RESEARCH PAPER

Accepted 3 January 2003
Revised 28 November 2002
Received 11 September 2001
Indian J. Pharm. Sci., 2003, 65(2): 152-160

Protransfersomes for Effective Transdermal Delivery of Norgestrel Preparation and *in vitro* Characterization

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The present study was aimed at formulation, performance evaluation and stability studies of new vesicular drug carriers system called protransfersomes bearing norgestrel. Protranfersomes of norgestrel were prepared and extensively characterized for drug loading, vesicular shape, vesicular size and size distribution, entrapment efficiency, degree of deformability, transit time, rate of hydration, drug diffusion across rat skin and stability study at 4° and at room temperature. The effects of various parameters such as type of alcohol, type of surfactant and amount of drug of formulation on transdermal permeability profile were assessed. *In vitro* flux, permeability coefficient and release rate pattern of norgestrel was calculated for transdermal delivery of norgestrel. In the release rate study, no lag phase could be detected and the release of norgestrel from proposed formulation through rat skin was found to be constant but slow (sustained release). Entrapment efficiency was found to be nearly 95% and it depends on the type of surfactant and concentration of surfactant. After one month storage of formulation in liquid crystalline nature, drug content and other characteristic parameters were not changed. Proposed system also compared for *in vitro* performance with well-known vesicular system proliposomes, and shows better skin permeation. Results indicate that the protransfersomal formulation for transdermal drug delivery of norgestrel provides effective contraception, better stability, higher entrapment efficiency, ability as a self penetration enhancer, easy to scale up and better for transdermal delivery as compared to proliposomes.

In the field of contraceptive delivery aiming at birth control, significant research studies have so for been progressed, but still there is a need for a safe, effective and convenient system. Oral deliveries of contraceptives are associated with many contraindicated manifestations such as GIT disturbances, weight gain, irregular bleeding, headache, and depression of mood; mainly due to excess drug level in blood. Omission of the minipill might result in failure of therapy and many other complications to user[1].

The transdermal route, besides being convenient and safe, offers several advantages over conventional one. The vesicular approach in transdermal drug delivery systems has been studied for the contraception purpose[2] but their unstable nature limits their use at clinical and industrial level. In order to increase the stability of liposome, concept of proliposomes has been proposed. This approach has been extended to niosomes, which exhibit superior stability as compared to liposome, and efforts have been made to further stabilize them and overcome their limitations by proniosomal approach. But all approaches because of their poor skin permeability, breaking of vesicles and leakage of drug aggregation and fusion of vesicles are not much successful for effective drug delivery. Recently to overcome all above problem, specialized optimized, ultradeformable lipidic supramolecular aggregates called "transfersomes" have been proposed[3-8]. They offer a new approach to the problem

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of efficient dermal and transcutaneous drug delivery of high and low molecular weight substances. Specially optimized ultradeformable lipid supramolecular aggregates, transfersomes are able to penetrate the mammalian skin intact. Each transfersomes consists of at least one inner aqueous compartment that is surrounded by a lipid bilayer with specially tailored properties. These novel drug carrier are applied in the form of semi-dilute suspension without occlusion and allow the efficient dermal and transcutaneous drug delivery of high and low molecular weight substances. If properly made and optimally applied, this drug carrier can regularly bring more than 85-90% of the applied agent across the intact skin. But, the problem in this carrier is self-stability. So in present study liquid crystalline pro-ultraflexible lipid vesicles "protransfersomes" were proposed, that will be converted into the ultraflexible vesicles transfersomes *in situ* by absorbing water from the skin. Beside providing the controlled systemic transdermal delivery of contraceptive agent, the proposed system is more stable having higher entrapment efficiency, can be used as self penetration enhancer, easy to scale up, better for dermal and transcutaneous delivery[8].

In the present study, one of the potent contraceptive progestogen norgestrel was encapsulated in the protransfersomes for inhibition of ovulation. The daily dose of 75 μg/day of norgestrel is required for the purpose of contraception. Because of the complication and side effects associated with the other routes of drug delivery, the transdermal route appears suitable for the successful delivery of the norgestrel.

