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Research Article

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ENHANCED TRANSDERMAL DELIVERY OF INDINAVIR SULFATE VIA TRANSFERSOMES

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ABSTRACT

The aim of the present study was to investigate the potential of transfersomal formulations for transdermal delivery of indinavir sulfate and to evaluate Span 80, Tween 80, Sodium cholate and Sodium deoxycholate as edge activators. Vesicles containing phosphatidylcholine (PC) mixed with edge activators and indinavir sulfate were prepared by conventional rotary evaporation method and characterized for various parameters including vesicles shape and surface morphology, size and size distribution, entrapment efficiency, elasticity, turbidity, and in vitro drug release. The compositions of refined formulations were predicted, liposomes prepared, and tested against control. Skin permeation profile of transfersomal formulation bearing indinavir sulfate was observed and the investigations revealed an enhanced transdermal flux (8.91±0.9 μg/cm^2/hr) and decrease lag time (0.9 hr) for indinavir sulfate. The obtained flux was nearly 7.5 and 12.04 times higher than conventional liposomal formulation bearing indinavir sulfate and plain drug solution. These results suggested that transfersomes are potential vehicles for improved transdermal delivery of indinavir sulfate and Span 80, Tween 80 and Sodium cholate were equivalent to Sodium deoxycholate as edge-activators.

Keywords: Indinavir sulfate, Transfersomes, Transdermal delivery, Surfactant, Phosphatidylcholine.

INTRODUCTION

Indinavir sulfate was approved by the FDA (Food and Drugs Administration) on March 13, 1996, for use in combination (a dose of 800 mg orally every 8 hr) with other protease inhibitors, nucleoside analogues or reverse transcriptase inhibitors for the treatment against Acquired Immune Deficiency Syndrome (AIDS). Indinavir is prescribed as a sulfate salt due to superior gastrointestinal solubility and absorption when compared with the free base[1]. Clinical experience with indinavir has demonstrated that it has a relatively narrow therapeutic window and is frequently associated with nephrotoxicity, which may manifest as a syndrome of renal colic, tubulointerstitial nephritis or even acute renal failure. Prolonged use of indinavir is associated with chronic elevations in serum creatinine[2-6]. Transdermal delivery of indinavir sulfate is a better option to overcome problems associated with its oral delivery. The transdermal route, besides being convenient and safe, offers several advantages over conventional ones, such as avoidance of GI incompatibility, variable GI absorption, avoidance of first pass metabolism, reduced frequency of administration, improved patient compliance and rapid termination of drug input[7]. To overcome all these side effects it is needed to develop some newer, safer formulation of Indinavir sulphate to increase both depth of skin permeation and the amount of drug delivered to skin for better therapeutic effect for long duration of action. However, the barrier nature of the skin inhibits the penetration of most drugs. The strategy of using lipid vesicles to overcome this difficulty is gaining interest, but it remains controversial. Most relevant reports cite the localizing effect of liposomes with transport processes reported in few cases depending on the formulation[8-10].

To overcome the limitation of poor skin permeability of above-mentioned approaches we have recently reported the elastic vesicles; transfersomes has been recorded to penetrate intact skin if applied non- occlusively[11,12]. Elastic liposomes overcome the skin penetration difficulty, possibly by squeezing themselves along the intracellular sealing lipids of the stratum corneum. The reason for this is the high vesicle deformability, which permits them to respond to the mechanical stress of the surrounding in a self-adapting manner[13]. Transfersomes for potential transdermal application, contain a mixture of lipids and biocompatible membrane softners. The optimal mixture leads to flexibility of the elastic liposomes membranes and to the possibility of penetration through channels of the skin, which are opened by the carriers. Thus the present study were to refine the formulation of Transfersomes of indinavir sulphate that will release the drug over a prolonged period of time barring frequent dosing,

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improving patient compliance, provide more constant plasma level and in terms reduce the side effect and to evaluate Span 80, Tween 80, Sodium cholate and Sodium deoxycholate as edge activators[14].

