International Journal of Pharmaceutics 397 (2010) 164–172

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Role of edge activators and surface charge in developing ultradeformable vesicles with enhanced skin delivery

Ghada M. El Zaafarany, Gehanne A.S. Awad*, Samar M. Holayel, Nahed D. Mortada

Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University, Monazzamet El-wehda Elafrikeya Street, Abbasseya, Cairo, Egypt

ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form 12 June 2010
Accepted 19 June 2010
Available online 3 July 2010

Keywords:
Ultraflexible lipid vesicles
Transfersomes
Enhanced skin delivery
Deformability
Diclofenac sodium
Edge activators

ABSTRACT

Transfersomes are highly efficient edge activator (EA)-based ultraflexible vesicles capable of, non-invasively, trespassing skin by virtue of their high, self-optimizing deformability. This investigation presents different approaches for the optimization of Transfersomes for enhanced transepidermal delivery of Diclofenac sodium (DS). Different methods of preparation, drug and lipid concentrations and vesicle compositions were employed, resulting in ultraflexible vesicles with diverse membrane characteristics. Evaluation of Transfersomes was implemented in terms of their shapes, sizes, entrapment efficiencies (EE%), relative deformabilities and *in vitro* skin permeation. Transfersomes prepared with 95:5% (w/w) (PC:EA) ratio showed highest EE% (Span 85 > Span 80 > Na cholate > Na deoxycholate > Tween 80). Whereas, those prepared using 85:15% (w/w) ratio showed highest deformability (Tween 80 was superior to bile salts and spans). Transfersomes were proved significantly superior in terms of, the amount of drug deposited in the skin and the amount permeated, with an enhancement ratio of 2.45, when compared to a marketed product. The study proved that the type and concentration of EA, as well as, the method of preparation had great influences on the properties of Transfersomes. Hence, optimized Transfersomes can significantly increase transepidermal flux and prolong the release of DS, when applied non-occlusively.

1. Introduction

The strategy of using lipid vesicles has being gaining interest over the past decade to trespass the stratum corneum which is the main obstacle to topical drug delivery. In order to cross intact skin, drug carriers must pass through a series of very fine pores with an average diameter typically around 30 nm or less, under the influence of a suitable transdermal gradient (Trotta et al., 2002).

Conventional liposomes have been generally reported to remain confined to the upper layer of the stratum corneum and to accumulate in the skin appendages, with minimal penetration to deeper tissues, owing to their large minimum size and lack of flexibility (Li and Hoffman, 1997; Verma et al., 2003; Manosroi et al., 2008).

Recent approaches in modulating vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macromolecules to deeper tissues. These approaches have resulted in the design of two novel vesicular carriers, ethosomes and ultraflexible lipid-based elastic vesicles (Transfersomes). Ethosomes are composed of phospholipids, water and ethanol (Touitou et al., 2000; Elsayed et al., 2007). Ethanol allows them to penetrate more easily into the deeper layers of the skin by fluidizing the lipid domain of the stratum corneum (Bahia et al., 2010).

Transfersomes are the first generation of elastic vesicles, which were first introduced in the early 1990s by Cevc and Blume (1992). They have been reported to be able to penetrate intact skin, carrying therapeutic concentrations of drugs when applied under non-occlusive conditions (Cevc et al., 1996; El Maghraby et al., 2000a,b; Cevc and Blume, 2001; Trotta et al., 2004; Honeywell-Nguyen and Bouwstra, 2005). They are liquid-state vesicles with a highly deformable membrane (Cevc and Gebauer, 2003; Manconi et al., 2009; Montanari et al., 2009) which permits their easy penetration through skin pores much smaller than the vesicles' size. They have been proven to be superior to conventional gel-state and even liquid-state vesicles in terms of both, the enhancement of drug permeation and interactions with human skin (Van den Bergh et al., 2001; Dragicevic-Curic et al., 2010).

From the composition point of view, Transfersomes are self-regulating, mixed lipid aggregates containing edge activators within a phospholipid matrix so as to drastically reduce the value of its elastic module. Applied on the skin surface, these elastic vesicles are able to squeeze through intercellular regions of the stratum corneum under the influence of the transepidermal water-activity gradient. Phospholipid hydrophilicity leads to xerophobia (tendency to avoid dry surroundings), thus, for the vesicles to remain

* Corresponding author. Tel.: +20 2 24051120; fax: +20 2 24051107.
E-mail addresses: gehaneas.awad@yahoo.com, gehanawd@link.net (G.A.S. Awad).

