the following assumptions:

1. The receptor phase is a perfect sink.
2. Depletion of donor phase is negligible.
3. The membrane is a homogeneous slab.

In vitro systems range in convolution from a simple two-compartment "static" diffusion cell to multi-jacketed "flow-through" cells. Construction materials must be an inert substance, and glass is most common. Excised skin is always mounted as a barrier between, a donor chamber and the receptor chamber, and the amount of compound permeating from the donor to the receptor side is determined as a function of the time. Efficient mixing of the receptor phase (and sometimes the donor phase) is vital, and removal of sample should be simple. Static diffusion cells are usually of the upright ("Franz") or side-by-side type, with receptor chamber of volumes of about 2–10 ml and surface areas of exposed membranes of near 0.2–2 cm^2.

Receptor Chamber and Medium

Receptor chamber dimensions were constrained by the conflicting requirements of guaranteeing that the receptor phase can act as a sink, while ensuring that the sample dilution does not preclude analysis. A large receptor volume may ensure sink conditions, but it will reduce analytical sensitivity unless large samples can be taken and subsequently concentrated. As a common rule, the concentration of the permeant in the receptor fluid should not be allowed to exceed approximately 10% of the saturation solubility. Excessive receptor-phase concentration can lead to a decrease in the rate of absorption, which may result in an underestimate of the bioavailability. pH 7.4 phosphate buffered saline (PBS) is the most commonly used receptor fluid , although this is not always the most appropriate material. It has been assumed that if a compound has a water solubility of less than about 10 μg/ml, then a wholly aqueous receptor phase is unsuitable, and the addition of solubilizers becomes necessary. There are many commercially available membranes used to carry out the diffusion studies, among them the three major membranes which are widely used were selected for the study.

Dialysis membrane

A regenerated seamless cellulose tubing, membrane is partially permeable and has molecular weight cut off 12000 to 14000. Pore size is 2.4 nanometres; ideal for filtration and osmosis work.

The dry membrane should be stored away from direct sunlight in a minimum relative humidity of 35% at a temperature of 23°C. Membranes keep well under these conditions but should always be examined to ensure no drying out has occurred.

Polytetrafluoroethylene (PTFE)

PTFE membranes are hydrophobic in nature having the pore size of 450 nm and are effective barriers to microbes and particulate matter.

Material characteristics include broad chemical compatibility, excellent particle retention, easy handling compatibility and sealing with various sterilizing methods, making it a versatile material for applications which require a hydrophobic barrier.

Polyvinylidenedifluoride (PVDF)

Polyvinylidenedifluoride (PVDF) membranes are hydrophobic having the pore size of 450 nm and have high binding affinity for proteins and nucleic acids.

MATERIALS AND METHODS

Materials

All chemicals used were of either analytical or pharmaceutical grade.

For Niacinamide Cream(Table 1)

Glyceryl monostearate SE, (Gifted by, Inolex, USA)
Cetyl alcohol, (LobachemiePvt. Ltd.)
Sorbitan monostearate, (LobachemiePvt. Ltd.)
Isopropyl myristate, (LobachemiePvt. Ltd.)
Methyl paraben, (Merck Pvt. Ltd)
Propyl paraben, (HimediaPvt. Ltd)
Avobenzone, (Gifted by, Chemspec Chemicals Pvt. Ltd., Mumbai)
Octyl methoxycinnamate, (Chemspec Chemicals Pvt. Ltd., Raigad)
Niacinamide, (Veer Chemie and Aromatics Pvt. Ltd., Hyderabad)
EDTA disodium salt, (Reachem Laboratory Chemicals Pvt. Ltd., Chennai)
Propylene glycol, (LobachemiePvt. Ltd.)