MATERIAL AND METHOD

Material

Indinavir sulfate was a gift sample from Ranbaxy Research Labs (Gurgaon, India). Sodium deoxycholate was a gift sample from Himedia laboratories, Nashik, Mumbai. Soyaphosphatidylcholine (PC), Triton X-100, Cellophane membrane (molecular weight cut off, 12000–14000) and Sodium cholate were purchased from Himedia laboratories, Mumbai. N-Octanol was procured from Loba Chemicals, Mumbai. Span 80 and Tween 80 were procured from CDH, India. Ethanol, chloroform, and methanol were procured from E. Merck (Mumbai, India). All other reagents were of analytical reagent grade and double distilled water was used in all experiments. Swiss albino mice and white rabbits were used for all the animal experiments.

Method

Preparation of Vesicular Formulations

Transfersomes were prepared by conventional rotary evaporation sonication method described by Cevc et al.[15], Precisely, phospholipid mixed with 5, 10, 15, 20 and 25 % w/w surfactants (sodium cholate, Span 80, Tween 80 and Sodium deoxycholate) and 0.05 % w/w dicetylphosphate were taken in a clean, dry, round bottom flask and the lipid mixture was dissolved in methanol or chloroform: methanol, 3:1 v/v. Drug was added to furnish the desired concentration (0.3% w/v) in the final preparation, Table **1**. The organic solvent was removed by rotary evaporator above the lipid transition temperature (Bucci type rotary evaporator, Zenith engg, India). Final traces of solvent were removed under vacuum overnight. The deposited lipid film was hydrated with 10% v/v ethanol in PBS (pH 6.5) by rotation at 60 rpm for 1 h at room temperature. The resulting vesicles were swollen for 2 hr at room temperature to get large multilamellar vesicles (LMLVs). The thick suspension thus obtained was broken by vortexing (Vortex shaker, Jyoti scientific industry, India). The resulting suspension was sonicated (Soniwel, India) for 35 min at 4°C for achieved desired vesicle size. The sonicated vesicles were extruded through a sandwich of 200 and 450nm polycarbonate membranes (Millipore).The liposomes (Phosphatidylcholine: cholesterol; 7:3) that act as a control in the present study were prepared by the same method as described above.

Incorporation of Indinavir sulfate into Vesicle Dispersion at Saturated Concentration

To determine the maximum amount of drug that could be added described by Mishra et al.[16]. Indinavir sulfate was incorporated into all vesicle formulations at saturated concentration to obtain equal thermodynamic activities. To determine the maximum amount of drug that could be added, increasing amounts of indinavir sulfate were added during preparation of transfersomal formulation. It was assumed that the presence of indinavir sulfate crystals would indicate that the formulation was saturated with indinavir sulfate. Therefore, all indinavir sulfate -loaded vesicle formulations were examined over a period of 14 days using Optical Microscope, (Olympus Pvt. Ltd., India).

Vesicle Characterization Vesicle Morphology

Visualization by transmission electron microscopy (TEM): A drop of transfersomes sample was placed on a carbon coated grid to leave a thin before the film dried on the grid; it was negatively stained with 1% phosphotungstic acid (PTA. A drop of staining solution was added on the film and the excess of the solution was drained off with a filter paper. The grid was allowed to air dried thoroughly and sample was viewed in a transmission electron microscope (AIIMS, New Delhi) at 80 kV.

Vesicle Size Distribution

Vesicle size, size distribution and zeta potential were determined by Dynamic Light Scattering Method (DLS) (Department of nanotechnology, National Institute of Pharmaceutical Education and Research, Mohali, Chandigarh), using a computerized inspection system by Malvern Zetasizer, (Malvern Instruments, Malvern, UK).

Entrapment Efficiency Measurement

Vesicle preparations were kept overnight at 4°C and ultracentrifuged (Remi Equipments, Mumbai, India) at 22000 rpm at a temperature of 4°C for 2 hours, where upon the pellets of transfersomes and the supernatant containing free drug were obtained. The transfersomes pellets were washed again with distilled water to remove any unentrapped drug by centrifugation. The combined supernatant was analyzed for the drug content after suitable dilution with saline solution by measuring absorbance at 259 nm using Shimadzu U-V 1700 (U-V Spectrophotometer). Encapsulation efficiency was calculated according to equation.