0378-5173/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2010.06.034

Table 1
Composition, entrapment efficiency, relative deformability and flux at 24 h of Diclofenac sodium Transfersomal formulations prepared by the rotary evaporation–sonication method.

Transfersomal formulation code[a]	Formulation composition[b] PC:EA (w/w)	Entrapment efficiency (EE%) (% ± SD)[c]	Relative deformability (sec ± SD)[c]	Flux (J) (μg/cm² h ± SD)[c]
SC-1	98:2	50.13 ± 3.27	48 ± 0.87	5.65 ± 0.22
SC-2	95:5	53.63 ± 1.52	33 ± 2.52	5.96 ± 0.33
SC-3	85:15	48.82 ± 0.092	13.3 ± 1.91	7.09 ± 0.20
SC-4	75:25	42.37 ± 0.65	50 ± 1.52	5.63 ± 0.13
SDC-1	98:2	49.96 ± 4.3	68 ± 2.03	5.42 ± 0.25
SDC-2	95:5	52.70 ± 0.42	60 ± 0.97	5.61 ± 0.42
SDC-3	85:15	48.07 ± 1.21	13 ± 1.00	7.60 ± 0.68
SDC-4	75:25	41.25 ± 0.61	73 ± 1.54	5.36 ± 0.14
TW80-1	98:2	50.68 ± 2.4	31 ± 1.33	6.31 ± 0.28
TW80-2	95:5	50.84 ± 1.34	20 ± 2.51	6.95 ± 0.19
TW80-3	85:15	49.19 ± 0.73	4 ± 0.98	13.14 ± 0.62
TW80-4	75:25	48.71 ± 0.58	35 ± 1.28	5.78 ± 0.043
SP80-1	98:2	50.73 ± 3.79	149 ± 2.44	3.70 ± 0.36
SP80-2	95:5	55.19 ± 0.57	120 ± 2.01	4.80 ± 0.15
SP80-3	85:15	44.93 ± 0.09	55 ± 1.65	5.62 ± 0.17
SP80-4	75:25	42.80 ± 0.64	166 ± 2.39	3.40 ± 0.054
SP85-1	98:2	43.36 ± 1.59	336 ± 3.34	1.78 ± 0.068
SP85-2	95:5	61.92 ± 1.89	300 ± 4.02	1.90 ± 0.22
SP85-3	85:15	48.65 ± 3.73	90 ± 1.85	5.18 ± 0.082
SP85-4	75:25	47.06 ± 3.45	352 ± 3.96	1.48 ± 0.03

[a] PC: phosphatidylcholine, EA: edge activator, SC: sodium cholate, SDC: sodium deoxycholate, TW80: Tween 80, SP80: Span 80, SP85: Span 85.
[b] Final lipid concentration in all formulations = 5% (w/w).
[c] Average of three determinations ± standard deviation (SD).

maximally swollen on skin surface, they try to follow the local hydration gradient, moving into deeper skin strata. The process of vesicle skin penetration is attributed to the high deformability, resulting from EA molecules accumulating at the site of high stress, due to their raised propensity for greatly curved structures (El Maghraby et al., 2008). Thus, Transfersomes undergo a series of stress-dependant adjustments of the local carrier composition, to minimize the resistance to their motion through the otherwise confining channel, which allows them to transport drugs non-invasively and reproducibly (Cevc and Blume, 2003; Song and Kim, 2006).

The ultraflexible carrier's hydration sensitivity and its unique driving force create an unprecedented opportunity to control the depth of the carriers' migration, by selecting different drug dose and/or carrier dose per area (Cevc, 2003). Transfersomes can effectively protect the drug against undesired rapid clearance into cutaneous blood vessels and are capable of retaining the drug long enough, on, in and below the skin barrier. Furthermore, they can cross the stratum corneum independent of drug concentration (Cevc et al., 2008). Transfersomes have been used as carriers for various compounds, amongst which are, proteins (Paul et al., 1995; Paul et al., 1998), insulin (Cevc et al., 1998; Cevc, 2003), corticosteroids (Cevc et al., 1997; Jain et al., 2003; Cevc and Blume, 2004), ketoprofen (Cevc et al., 2008) and anticancer drugs (Trotta et al., 2004; Hiruta et al., 2006).