For Diclofenac sodium gel

Diclofenac sodium, (Gifted by Medrich, Bengaluru)
Carbopol 934, (LobachemiePvt. Ltd.)
Propylene glycol, (LobachemiePvt. Ltd.)
Methyl Paraben, (Merck Pvt. Ltd)
Propyl Paraben, (HimediaPvt. Ltd)
Triethanolamine, (Merck Pvt. Ltd)

Table 1: Formula chart for Niacinamide Cream

Ingredients	Percentage (%w/w)
Glycerylmonostearate SE	4
Cetyl Alcohol	3
Sorbitanmonostearate	2
Isopropyl myristate	5
Methyl paraben	0.25
Propyl paraben	0.15
Avobenzone	0.7
Octylmethoxycinnamate	1.5
Niacinamide	1.5
EDTA disodium salt	0.05
Propylene glycol	5

Table 2: Formula chart for Diclofenac sodium gel

Ingredients	Percentage (%w/w)
Diclofenac sodium	1
Carbopol 934	2
Propylene glycol	5
Methyl paraben	0.2
Propyl paraben	0.15
Triethanolamine	1

Table 3: Percent drug diffused after 120 min.

Sl. no	Time (min)	Membranes	% drug released
1	120	Polyvinylidene fluoride (PVDF)	54.15
2		Dialysis membrane	21.6
3		Polytetrafluroethylene (PTFE)	1.5

METHODS

Preparation of Standard solution for Niacinamide[12]

10 mg of Niacinamide was accurately weighed and transferred into 10 ml clean dry volumetric flask; 7 ml of water was added and sonicated for 5 mins. Finally, the volume was made up to 10 ml with water.

Into a 10 ml volumetric flask, 1 ml of the stock solution was taken and volume was made up with water.

From the above solution 0.25, 0.5, 0.75, 1, 1.25, 1.5 ml were withdrawn and transferred into 10 ml volumetric flask and volume was made up to 10 ml.

Preparation of 20% sodium chloride solution

Accurately Weighed and transferred 20 g of sodium chloride to the 100 ml volumetric flask, then the volume was made up with water. Sonicated the solution and filtered through 0.45 µ vacuum filter.

For Cream samples

0.2 g of the cream was measured directly into a centrifuge tube, 5 ml of 20% (w/v) solution of sodium chloride was added, and the mixture was shaken and centrifuged. Niacinamide was extracted into the water layer, which was then collected and injected into the HPLC instrument.

Preparation of Niacinamide cream

GMS, Cetyl alcohol, Span 60 were weighed into a beaker and melted in a water bath of temperature around 70°C to this required quantities of parabens were added. Finally IPM was added to the same oil phase. After this mix has melted AVO and OMC were added in the quantities mentioned above, and are kept on water bath till they dissolve. Simultaneously in another beaker distilled water, propylene glycol and EDTA disodium salt were heated to the same 70°C. The required amount of niacinamide was added to the aqueous phase and dissolved. The oil phase is added to aqueous phase to form an oil-in-water emulsion and is homogenized for 15 min at 8000 rpm and cooled to room temperature.

Preparation of Standard solution for Diclofenac

Table 4: Percent drug diffused from Dialysis membrane

Sl. no	Time (min)	Absorbance	Dilution factor	Amount of drug release (mg)	% drug released
1	0	0	0	0	0
2	30	0.09	10	0.048	4.8
3	60	0.104	10	0.055	5.74
4	90	0.123	10	0.066	7.11
5	120	0.151	10	0.081	7.44
6	150	0.129	10	0.069	9.34
7	180	0.184	10	0.098	9.8

Table 5: Percentage drug diffused from Polyvinylidene fluoride (PVDF)

Sl. no	Time(min)	Absorbance	Dilution factor	Amount of drug release (mg)	% drug released
1	0	0	0	0	0
2	30	0.140	10	0.075	7.5
3	60	0.181	10	0.097	9.7
4	90	0.205	10	0.110	11.021
5	120	0.216	10	0.120	13.41
6	150	0.228	10	0.122	14.21
7	180	0.221	10	0.118	14.42

Table 6: Percent drug diffused from Polytetrafluoroethylene (PTFE)