MATERIALS AND METHODS

Soyaphosphatidyl choline (PC), cholesterol, sodium cholate, sodium deoxycholate, Sephadex-G-50, stearyl amine, phosphotungstic acid, Triton X-100 were purchased from Sigma Chemicals, St. Louis, MO. Polyethylene glycol-200 and Briz-35 were obtained from Loba Chemie, Mumbai. Ethanol, isopropyl alcohol, butanol and chloroform were procured from E. Merck, Mumbai. All other reagents were of AR grade. Double distilled water was used for all experiments.

Preparation of protransfersomes:

Protransfersomes were prepared by the method reported by Perrett *et al*[9], for preparation of proliposomes. Precisely, phospholipid, surfactant, alcohol and drug were taken in a clean, dry, and wide mouth small glass tube. After mixing all the ingredients, the open end of the glass tube was covered with a lid to prevent the loss of solvent from it. The tube was warmed on a water bath at 60-70°, till the ingredients were dissolved. The aqueous phase (0.1% glycerol solution) was added and warmed on a water bath till clear solution was formed, which on cooling converted into protransfersomal gel. Different formulations containing different type and concentration of surfactant and alcohol were prepared. The proposed protransfersomal formulations were optimized on the basis of degree of deformability, entrapment efficiency and *in vitro* performance and stability of formulation and comparison with proliposomal formulation.

Visualization of vesicles:

Reconstitution of transfersomes from protransfersomes following hydration was confirmed by TEM. Samples were prepared by adding a drop of distilled water to protransfersomes gel and shaking the mixture manually for 1 min, twice with a 15 min. interval. A drop of the sample was placed on to a carbon coated copper grid and negatively stained with 1% aqueous solution of phosphotungstic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscope. A thin layer of protransfersomal gel was spread on a slide and after placing coverslip, observed under the microscope with and without polarized light. A drop of water was added through the side of the cover slip into the cavity slide and again observed. Photomicrographs were taken at suitable magnification before and after addition of water for both plain and polarized light.

Vesicle size and size distribution:

Size and size distribution studies were done by two methods i.e. hydration with agitation and hydration without agitation. Protransfersomal gel (100 mg) was hydrated using 10 ml of saline solution (0.154 M sodium chloride) with manual shaking for 5 min. The dispersion was observed under optical microscope (X450). Size and size distribution was noted using calibrated stage and ocular micrometer. Similarly, size was noted after hydration without agitation in a cavity slide[10].

Rate of hydration (Spontaneity):

Rate of hydration of protransfersomal formulation is described as number of transfersomes formed after hydration of protransfersomes for 15 min. This study was first described by Payne *et al*[13] for proliposomal system. About 10 mg of protransfersomal gel was transferred to a small glass tube. One ml of saline (0.154 M sodium chloride) was added along the walls of the tube and kept aside without agitation. After 15 min, a drop of aqueous layer was withdrawn and

placed on Neubaurs chamber (Fein-optik, Germany) and examined under optical microscope. The numbers of transfersomes formed from protransfersomes were counted.

Entrapment efficiency:

Entrapment efficiency was determined by first separation of the unentrapped drug by placing formulation in cellophane tubing and dialyzing exhaustively against 400 ml of 0.154 M sodium chloride for 24 h. The vesicles were resuspended in 20 ml of polyethylene glycol-200 (30% v/v) and 1 ml of Triton X-100 (1% v/v) was added to disrupt the vesicles. The solution was filtered and analyzed for drug content spectrophotometrically (Shimadzu 1601, Japan). Entrapment efficiency was expressed as % of drug entrapped[14].

Degree of deformability:

The deformability study was done for the protransfersomal formulation against the standard proliposomal preparation. All preparations were passed through the G_4 glass crucible (Borosil, India) and size and size distributions were monitored after each pass up to three pass[11,12].

Transit time:

Transit time is the measure of elasticity of membrane vesicles, the time taken by the formulation for passing through the membrane filter. Formulation PTF-E_1, PTBF-E_1 proliposomes formulations (10 mg-10 ml dilution) were passed through the membrane filter and time taken for passing was noted down for each formulation.

Fabrication of transdermal patch:

A circular aluminum foil was used as a backing membrane on which a plastic sheet of 1mm thickness was stuck. The circle of diameter 1.31 cm (area is 1.36 cm^2) was cut on the plastic sheet and the protransfersomal gel was evenly spread over this area and covered with fine nylon mesh. The liquid crystalline protransfersomal gel acts as a reservoir for the transdermal delivery of norgestrel.