Entrapment efficiency = [(A1 – A2) ÷ A1] ×100

Where,

A1= Amount of indinavir sulfate added initially,

A2= Amount of indinavir sulfate determined in the filtrate by spectrophotometrically,

(**A1 – A2**) = represents the amount of indinavir sulfate entrapped in the formulation.

Vesicles Elasticity measurement

The deformability study was done for the elastic liposomal formulation against the standard liposome preparations using a home-built device as described[13-17]. The elasticity of transfersomes vesicles were measured by extrusion method. The transfersomes formulation were extruded through filter membrane (pore size diameter 100 nm), using a stainless steel filter holder having 50 mm diameter, by applying a pressure of 2.5 bar. The quantity of vesicles suspension, extruded in 5 minutes was measured.

$$E = J * (rv / rp)^2$$

Where,

E = Elasticity of vesicles membrane,

J = Amount of suspension extruded in 5 minutes,

r_v = Vesicles size, r_p = pore diameter.

In Vitro Skin Permeation Studies[18]

The permeation of indinavir sulfate from transfersomes through hairless rat skin was determined by using locally fabricated Franz diffusion cell. Hairless rat abdominal skin (removal of skin was done by scalp removal method) was clamped between the donor and receptor compartment of diffusion cell (1.32 cm^2). The membrane was carefully mounted onto the diffusion cell with the stratum corneum

side facing the donor compartment and dermal side bathed in receptor media. The transfersomes, conventional liposome and plain solution bearing indinavir sulfate, 0.5 ml were placed into the donor compartment over skin while the receptor compartment (25 ml) was filled with phosphate buffer saline (PBS, pH 6.5) containing 2 % methanol. When PBS, pH 6.5 along was used, indinavir sulfate was barely detectable because it has poor solubility at higher pH. Temperature of the cell assembly was maintained at 37±1°C and contents of the receptor compartment were stirred using a magnetic stirrer. Samples were withdrawn (2 ml) through the sampling port at regular intervals (0.5, 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours) and replaced with equal amount of fresh diffusion medium. Sink condition was maintained throughout the experiment. The samples were filtered through a 0.45-membrane filter, and analyzed for drug content by HPLC. Triplicate experiments were conducted for each study. At the end of this stage (24 hour), the skin mounted on the diffusion cell was removed carefully and skin surface were washed five times with warm (45°C) receptor medium. The second stage was employed to determine skin deposited drug. The above cleaned skin was cut into small pieces, mashed completely in a beaker and 10 ml 50% ethanol in distilled water added to this mass and mechanically shaken in a water shaker bath at 37°C for 2 hour for complete extraction of drug. The above dispersion was first filtered through Whatman filter paper no. 1 and then further filtered through 0.45 µ membrane filter. After suitable dilution, the indinavir sulfate contain in the filtrate was determined by HPLC[16]. The cumulative amount of drug permeated per unit area was plotted as a function of time, the steady-state permeation rate (Jss) and lag time (LT, hrs) were calculated from the slope and X-intercept of the linear portion, respectively. The permeability coefficient (Kp) was calculated by dividing transdermal flux values (Jss) by the initial concentration of drug in the donor cell (Co)

$$Kp = Jss / Co$$

Enhancement ratio (Er) was calculated by dividing the Jss of the respective formulation by the Jss of the plain drug formulation

$$Er = Jss \text{ of formulation} / Jss \text{ of plain drug}$$

Physical Stability of Transfersomes

After measuring the initial percentage entrapment of the drug in the various formulations, the three batches of the same formulation were stored in sealed glass ampoules (one each) at refrigeration temperature (4±2°C), room temperature (25±2°C) and body temperature (37±2°C) for a period of at least 3 months. After every 1month, percentage entrapment of the drug was determined in the formulations to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100%.

***In Vivo* Studies**

In-vivo study of optimized transfersomal gel formulation was performed on male white rabbits. This study was approved by the ethical committee CPCSEA (Committee for the Purpose of Control and Supervision of experiments on animal, Ministry of Culture, Government of India), approval no: BU/ Pharm /IAEC/08/018.

Animals

Six male white rabbits (mean ± weight 2.0 ± 0.05 Kg) obtained from the Animal house, Institute of pharmacy, Bundelkhand University were used in this study. Animals were not studied until after two-week environmental adjustment period. Rabbits were kept individually in metal cages, fitted with wire floors to reduce coprography. Food and water were supplied *ad libitum* to the animals.