The aim of the present study was to refine the formulation of ultraflexible lipid vesicles for enhanced skin delivery of a model hydrophilic drug, Diclofenac sodium (DS). DS is a non-steroidal anti-inflammatory drug (NSAID) used for the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and a variety of non-rheumatic inflammatory conditions (Manconi et al., 2009). DS is usually administered orally but topical dosage forms are desirable for chronic treatment, especially in case of rheumatic symptoms (Manosroi et al., 2008), in order to alleviate the well-known gastropathy (Bartolomei et al., 2006) and avoid the hepatic first pass metabolism (Boinpally et al., 2003).

Pharmaceutically acceptable bile salts as well as non-ionic surfactants (edge activators) were utilized in the preparation of the DS vesicles. The vesicle sizes and morphologies, entrapment efficiencies, drug release and relative vesicle deformabilities were determined. Skin permeation and deposition of the optimized formulation in comparison to a marketed product were evaluated as well. The effect of several variables such as: method of preparation, drug concentration, lipid concentration and vesicle composition on the entrapment efficiencies was studied.

2. Materials and methods

2.1. Materials

Diclofenac sodium (DS) was a kind gift from E.P.I.Co Pharmaceutical Co. (Cairo, Egypt). Egg phosphatidylcholine (type X-E) (PC), stearylamine (SA), sephadex-G-100, sodium cholate (SC), sodium deoxycholate (SDC) and sodium azide were purchased from Sigma Chemical Co. (St. Louis, USA). Tween 80 (TW80), span 80 (SP80), span 85 (SP85), chloroform, methanol and isopropyl alcohol were purchased from ADWIC, El-Nasr Pharmaceutical Co. (Cairo, Egypt). Cellophane membrane (12,000–14,000 molecular weight cut off) was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, Canada).

2.2. Preparation of transfersomes

2.2.1. Vortexing-sonication method

Transfersomal formulations SC-2, SDC-2, TW80-2, SP80-2 and SP85-2 (compositions mentioned in Table 1) were prepared using the vortexing-sonication method for comparison to the conventional method. The mixed lipid (phosphatidylcholine + edge activator (EA)) and the drug were mixed in phosphate buffer (pH 7.4) and vortexed until a milky suspension was obtained. The mixture was then sonicated, in a bath sonicator, for 30 min at room temperature and then extruded through polycarbonate membranes (450 and 220 nm) (Kim et al., 2004; Hofer et al., 2004).

2.2.2. Rotary evaporation–sonication method

The method described by Cevc et al. (1997) and Jain et al. (2003) was adopted, but with some modifications. The mixed lipid consisted of phosphatidylcholine and edge activator (500 mg), which

was dissolved in an organic solvent mixture of chloroform and methanol (2:1, v/v) then placed in a clean, dry round bottom flask. The organic solvent was removed by rotary evaporation (Model RVO5, Janke and Kunkel, IKA Laboratories, Staufen, Germany) under reduced pressure at 40 °C. The deposited film was hydrated with a solution of DS dissolved in phosphate buffer (pH 7.4) by rotation for 1hr at room temperature. The resulting vesicles were swollen for 2 h at room temperature then sonicated for 30 min in a bath sonicator (Model 275T, Crest Ultrasonics Carp, Trenton, USA) to reduce the size of the vesicles. The sonicated vesicles were extruded through a sandwich of 450 and 220 nm polycarbonate membranes then stored at 4 °C. Different edge activators (in several ratios) were used for the preparation Transfersomes, the composition of these formulations is shown in Table 1.

2.3. Measurement of elasticity (relative deformability)

Comparative measurement of elasticity of the bilayer of different Transfersomal formulations was carried out by extrusion measurement (Van den Bergh et al., 2001) through a locally fabricated stainless steel pressure filter holder. The vesicles were extruded through polycarbonate filter with a pore size of 220 nm at a constant pressure of 0.17 MPa. The elasticity was measured as a function of time (i.e. the time taken for the extrusion of 10 ml Transfersomal suspension) (Cevc et al., 1995; Cevc and Gebauer, 2003). The experiments were carried out in triplicates to obtain an average value.