***In vitro* drug release:**

The *in vitro* release of norgestrel from protransfersomal system was studied using locally fabricated Keshary-Chein diffusion cell through the excised rat skin. The hair of the female albino rat was removed by applying depilatory (Anne-French) on the skin for 10 min and then wiped off with cotton. After cleaning the skin with water the animal was returned to the cage. This animal was sacrificed next day by excessive chloroform inhalation. An incision was made on the flank of the animal and the skin was separated from the underlying connective tissue. The prepared skin was then washed with saline (0.15 M sodium chloride) and stored at 0-4° after applying gentamicin (as preservatives) on it and keeping it between the glass plates. It was used within 2 d[14]. Locally fabricated Keshary-Chein type diffusion cell was used for *in vitro* release studies. The patch containing protransfersomal formulation with 1mg drug was applied to the epidermal side of the skin. The receptor medium was 20 ml 30% v/v PEG-200. The receptor compartment was surrounded by a water jacket for maintaining the temperature at 37±1°. The receptor fluid was stirred by magnetic bead operated on a magnetic stirrer. Samples withdrawn were analyzed spectrophotometrically and amount of drug diffused at various time intervals was determined. *In vitro* release rate studies were done for different formulations and effect of variation in composition; alcohols and amount of drug on release rate were studied.

Stability studies:

The formulation PTF-E_1 was selected on the basis of the *in vitro* characterization. The formulations were stored in glass tubes covered with aluminum foil at room temperature and at 4° for a month and were observed visually and under optical microscope for the change in consistency, liquid crystalline structure and appearance of drug crystals. Transfersomes formed from protransfersomes were also characterized for size and size distributions, after hydration with and without agitation under optical microscope, rate of hydration and drug content.

RESULTS AND DISCUSSION

The method of preparation involves the principle of coacervation phase separation[15]. The method is based on the simple idea that the mixture of surfactant, alcohol, and aqueous phase can be used to form the concentrated protransfersomal gel, which can be converted to stable transfersomal dispersion by dilution with excess aqueous phase. The composition of formulations were taken and optimized for protransfersomal formulation using the principle of three-phase diagram. Different formulations using different surfactants, compositions and alcohols were prepared. The biosurfactant like sodium cholate, sodium deoxycholate were used because of their biocompatibility in comparison to other surfactants (Table 1).

The protransfersomal formulation when observed under cross polarizer show birefringent streaks lamellar structures in liquid crystalline form. When this gel was hy-

drated transfersomes formed from it were multilamellar, and spherical and somewhat elongated in shape (photomicrographs shown in fig. 2. The transformation of lamellar liquid crystalline protransfersomes to transfersomes may be explained by different degree of hydration of surfactant and phospholipid molecules and simultaneously by change in shape of the hydrated molecules characterized by their packing parameter. Initially, Due to the limited solvent present, the protransfersomes formed were the mixture of lamellar liquid crystals resembling palisades and vesiculating lamellas stacked together, which may be termed as compact transfersomes. Further addition of water leads to swelling of bilayers as well as vesicles due to interactions of water with polar groups of surfactants. Above a limiting concentration of solvent, the bilayers tend to form spherical structures randomly giving rise to multilamellar structures. When shaken with water i.e. with excess aqueous phase, complete hydration takes place leading to the formation of transfersomes. For the morphological characterization the protransfersomes were also visualized by transmission electron microscopy. It appeared as multilamellar vesicles (fig. 3). The lamellae of vesicles were evenly spaced to core.

TABLE 1: COMPOSITION OF DIFFERENT PROTRANSFERSOMAL FORMULATION.

Formulation	Composition [PC:S] (W/W ratio)	Alcohol used
1.With sod. cholate		
a. PTF*-E_1	90:10	Ethanol
b. PTF-I_1	90:10	Isopropanol
c. PTF-B_1	90.10	Butanol
2.With Brij 35		
a. PTBF**-E_1	90:10	Ethanol
b. PTBF-I_1	90.10	Isopropanol
c. PTBF-B_1	90.10	Butanol
3.With sod. deoxycholate		
a. PTDF***-E_1	90:10	Ethanol
b. PTDF-I_1	90:10	Isopropanol
c. PTDF-B_1	90:10	Butanol

PTF* is protransfersomal formulation containing sodium cholate, PTBF** is protransfersomal formulation containing Briz 35, PTDF***is pProtransfersomal formulation containing sodium deoxycholate, PC stands for phosphotidyl choline (Soya) and S denotes surfactants.