Sl. no	Time(min)	Absorbance	Dilution factor	Amount of drug release (mg)	% drug released
1	0	0	0	0	0
2	30	0.039	10	0.020	0.69
3	60	0.018	10	0.009	0.96
4	90	0.015	10	0.008	0.80
5	120	0.026	10	0.013	1.39
6	150	0.026	10	0.013	1.39
7	180	0.122	10	0.065	2.42

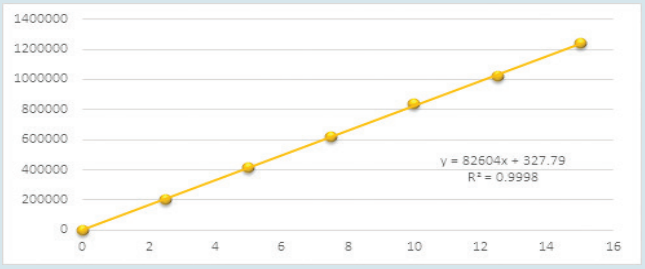

Figure 1: Standard calibration curve of Niacinamide.

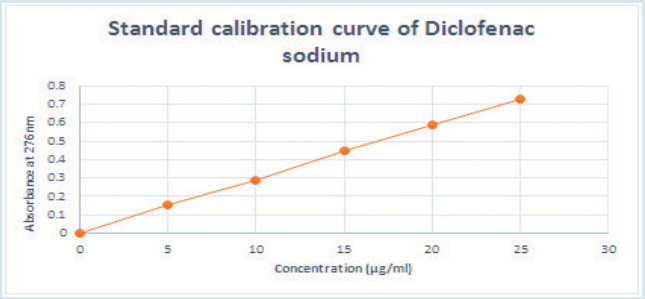

Figure 2: Standard calibration curve of Diclofenac sodium.

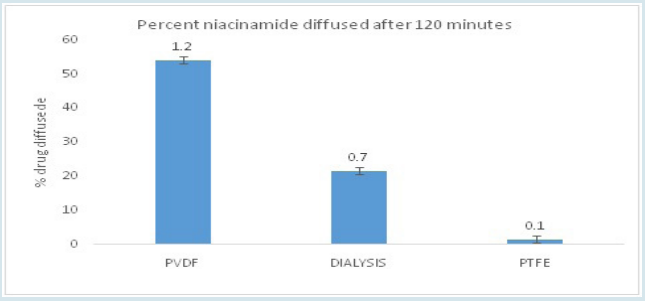

Figure 3: Effect of diffusion membranes on penetration of Niacinamide cream.

sodium

100 mg of Diclofenac sodium was exactly weighed and transferred into 100 ml clean dry volumetric flask. Finally, the volume was made up to 100 ml with water.

10 ml from the above stock solution was taken into a 100 ml volumetric flask and volume was made up with water.

From the above solution 0.5, 1, 1.5, 2, 2.5, 3 ml were withdrawn and transferred into 10 ml volumetric flask and the volume was made up to 10 ml.

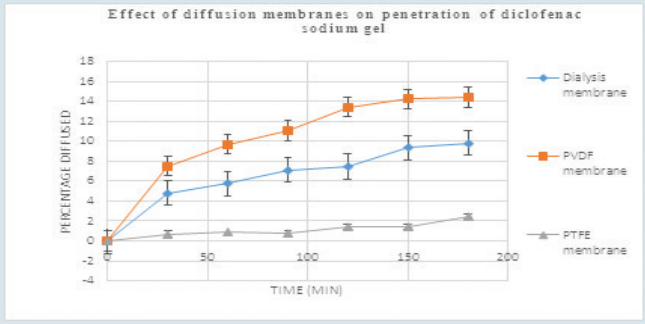

Figure 4: Effect of diffusion membranes on penetration of diclofenac sodium gel.