Plasma Drug Concentration Study

This crossover study required three rabbits were used in a group for 3 groups, namely: Group I received topical plain Indinavir sulfate gel, Group II received liposomal gel, Group III received transfersomal gel. Before, investigation each rabbit was housed individually in a metal cage with a wire floor. During initial catheterization and dosing each rabbit was placed briefly in rabbit holder and then returns to its cage. Two day before each study, a 10 cm × 10 cm area on the back of each rabbit was shaved using an electric shaver. One day before each study, a depilatory was applied for 15 minutes to shave the area on back and to ears, and then thoroughly washed off, to ensure complete removal of hairs. All gel formulations, an equivalent amount of 5 mg Indinavir sulfate were applied to the definite area on the rabbit back (2 cm x 2 cm) for 24 hrs and the blood samples were taken at 1, 2, 4, 6, 8, 12, 18 and 24 hr after application. The skin was carefully wiped with 70% ethanol prior to application of vesicular formulation. The same experiment was carried out in triplicate on the same rabbits, in which at least one week passed between each application in order to obtain complete washout of the drug. For blood sampling, a catheter was inserted into the ear artery. After 0.5 ml of blood was withdrawn and discarded, a 1.5 ml sample was collected as a predose control and placed in a centrifuger with no additives. The catheter was flushed with 2 ml of 0.9% sodium chloride followed by 0.2 ml heparin solution to avoid the coagulation. The serum was separated by placing the tubes in a centrifuge 15 minutes at 3000 rpm. The plasma drug concentration of indinavir sulfate was analyzed by HPLC.

HPLC assay

The quantitative determination of indinavir sulfate in plasma and in skin permeation study was performed by HPLC (Shimadzu, Tokyo, Japan , C18 column Phenomenex, 5 µm, 5x150 mm; flow rate 1.2 mL/min at room temperature; UV–visible detector at λ=220 nm); using, mobile phase was a mixture of acetonitrile, sodium phosphate buffer pH 7.5 and water (30:5:65). The retention time of indinavir was 5 min. A calibration curve with a concentration range from 0.3, 0.4, 0.5, 0.6, 0.7 mg/mL was used to measure the indinavir sulfate concentration of the samples. The calibration curve was prepared using rabbit serum and a blank (containing phosphate buffer solution and serum) was also prepared to check any interference of protein in final estimation and no interference was observed during estimation. Results are expressed as mean ± SD (n=5).

Statistical Analysis

The mean and standard deviation was calculated by Graph Pad Instat 3.0. The statistical analysis was carried out employing analysis of variance (ANOVA) by using the software PRISM (Graph Pad) 5.0. Differences were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The conventional rotary evaporation sonication method reported by Cevc et al.[15] was used to prepare the transfersomal formulation. Formulations were prepared by using different types and concentrations of surfactant. The biosurfactant sodium deoxycholate was used because of its biocompatibility; Tween-80 and Span-80 were selected because they are pharmaceutically acceptable.

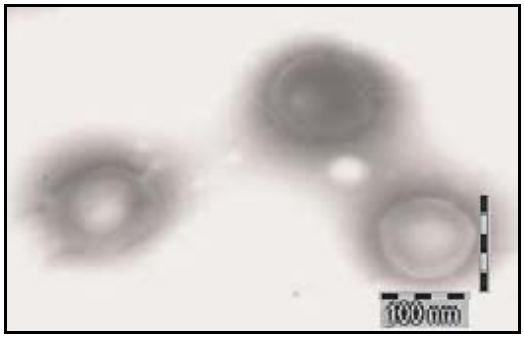
Table 1. Composition and Characterization of Transfersomal Formulations