2.4. Entrapment efficiency (EE%)

The EE% of Transfersomes was determined after separation of the non-entrapped drug using the mini-column centrifugation method (El Maghraby et al., 2000a,b). Sephadex G-100 was swollen in distilled water at room temperature with occasional shaking for at least 5 h, and stored at 4 °C. To prepare the mini-columns, Whatman filter pads were inserted in the bottom of eppindorf tubes which were then filled with the sephadex gel. Excess water was centrifuged off at 6000 rpm for 10 min (Heraeus Centrifuge, Germany) and 300 μl of Transfersomes were added dropwise to the center of the column, followed by centrifugation as before. Vesicles were recovered and no washing was required. When a saturated drug solution was used instead of the transfersome suspensions, all the drug remained bound to the gel. This confirmed that there would be no free drug present after recovering the vesicles. The amount of drug entrapped in the vesicles was determined by disrupting the vesicles using 50% isopropyl alcohol, and then analyzing the drug content spectrophotometrically at a wavelength of 283 nm (U.V. visible spectrophotometer, model UV-1601 PC, Shimadzu, Kyoto, Japan). The amount of entrapment drug expressed as % was calculated from the following equation:

$$EE\% = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100 \quad (1)$$

Determination of EE% was conducted for different Transfersomal formulations and the effects of variables, such as: method of preparation, drug and lipid concentrations, vesicle composition (PC:EA ratio, edge activator type and the incorporation of charge-inducing agent, stearylamine) were studied.

2.5. Differential scanning calorimetry measurements (DSC)

DSC experiments were performed with differential scanning calorimeter (Model TA-50WSI, Schimadzu, Japan) calibrated with indium. Samples of Transfersomes in the ratio 95:5% (w/w) (PC:EA) with and without stearylamine were submitted to DSC analysis. The analyses were performed on 40 μl samples sealed in standard aluminium pans. Thermograms were obtained at a scanning rate of 10 °C/min using dry nitrogen flow (25 ml/min). Phosphate buffer (pH 7.4) was employed as reference. Each sample was scanned between zero and 400 °C (Hathout et al., 2007).

2.6. In vitro drug release (flux studies)

The permeation of DS-bearing Transfersomes through an artificial cellophane membrane was performed in Franz-type diffusion cells (Variomag, Telesystem, H + P Labortechnik, Germany) with a diffusion area of 1.77 cm^2 (Gupta et al., 2005). The receptor medium was 7.5 ml phosphate buffer (pH 7.4) which was constantly stirred at 100 rpm with a small magnetic bar. The receptor compartment was maintained at 37 ± 0.2 °C by a circulating water jacket. An amount of Transfersomes equivalent to 600 μg drug was placed in the donor compartment. Samples of 500 μl were withdrawn from the receptor compartment via the sampling port at 0.5, 1, 2, 4, 6, 8 and 24, and immediately replaced with an equal volume of fresh receptor solution. Triplicate experiments were conducted for each study and sink conditions were always maintained. All samples were analyzed for DS content spectrophotometrically at a wavelength of 276 nm. The obtained data were kinetically treated to determine the order of release. The flux at 24 h (J) was assessed and the release profile curves were constructed for all formulae.

$$J = \frac{\text{Amount of permeated drug}}{\text{Time} \times \text{Area of release membrane}} \quad (2)$$

2.7. Vesicle size analysis

The particle size of freshly prepared vesicles was determined by light scattering based on laser diffraction using the Malvern Mastersizer (Malvern Instruments Ltd., Worcestershire, UK) (Guinedi et al., 2005). It consisted of a He–Ne laser (5 mW) and a small volume sample-holding cell, with a stirrer so that the sample, diluted with distilled water, was stirred all over the period of measurement. Measurements were performed using a 45 mm focus objective and a beam length of 2.4 mm.

2.8. Physical stability studies

Selected Transfersomal suspensions were stored in glass vials at 4 °C for up to 3 months. Samples from each Transfersomal formulation were withdrawn at definite time intervals (30, 60 and 90 days) and characterized for their vesicle size and drug leakage (Nasr et al., 2008).

2.9. Photomicroscopic analysis and transmission electron microscopy (TEM)

A selected Transfersomal formulation was chosen for microscopic investigation due to its optimum relative deformability. The vesicles were examined under a binocular optical microscope (Carl Zeiss, Berlin, Germany) and photographed at a magnification of 100× by means of a fitted camera (Panasonic, Japan).