Size of the vesicles was measured under optical microscope in two conditions, with and without agitation. Hydration without agitation results in the larger vesicle size. Whereas the application of energy i.e. hydration with agitation results in the vesicles of smaller size because energy applied during agitation causes their breakage into smaller vesicles. Vesicles formed from different alcohols are of different size, they follow the order: ethanol>butanol >isopropanol. Vesicles with ethanol are of highest size may be because of its greater solubility in water, which causes the slowest phase separation with this alcohol. Isopropanol gives vesicles of smaller size may be due to the branched

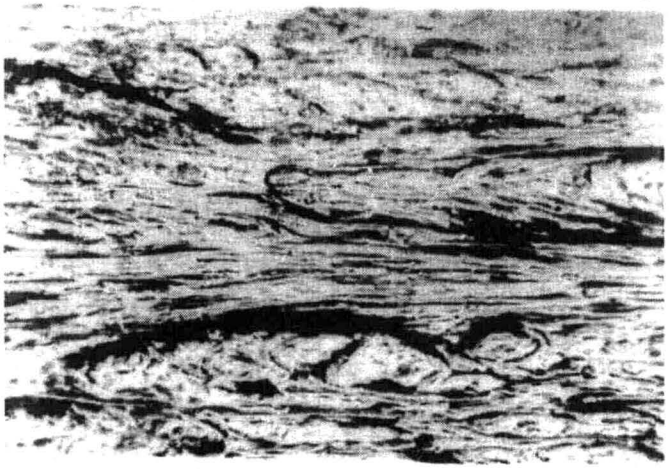

Fig. 1: Lamellar liquid crystalline structure of protransfersomes (Photomicrograph x 100)

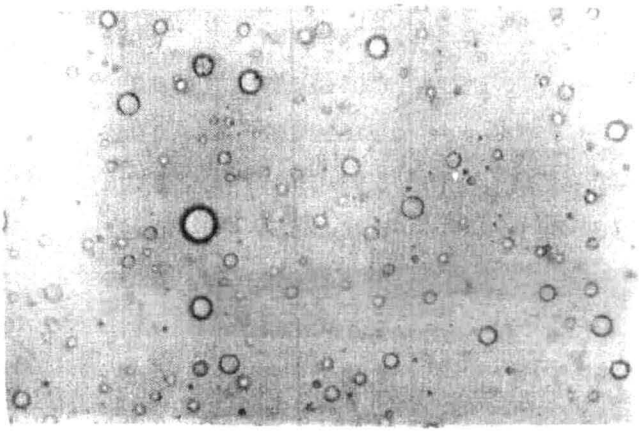

Fig. 2: Vesicular structure of transfersomes formed upon hydration of protransfersomes (Photomicrograph x 450)

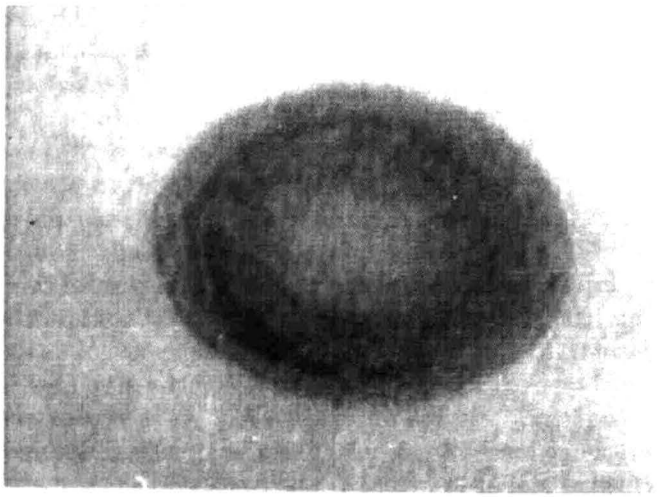

Fig. 3: Transmission electron photomicrograph (x 40,000)

chain present in it[15]. The polydispersity index which is the ratio of the standard deviation to vesicle size was also low, which indicates that this method of preparation results in vesicle of uniform size (Table 2). Entrapment efficiency of the gel formulation was nearly 95% and transfersomes formed from it also exhibit good entrapment efficiency due to lipophilic nature of drug and multilamellar nature of vesicles (Table 2).