Formulation	Composition PC:S[a] (%W/W)	Vesicle size (nm)	Entrapment efficiency (%)	Deformability Index	Characteristic
TF-S801	95:5	314	54.3±0.17	24.5 ± 2.2	+++
TF-S802	90:10	281	51.8±0.23	27.5 ± 2.5	+++
TF-S803	85:15	266	48.3±0.31	33.8 ± 2.0	++++
TF-S804	80:20	257	45.6±0.24	13.4 ± 2.8	++
TF-S805	75:25	248	41.7±1.43	8.7 ± 1.2	+
TF-TW801	95:5	332	50.6±0.54	11.8 ± 1.1	+++
TF-TW802	90:10	325	45.8±1.26	13.4 ± 1.4	+++
TF-TW803	85:15	321	41.8±0.84	16.2 ± 1.8	++++
TF-TW804	80:20	314	38.7±2.10	8.8 ± 1.5	++
TF-TW805	75:25	301	35.4±1.37	5.2 ± 0.8	+
TF-SDC1	95:5	353	58.6±1.65	21.8 ± 1.2	+++
TF-SDC2	90:10	344	54.9±0.54	32.3 ± 1.5	+++
TF-SDC3	85:15	282	52.3±2.43	34.4 ± 2.8[b]	++++
TF-SDC4	80:20	265.2	49.8±2.12	22.8 ± 1.8	++
TF-SDC5	75:25	262	46.2±1.29	11.3 ± 0.5	+
TF-SC1	95:5	358	55.1±0.25	24.5 ± 2.3	+++
TF-SC2	90:10	342	53.3±0.15	30.5 ± 2.3	+++
TF-SC3	85:15	317	50.8±1.24	31.5 ± 2.8	++++
TF-SC4	80:20	272	48.4±2.21	23.1 ± 2.4	++
TF-SC5	75:25	265	43.7±0.86	20.3 ± 1.5	+
Liposome		454	33.1±1.76	1.7 ± 0.2	+++

Values represented as mean ± S.D. (n = 3).
+: Clear to colloidal; ++: Less turbid and colloidal; +++: Turbid and colloidal; ++++: Dense and colloidal.
a Phosphatidyl choline: Surfactant.
b Denotes maximum deformability Index.

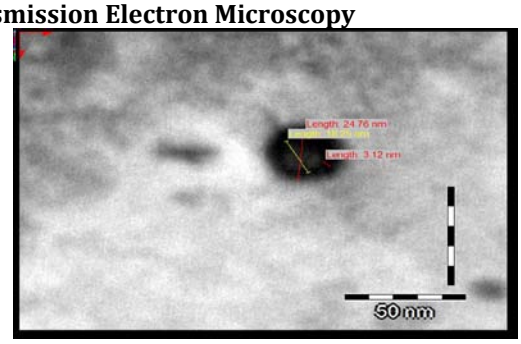
Visualization by transmission electron microscope and scanning electron microscope showed that transfersomes has a lamellar vesicular structure. (Figure **1**&**2**), and this confirms the existence of vesicular structure. The surface morphology and three dimensional natures of transfersomes were studied by further analysis of preparation by SEM, confirmed the vesicular characteristics possessed by this novel carrier. Transmission Electron Microscopic studies shows that transfersomes appeared as multilamellar vesicles, with the lamellae of vesicles evenly spaced to the core.

Figure 1. Visualization of Transfersomal Vesicles by Transmission Electron Microscopy



TF-SDC: Transfersomes with Sodium deoxycholate.
TF-SC: Transfersomes with Sodium cholate.
TF-S80: Transfersomes with Span-80.
TF-TW80: Transfersomes with Tween-80.

Figure 2. Visualization of Transfersomal Vesicles by Transmission Electron Microscopy



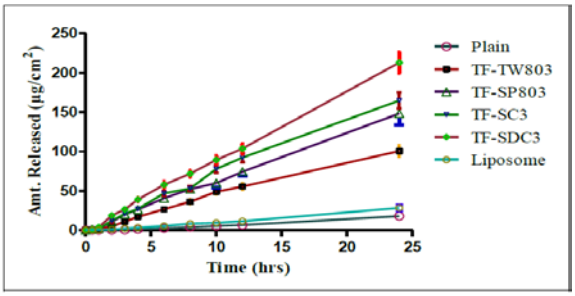
Transfersomes size was measured for refined formulations (Table **1**) the vesicles size of all transfersomal formulations ranged between 248 and 358 nm. There were no significant differences in size between transfersomal formulation containing different surfactants. However, Results showed that decreasing phospholipids concentration and increasing surfactant concentration vesicle size decreases. A reduction of vesicle size was observed when surfactant concentration increased above 15% w/w. This is due to the formation of a micelles structure instead of the vesicles, which are relatively smaller in size. These results are in accordance with the findings of other authors[20] who concluded that