For morphological examination of the vesicles, TEM (Model JEM-1230, JOEL, Tokyo, Japan) was used (Maestrelli et al., 2005). The sample was negatively stained by placing a drop of 1 μl of the vesicular suspension on a carbon coated grid. The suspension was left for 2 min, to allow its absorption in the carbon film, and the excess liquid was drawn off with filter paper. Subsequently, a drop of 1% phosphotungstic acid was placed on the grid. The excess was removed with distilled water and the samples were examined by TEM at 20 and 25 kV (Manconi et al., 2003, 2009).

2.10. In vitro skin permeation and skin deposition studies

2.10.1. Preparation of rabbit skin

Albino male rabbits (2–2.5 kg) were obtained from Faculty of Agriculture, Zagazig University, Egypt. Hair was removed from the abdominal skin with the aid of an electric animal clipper and shaver. Care was taken not to damage the skin surface. Rabbits were sacrificed by inhalation of excess chloroform and the abdominal skin was separated. Fatty and connective tissue layers were removed by rubbing with a cotton swab. The skin was stored at −20 °C and used within three days for the permeation study. The protocol of the present work was approved by Experiments and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

2.10.2. Skin permeation study

A selected Transfersomal formulation was chosen (due to its high EE% and optimum deformability) for the study. Results were compared to that of a marketed product (Olfen® gel manufactured by Mepha Ltd., Switzerland). The skin hydration protocol reported by El Maghraby et al., 2000a,b was followed to maintain the reported *in vivo* transepidermal hydration gradient which has been claimed to generate the driving force for Transfersomes-skin penetration (Cevc and Blume, 1992). The skin was floated, with the stratum corneum side up, on receptor solution (phosphate buffer pH 7.4 + 0.002% sodium azide) and the upper surface was left open to the atmosphere for 24 h (open hydration protocol). Subsequently, the skin was mounted onto Franz diffusion cells (specifications previously mentioned in *in vitro* drug release section), with the dermal side facing the receptor solution. Amounts equivalent to 1 mg drug of the tested Transfersomal formulation (TW80-3) composed of PC:TW80 (85:15%, w/w) and from Olfen® gel, were applied to the skin surface (non-occluded open application). 500 μl samples were withdrawn at the predetermined time intervals and analyzed spectrophotometrically for drug content to determine the amount of DS that permeated the skin.

Permeation rate parameters such as maximum flux (J max), permeability coefficient (k_p) and enhancement ratio (ER) for the transport of DS from TW80-3 in comparison to Olfen® gel, were calculated.

$$k_p = \frac{J\,\text{max}}{\text{Initial drug concentration in the donor compartment}} \quad (3)$$

$$\text{ER} = \frac{J \text{ max of test formulation}}{J \text{ max of market product}} \quad (4)$$

2.10.3. Skin deposition study

At the end of the permeation study (24 h), the skin was removed from the Franz cell and the formulation remaining on the skin surface was washed five times with warm receptor medium (45 °C) (Elsayed et al., 2006), then with distilled water. The permeation area of the skin was then excised and soaked in alcohol for 6 h to extract the deposited drug. The mixture was centrifuged (at 3000 rpm) and the DS content was measured spectrophotometrically (Escribano et al., 2003).

2.11. Statistical analysis

All data were expressed as a mean ± standard deviation ($n = 3$). Significant differences in the mean values were evaluated by unpaired test or one-way analysis of variance (ANOVA), using a computer based program (Graph Pad). A p-value of less than 0.05 was considered to be significant.

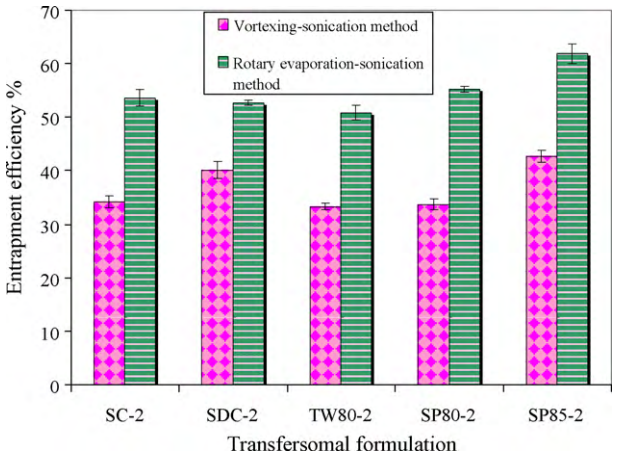

Fig. 1. Effect of method of preparation on the entrapment efficiencies of Transfersomes.