Rate of hydration studies indicates that preparations containing isopropanol are formed more spontaneously than preparation containing butanol and ethanol and this may be due to faster phase separation of isopropanol due to its lower solubility in water (Table 2). The rate of hydration was found to be in order of: isopropanol>butanol>ethanol.

Degree of deformability is the most important parameter of protransfersomal formulation because, this parameter differentiate protransfersomes from other vesicular carriers like liposomes that are not able to cross the stratum corneum intact. Deformability of protransfersomes membrane is due to the presence of surface-active agent in proper ratio. The resulting deformability of protransfersomes membrane minimizes the risk of complete vesicle rupture in the skin. Deformability study of different formulations against standard proliposomal formulations shows the elastic nature of the vesicles. The negligible difference in size after passage through G_4 filter indicated that these vesicles could deform or change their shape and the vesicle rupture of these vesicles during passage is minimum as compared to standard proliposomes preparation[16] (Table 3).

In vitro permeation studies gives valuable information about the product behavior *in vivo*. 20 ml of 30% v/v PEG-200 solution was used as a receptor fluid for the *in vitro* drug permeation studies based on the solubility consideration of norgestrel for maintain the sink condition. For the different protransfersomal formulations drug release profiles were studied in triplicate and standard deviation,

TABLE 2: CHARACTERIZATION OF TRANSFERSOMES FORMED FROM PROTRANSFERSOMES.

Formulation Code	Size (μm)				Rate of Hydration	% Entrapment
	Without agitation		With agitation			
	VS*±SD**	PI***	VS±SD	PI		
PTF-E_1	6.39±0.184	0.028	3.79±0.102	0.026	$8.22x10^3$	95.7±0.59
PTF-B_1	6.05±0.237	0.039	3.74±0.179	0.047	$8.04x10^4$	92.3±0.83
PTF-I_1	6.16±0.267	0.043	3.64±0.054	0.014	$8.36x10^4$	96.4±0.86
PTBF-E_1	6.93±0.106	0.015	5.10±0.11	0.021	$7.98x10^3$	91.1±0.85
PTBF-B_1	6.48±0.138	0.021	5.22±0.057	0.010	$8.09x10^4$	91.5±0.055
PTBF-I_1	6.08±0.069	0.011	5.06±0.070	0.013	$8.14x10^4$	95.3±1.449
PTDF-E_1	6.30±0.064	0.010	5.44±0.294	0.054	$9.23x10^3$	91.8±0.758
PTDF-B_1	6.49±0.131	0.020	5.41±0.247	0.045	$8.12x10^4$	91.8±0.758
PTDF-I_1	5.95±0.057	0.009	4.35±0.192	0.044	$8.16x10^4$	93.8±0.873

All values are expressed as mean±SE (n=3). VS*stands for mean vesicle size, SD** denotes standard deviation and PI means polydispersity index, which is SD/VS.

TABLE 3: DEFORMABILITY STUDIES OF DIFFERENT PROTRANSFERSOMAL FORMULATIONS AGAINST PROLIPOSOMAL PREPARATION.

	Proliposomes (μm)	PTF-E_1 (μm)	PTBF-E_1 (μm)
Without agitation	8.40 ± 0.32	6.57 ± 0.14	6.99 ± 0.13
After 1st pass	4.02 ± 0.36	4.58 ± 0.33	4.02 ± 0.14
After 2nd pass	2.82 ± 0.21	3.82 ± 0.23	3.02 ± 0.14
After 3rd pass	1.07 ± 0.24	3.37 ± 0.11	2.57 ± 0.23
Transit time (min)	25 ± 1.0	16 ± 0.5	15 ± 0.6

All values are expressed as mean±SE (n=3).

transdermal flux, permeation coefficient, and regression coefficient values were calculated from the data[17] and data of each permeation profile of norgestrel through excised rat skin were linearly regressed and fitted into the straight-line equations. The regression coefficient values for the formulations were very close to one showing the nearly zero order release profile (Table 4).