Preparation of diclofenac gel (Table 2)

2 gm of carbopol was dispersed in 100 ml of distilled water. From this dispersion 50 ml was taken and to this 5 ml of propylene glycol, 0.2 gm of methyl paraben and 0.15 gm of propyl paraben was added. To this half the quantity of triethanolamine was added to neutralize the carbopol gel. To this gel 1 gm of diclofenac sodium was dissolved and water was added to make up the volume to 100 gm. The remaining quantity of triethanolamine was added and mixed well.

***In vitro** release testing method*[11]

Franz diffusion cells with a receiver compartment volume of 12 ml and effective diffusion area of 2.84 cm^2 were used to evaluate drug delivery characteristics. Dialysis membrane, Polytetrafluoroethylene (PTFE), Polyvinylidene fluoride (PVDF) were used as diffusion membranes. During the experiments receptor phase (PBS pH 5.5) was continuously stirred and kept at a temperature of 32± 0.5°C. In the donor compartment 0.1 gm of the gel was evaluated for Diclofenac sodium delivery and 0.2 gm of cream formulation was evaluated for niacinamide delivery. At appropriate time, 0.5 ml of the sample was withdrawn from the receiver compartment and the same amount of the fresh solution was added to maintain the volume constant. Each experiment was run in three independent cells. Analysis of the samples were done spectrophotometrically for the diclofenac sodium gel at a wavelength of 276 nm and the concentration of diclofenac sodium in each of the sample was determined from a standard curve (5–30 µg). The samples of niacinamide were withdrawn after two hours and were analyzed by HPLC method. Each data point represented the average of three determinations. *In vitro* release studies were recorded for 180 min for diclofenac gel and at 120 min for niacinamide cream.

RESULTS AND DISCUSSION

Standard calibration curve for niacinamide and diclofenac sodium are shown in the Figure 1,2.

In vitro release of Niacinamide and the Diclofenac sodium formulations were studied for their permeation through the various membranes like Polytetrafluoroethylene (PTFE), Polyvinylidene fluoride (PVDF) and dialysis membrane. The amount of the drug permeated from the preparation was estimated by HPLC and UV method and the results are represented in Tables (3, 4, 5 and 6).

For Niacinamide cream (Table 3, Figure 3)

The percentage drug diffused after 120 min was found to be PTFE (1.5%), PVDF (54.15%) and Dialysis membranes (21.6%). The maximum percentage drug release was found to be in Polyvinylidene fluoride (PVDF).

For diclofenac sodium gel (Table 4, 5, 6, Figure 4)

The percentage drug diffused after 180 min from the membranes was found to be PTFE (2.42%), PVDF (14.42%) and Dialysis membranes (9.8%). The maximum percentage drug release was found to be in Polyvinylidene fluoride (PVDF). Even though the drugs are water soluble the maximum drug release was found in the hydrophobic membrane. This study states that the membranes having different characteristic properties shows different diffusivity.

CONCLUSION

Membranes' having different characteristic property shows different diffusivity. Therefore interpretation of *in vitro* diffusion data to correlate with *in vivo* conditions need further in-depth study.

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CONFLICT OF INTEREST

The authors have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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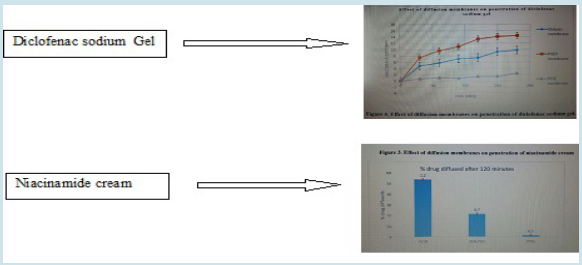
PICTORIAL ABSTRACT



SUMMARY

- A cream with 1.5% niacinamide was formulated.
- A Gel with 1% diclofenac sodium was formulated
- The cream and the gel were tested for diffusion of actives using diffusion cell and three different membranes.
- The three membranes showed three different values that were significantly different for both the actives.
- *In vitro* diffusion studies require more refined technique to use the data to predict in vivo dermal diffusion.

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