reduction in the vesicle sizes is due to the decreasing phospholipid concentration and increasing surfactant concentration. As shown in Table **1**, the maximum entrapment efficiency obtained was 58.6±1.65% for transfersomal formulation TF-SDC1. It was observed that with increased surfactant concentration in the lipid components of the vesicles, the entrapment efficiency of the indinavir sulfate decreased (Table **1**). This is due to the possible coexistence of mixed micelles and vesicles at higher concentration of surfactant, with the consequence of lower drug entrapment in mixed micelles. However, it is difficult to consider the effect of any one variable (surfactant or PC concentration) in the absence of the others. [21-22]

The crucial feature of all transfersomal drug formulations, in comparison with the standard liposomes and other types of the drug-laden lipid suspensions, is the flexibility of the transfersomes. The topical carrier system should be deformable so that it can pass easily through the minute pores present in the epidermis. Prepared transfersomal formulations were subjected to deformability study by extrusion measurement. The results were expressed in terms of deformability index (Table **1**). The results indicate that elasticity of vesicles depends on both surfactant concentration and type. Different component in the transfersomes are chosen to be able to accommodate to a confining pore and thus trespass such pores. Deformability was found to be initially increases, as concentration of surfactant increases. Deformability was found to be maximum 34.4 ± 2.8 with PC: Sodium deoxycholate ratio of 85:15 (TF-SDC3). Further increase in concentration of surfactants resulted in lowering of deformability. This type of behaviour may be attributed to the formation of mixed micelles at higher concentration of surfactant. Maximum deformability was found with the formulation TF-SDC3. This formulation also has suitable entrapment efficiency, thus this formulation was selected for *in-vivo* studies.

In-vitro permeation studies give us valuable information about the product behavior *in-vivo*. The drug permeated dictates the amount of drug available for absorption. Four surfactants (Sodium deoxycholate, sodium cholate, Span 80 and Tween 80) and liposomal formulation were studied, with saturated aqueous indinavir sulfate as control. For the different transfersomal formulations drug release profiles were studied in triplicate (Figure **3**, Table **2**).

Figure 3. Comparative % cumulative Drug release of Indinavir form different Formulation



The results of skin permeation study through rat skin from transfersomal formulations, liposomal formulation, and plain drug at the same drug concentration has been depicted in Table **2** and showed the results of transdermal flux values (Jss) of different vesicle formulation compared with liposome and plain drug. i.e. 0.74 (µg/cm^2/hr) for Plain drug, 1.174 (µg/cm^2/hr) for liposome, 7.122 (µg/cm^2/hr) for TF-SC3, 1.174 (µg/cm^2/hr) for TF- SDC3, 4.393 (µg/cm^2/hr) for TF-TW803, 6.241 (µg/cm^2/hr) for TF-SP803. The maximum flux obtained from TF-SDC3 was substantially 12.04 times higher than that of plain drug solution and nearly 7.62 times higher than rigid liposomal formulation. Also, a minimum lag time (0.9 h) was obtained with TF-SDC3 formulation.

Table 2. Permeation parameters of indinavir sulfate loaded formulations across rat skin after 24 hours

Formulation code	Transdermal Flux (Jss) (µg/cm^2/hr)	Lag time (hr)	Enhancement ratio (Er)	% Skin drug deposition
Plain drug	0.74±0.2	3.6	----	1.10±0.12
Liposome	1.174±0.7	2.4	1.58	3.28±0.36
TF-SDC3	8.91±0.9	0.9	12.04	7.81±0.85
TF-TW803	4.393±0.6	1.7	5.93	4.28±0.47
TF-SP803	6.24±0.6	1.4	8.43	5.32±0.58
TF-SC3	7.122±1.2	1.1	9.62	6.99±0.76

Better transdermal flux and lower lag phase with transfersomal formulation was perhaps due to combination of one or more of following mechanisms: (1) increase in thermodynamic activity, (2) increased skin vehicle partitioning of drug, (3) altering the barrier properties by interacting with skin component, and (4) elasticity of vesicle membrane. With respect to drug delivery from the vesicles, transdermal flux first increased with increasing surfactant concentration and then decreased, a common phenomenon seen with all four surfactants. % drug deposition in the skin from various formulations and data obtained from transdermal flux of different formulation shows linear relation between transdermal flux and % drug deposition in the skin for that formulation. The decrease in transdermal flux and skin deposition at higher concentration was due to decrease in vesicle elasticity at higher concentration of surfactant.