Protransfersomes gel when applied under suitable conditions can transfer 6 to 12 μg of drug h/cm^2 area across the intact skin and also with no lag phase. Better transdermal flux and no lag phase with our formulation was perhaps due to the penetration enhancing properties of the alcohols and surfactants and increase in solubility of free drug in stratum corneum lipid, and most importantly the deformability of the protransfersomes. The extremely high flexibility of their membrane permits protransfersomes to squeeze themselves even through pores much smaller than their own diameter. This is due to high flexibility of the protransfersomes membrane and is achieved by judiciously combining at least two lipophilic/amphiphilic components (phospholipid plus biosurfactant), with sufficiently different packing characteristics into a single bilayer. The high resulting aggregate deformability permits protransfersomes to penetrate the skin spontaneously. This tendency is also supported by the high surface hydrophilicity that enforces the search for surrounding of high water

TABLE 4: STEADY STATE TRANSDERMAL FLUX, PERMEABILITY CO-EFFICIENT, SLOPE OF STRAIGHT LINE FOR THE TRANSPORT OF NORGESTREL ACROSS RAT SKIN.

Formula Code	Steady state Transdermal flux* μg/cm^2.hr.	Permeability co-efficient** cm h^{-1}	Regression co-efficient
Optimization of type of surfactant			
PTF-E_1	8.37±0.031	6.39x10^{-3}	0.834
PTDF-E_1	7.26±0.012	5.54x10^{-3}	0.882
PTBF-E_1	6.16± 0.046	4.70x10^{-3}	0.843
Amount of drug			
PTF-E_1 0.5 mg	1.91±0.063	1.46x10^{-3}	0.812
PTF-E_1 1.0 mg	8.37±0.031	6.39x10^{-3}	0.834
PTF-E_1 1.5 mg	14.81±0.074	11.30x10^{-3}	0.830
PTF-E_1 2.0 mg	19.77±0.04	15.09x10^{-3}	0.787
Optimization of type of alcohol			
PTF-E_1 Ethanol	8.371±0.031	6.390x10^{-3}	0.834
PTF-B_1 Butanol	9.52±0.083	7.27x10^{-3}	0.870
PTF-I_1 Isopropanol	12.095±0.047	9.23x10^{-3}	0.798

All values are expressed as mean±SE (n=3). * is the amount of drug/time x area of patch. ** is the amount of drug x saturation solubility of drug in receptor fluid.

activity. It is almost certain that the high penetration potential of protransfersomes is not primarily a consequence of stratum corneum fluidization by the surfactant because micellar suspension contains much more detergent than transfersomes (PC/sodium cholate 65/35 w/w %, respectively). Thus, if the penetration enhancement via the solubilization of the skin lipids were the reason for the superior penetration capability of protransfersomes, one would expect an even better penetration performance of the micelles. In contrast to this postulate, the higher detergent concentration in the mixed micelles does not improve the efficacy of material transport into the skin[18-20]. This hypothesis is also supported by repot of Cevc[5] who compared the penetration ability of transfersomes, liposomes and mixed micelles by Confocal Laser Scanning Microscopy (CLSM) and observed that mixed micelles were restricted to the top most part of stratum corneum and transfersomes penetrate to deeper skin layer.

Fig. 4 shows the effect of different amounts of drug present in the transfersomal formulation on the skin permeation profile. Almost linear correlation was observed between concentration of drug and transdermal flux i.e. as the concentration of drug increases the steady state transdermal flux increases. Fig. 5 shows the effect of different alcohols on release rate and found in the order: isopropanol>butanol>ethanol. Formulations containing isopropanol gave maximum drug release among the different alcohols. These observations not correlate well with the previous reports by Friend *et al.*[21-22], that permeation increases as the chain length increase from C_2 to C_4 (ethanol to butanol) because drug permeation was observed maximum for isopropanol formulation which is not in agreement with the findings, which is possibly due to branched chain structure of isopropanol which acts as co-surfactants and might loosen the bilayer packing resulting into the increased release of drug. The enhancement in permeation may also be due to mixed action involving the effect of lecithin, surfactants and alcohols.