The studies indicated that the transfersomes bearing indinavir sulphate followed a mixed order release rate pattern. Initially, the permeation rate was higher (up to 8 hr), due to the release of the surface-absorbed drug, followed by nearly zero order release (up to 24 hr). This is possibly due to better reservoir properties of the dermis region of rat skin; where-upon, transfersomes could behave as a depot, resulting in a delayed release. The skin deposition study supported this fact. The value of a dose deposited is nearly 7 times higher in the case of transfersomal formulation (7.81±0.85%) than plane drug solution (1.10±0.12%), which could be attributed to the difference in the mechanism of drug transport across the skin for the vesicles and the plane drug solution of indinavir sulphate. This difference is due to the fact that, in

contrast to a indinavir sulphate molecule, transfersomes are too large to enter into the cutaneous blood circulation directly; locally, they bypass the cutaneous capillary bed and get to the subcutaneous capillary bed and to the subcutaneous tissue, and here they act as a depot and sustain the drug release. The drug from transfersomes is consequently leered less efficiently by the intradermal capillary plexus, allowing more of the drug to reach the deep subcutaneous tissue. In a plane drug solution free drug movement occurs, allowing drug absorption by intradermal capillary plexus once it reaches into the dermis region of the skin and then into the systemic circulation.

Transfersomes, when applied under suitable conditions can transfer high amount of drug across the intact skin (Table **2**). The probable reason for this high flux rate is a naturally occurring transdermal osmotic gradient, i.e., another much more prominent gradient is available across the skin. This osmotic gradient is developed due to the skin penetration barrier, which prevents water loss through the skin and maintains a water activity difference in the viable part of the epidermis (75% water content) and nearly completely dry stratum corneum, near to the skin surface (15% water content). This gradient is very stable because ambient air is a perfect sink for the water molecule even when the transdermal water loss is unphysiologically high. All polar lipids attract some water due to the energetically favorable interaction between the hydrophilic lipid residues and their proximal water. Most lipid bilayers thus spontaneously resist an induced dehydration. Consequently, all lipid vesicles made from the polar lipid vesicles move from the rather dry location to the sites with a sufficiently high water concentration. So when a lipid suspension (transfersomes) is placed on the skin surface, which is partly dehydrated by the water evaporation loss, the lipid vesicles feel this osmotic gradient and try to escape complete drying by moving along this gradient. They can only achieve this if they are sufficiently deformable to pass through the narrow pores in the skin, because transfersomes are composed of surfactant and have better rheologic and hydration properties, which are responsible for their superior skin penetration ability. Less deformable vesicles, including standard liposomes are confined to the skin surface where they dehydrate completely and fuse, so they have less penetration power than transfersomes. Transfersomes are optimized in this respect and thus attain maximum flexibility and, hence, can take full advantages of the transepidermal osmotic gradient (water concentration gradient).