Fig. 6 shows the effect of different surfactant on release rate and found in the order: sodium cholate>sodium deoxycholate>Briz 35. Sodium cholate shows the better permeation in comparison to other surfactant possibly due to the better interaction of sodium cholate with phospholipid bilayers which provide better deformability to the vesicles and that is responsible for better permeation[23]. Fig. 7 compares the *in vitro* drug release profile of protransfersomal and Proliposomal formulation and histogram shows that protransfersomes formulation shows two to three times better skin permeation than proliposomal formulation. The reason for this better performance is deformability, hydrophilicity and ability to retain vesicle integrity while the aggregates undergoes a dramatic change in shape in comparison to proliposomes and these all characteristics allow the transfersomes to pass the skin that is much smaller than their own diameter[24].

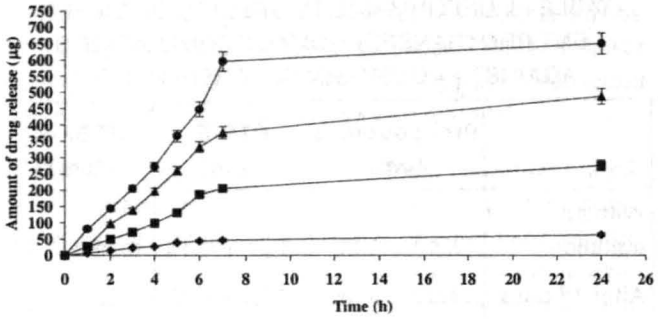

Fig. 4: Effect of amount of drug on permeation profile across the rat skin (formulation PTF-E_1).

The protransfersomal formulation were prepared using different amounts of drug. Formulation 1 (-●-), 2(-□-), 3 (-▲-), 4 (-X-) containing 0.5 mg, 1.0 mg, 1.5 mg, 2.0 mg of norgestrel and the effect of amount of drug on release profile of drug across the rat skin were studied. -●- 0.5 mg -□-1.0 mg -▲- 1.5 mg -X-1.5 mg.

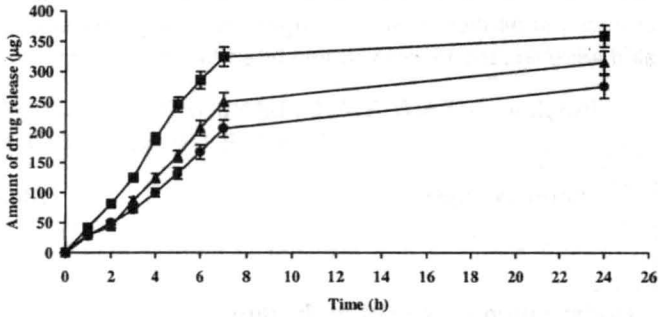

Fig. 5: Effect of different alcohol on drug permeation across rat skin from different protransfersomal formulations.

Protransfersomal formulations were prepared using different alcohols such as ethanol (PTF-E_1), formulation 1 (-●-), isopropanol (PTF-I_1) formulation 2 (-□-), butanol (PTF-B_1) formulation 3 (-▲-) and the effect of alcohol on drug release profile of these formulations were compared.

Stability of a product may be defined as the capability of a particular formulation in a specific container to remain

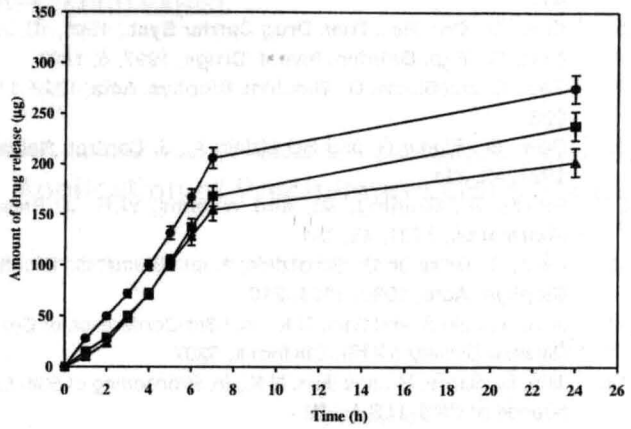

Fig. 6: Effect of different surfactant on drug permeation across rat skin from protransfersomal formulations.

Protransfersomal formulations were prepared using different surfactant such as sodium cholate (PTF-E_1), formulation 1 (-●-), sodium deoxycholate (PTDF-E_1), formulation 2 (-■-), Briz-35 (PTBF-E_1), formulation 3 (-▲-) and the effect of surfactant on drug release profile of these formulations were compared.