The stability studies was carried out by storing transfersomal formulation at different temperature i.e. refrigeration temperature (4±2°C), room temperature (25±2°C) and body temperature (37±2°C) for a period of at least 3 months. After every 1 month, percentage entrapment of the drug was determined in the formulations to know the amount of drug leaked out. % remaining drug entrapped (mean) at 4±2°C after 90 days was found to be 81.97, 92.93, 92.20, 92.78 and 89.64 for liposome, TF-SP803, TF-SC3, TF-SDC3 and TF-TW803 respectively. % remaining drug entrapped (mean) at 25±2°C after 90 days was found to be 77.73, 85.40, 88.14, 88.95, 86.71 for liposome, TF-SP803, TF-SC3, TF-SDC3 and TF-TW803 respectively. % remaining drug entrapped (mean) at 37±2°C after 90 days was found to be 78.16, 78.84, 79.97, 80.90 and 66.25 for LP, TF-SP803, TF-SC3, TF-SDC3 and TF-TW803 respectively. It is clear from the results obtained that the transfersomes have shown the minimum drug lost at refrigerated condition, and fairly high retention of drug inside the vesicles was observed. At this low temperature condition % remaining drug entrapped was good over a period of months. While, storage at higher temperature 25±2°C and 37±2°C leads to less % remaining drug entrapped over a period of 3 months respectively. The higher amount of drug leakage at elevated temperature may be due to the degradation of lipids constituting bilayers resulting in defects in membrane packing and loss of overall rigidity that makes them leaky. With the increase in temperature, there is also increase in the fluidity of lipid bilayers, due to phase transition phenomenon. So it can be inferred from the above discussion that the transfersomes formulation should be stored at lower temperature to minimize the drug loss.

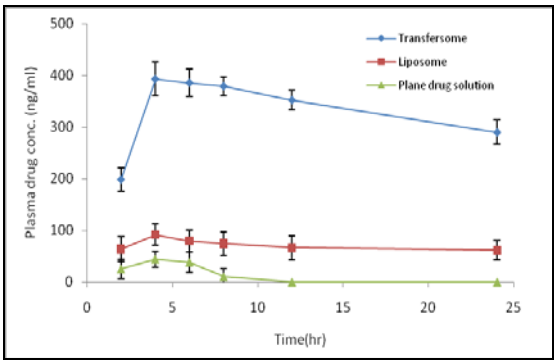
This *in-vivo* study of different formulations was carried out by means of plasma drug concentration measurement three formulations: topical plain Indinavir sulfate gel, transfersomal gel and liposomal gel (Table **3**), the transfersomal gel, liposomal gel, and plain drug gel showed the maximum blood level concentration 393 ng/ml, 85.56 ng/ml, 49.12 ng/ml. Transfersomal formulation showed nearly eight times better blood level concentration than plain Indinavir sulfate gel, representing greater permeation. Plasma levels of Indinavir sulfate after application of plain Indinavir sulfate gel were low (Cmax= 49.12 ng/ml) while those after application of the transfersomal gel, formulation gradually increased and reached a peak level of 393 ng/ml and maintained for longer times. The area under the curve (AUC) of the transfersomal gel was found to be 7310 ng.hr/ml (Table **3**) which was much larger than 261.8 ng.hr/ml being AUC of plain indinavir sulfate, Figure **4**.

Table 3. Pharmacokinetic parameter of indinavir sulfate

Formulation	Cmax (ng/ml)	AUC (ng.hr/ml)
Plain drug solution	49.12 ± 8.2	261.8 ± 28
Transfersome (TF-SDC3)	393 ± 32.4	7310 ± 159
Liposomes	85.56 ± 14.4	1202 ± 95

Values represented as mean ± SD (n=3).

Figure 4. Plasma concentration of indinavir sulfate after transdermal administration of different formulations



CONCLUSION

The results of the present investigation showed that the problems associated with the transdermal delivery of Indinavir sulfate could be overcome by incorporating it into the new ultraflexible drug carrier, transfersomes. Transfersomes are specially optimized vesicles, which can respond to an external stress by rapid and energetically inexpensive shape transformations. Transfersomes differ from conventional gel state noisome and liposomes by their characteristic fluid membrane with high elasticity. The elasticity of these vesicles is attributed to the simultaneous presence of different stabilizing (phospholipids) and destabilizing (surfactant) molecules and their tendency to redistribute in bilayers. Such highly deformable vesicles can thus be used to bring drugs across the biological permeability barriers such as skin. The lower penetration ability that is associated with the use of vesicular carriers such as liposomes can be overcome by entrapment of the drugs in the transfersomes. Transfersomes significantly improve the in vitro skin delivery of indinavir sulfate compared to saturated aqueous solution (maximum thermodynamic activity) when applied non-occlusively. There is a specific concentration for each surfactant for incorporation into lipid vesicles. Span 80 and Tween 80 and sodium cholate are as effective as sodium deoxycholate as edge activators in PC vesicles.

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