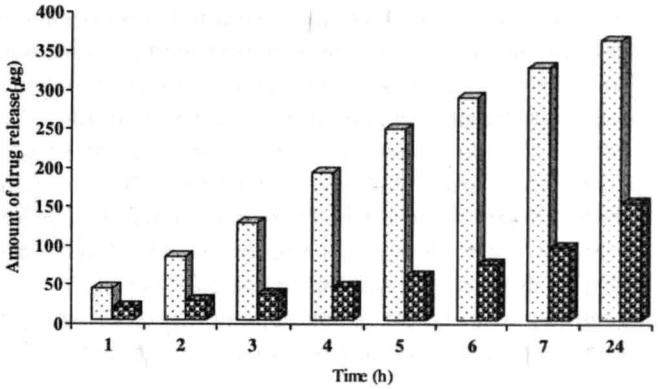

Fig. 7: Comparison in release rate across the rat skin.

Comparison in release rate across the rat skin between the optimized protransfersomal formulation (▭) and proliposomal formulation (▩) Histogram compares the *in vitro* drug release profile across the rat skin of the above mentioned two formulations.

with the physical, chemical, microbiological, therapeutic and toxicological specifications. After one month storage of formulations the liquid crystalline nature of the protransfersomal gel was not changed. Drug crystals were not observed after one month storage at both at room temperature and cold temperature. The consistency of the protransfersomal gel was increased may be because of the molecular interaction of polar head groups of surfactants with the solvent and permeation of solvent into bilayer. The solvent diffused into the bilayers does not disturb the liquid crystalline structure, rather results in the complete bilayer formation due to saturation of lipid polar heads and might lead to the increase in bilayer distance resulting in overall increase in consistency (Table 5).

Spontaneity of transfersomes formation from protransfersomal gel was decreased may be due to the in-

TABLE 5: CHARACTERIZATION OF TRANSFERSOMES FORMED FROM PROTRANSFERSOMES AFTER STORAGE

Time (D) Storage temp.	Size of transfersomes (μm)				Rate of Hydration	Drug content	Consistency	Liquid crystalline structure	Appearance of drug crystal
	Without agitation		With agitation						
	VS±SD	PI	VS±SD	PI					
10(1)	3.71±0.26	0.07	2.50±0.13	0.05	$8.13x10^3$	95.2±0.86	*	*	***
10(2)	3.95±0.07	0.01	2.96±0.05	0.01	$8.29 x10^3$	95.4±0.87	*	*	***
20(1)	4.42±0.27	0.06	2.95±0.07	0.02	$6.83 x10^3$	94.9±0.45	*	*	***
20(2)	4.81±0.21	0.04	3.01±0.08	0.02	$7.99 x10^3$	94.7±0.44	*	*	***
30(1)	5.60±0.07	0.01	3.17±0.05	0.01	$6.11 x10^2$	94.6±0.86	**	*	***
30(2)	5.99±0.06	0.01	3.26±0.08	0.02	$6.28 x10^2$	94.0±0.83	**	*	***

Formulation code PTF-E 1. Room temperature = 30±2° 2. Refrigerated temperature = 4±2°, * means no change, ** denotes increased, and *** stands for not appeared.

creased consistency. Size and size distribution studies on agitation showed that vesicle size increased whereas polydispersity index decreased indicating complete swelling of bilayer and hence formation of more uniform vesicles. The effect of storage temperature on vesicle size and polydispersity index was statistically insignificant. Rate of hydration was higher at low temperature may be due to less loss of alcohol and no change in membrane fluidity. The effect of aging on drug content was not too much. Only slight reduction in the drug content or entrapment was observed, may be due to leaching from the preparation[25] (Table 5).

It can be concluded from the results obtained that protransfersomal formulation for transdermal delivery of norgestrel provides effective contraception, better stability, higher entrapment efficiency, and ability as a self-penetration enhancer, easy to scale up and better for transdermal delivery, as compared to proliposomes. Thus it can be a logical conclusion that these vesicular carrier systems hold a promising future in effective transdermal delivery of bioactive agents and other problematic drug molecules. Their ultradeformable natures are overwhelming superiority over ther similar transdermal delivery systems.

NGEMENTS

o CSIR, New Delhi, India for providing hors (Subheet Jain).

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