3. Results and discussion

3.1. Optimization of Transfersomes preparation

3.1.1. Effect of the method of preparation

Transfersomes were prepared utilizing two methods, in an attempt to optimize the conditions of preparation. In both methods, Transfersomes were extruded after preparation through a sandwich of polycarbonate membranes in order to reduce their size. According to Cevc et al. (1995), Transfersomes have an inherent tendency to be small, consequently, they tend to diminish in size by fragmentation, upon passage through smaller constrictions.

Transfersomal formulations SC-2, SDC-2, TW80-2, SP80-2 and SP85-2 prepared using the vortexing-sonication method had EE% of 34.12, 40.14, 33.32, 33.64 and 42.64%, respectively. Upon preparation of these formulations using the rotary evaporation–sonication method, these EE% were raised to 53.63, 52.7, 50.84, 55.19 and 61.92%, respectively (Fig. 1). All differences were significant with $p \leq 0.0002$. This increase in EE% maybe attributed to the formation of a thin film with a large surface area in the rotary evaporation method which enabled the complete hydration of vesicles. On the other hand, during vortexing, visual observation revealed that lipids tended to aggregate and adhere to the walls of the vortexing vial, making hydration of the vesicles difficult. This vortexing method was incapable of completely dispersing the lipids, hence, resulted in lumpy dispersions, difficultly homogenized and prone to rapid sedimentation and aggregation. Consequently, the rotary evaporation method is recommended despite the simplicity and less time consumption of the vortexing method.

3.1.2. Effect of drug concentration

It was found that upon increasing DS concentration from 0.25 to 1 g%, in Transfersomes prepared using PC:SP85 (95:5%, w/w), the EE% significantly increased from 25 to 61.92% ($p < 0.001$). However, a further increase in drug concentration to 1.25 g% led to a significant decrease in EE% to 50.34% ($p < 0.001$). Drug crystals were also seen when observed under the optical microscope (results not shown). The amphiphilic properties of DS enable its encapsulation in both, bilayers and aqueous compartment of the vesicles (Lopes et al., 2004). When the lipid domains and aqueous compartment became saturated with the drug, the vesicles provided limited entrapment capacity (Ning et al., 2005). Hence, Transfersomes could entrap DS only to an optimum extent, after which, any further increase in drug concentration will lead to precipitation.

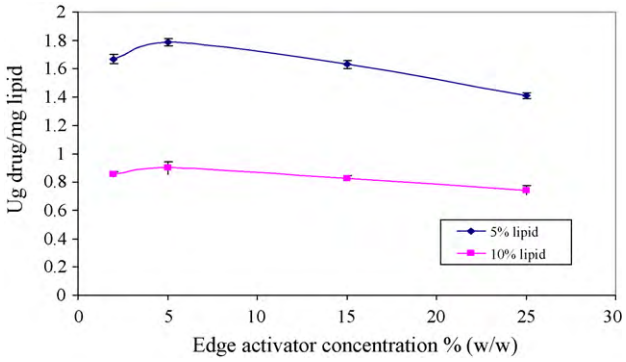

Fig. 2. Effect of total lipid concentration on entrapment of DS-bearing Transfersomes prepared using sodium cholate, expressed as μg drug/mg lipid.

3.1.3. Effect of total lipid concentration

Increasing sodium cholate concentration from 2 to 25%, w/w, no marked variation in DS EE% was seen by doubling the total lipid concentration from 5 to 10% (w/w) and the values were superimposed (results not shown). On the other hand, marked reductions ($p < 0.001$) in the entrapment, expressed as μg drug/mg lipid, were observed throughout all SC concentrations from 1.67 to 0.854, from 1.79 to 0.901, from 1.63 to 0.828 and from 1.41 to 0.742 μg, respectively (Fig. 2). These findings indicated that the fraction of lipid taking part in encapsulation was reduced upon increasing the total lipid concentration. Similar observations were obtained by Mokhtar et al. (2008) who reported an optimum lipid concentration for the encapsulation of flurbiprofen into proniosomes.

Hence, further work in this study was carried out using the more efficient rotary evaporation–sonication method with an optimum drug concentration of 1% and a lipid concentration of 5% (w/w). To investigate the effect of vesicle composition on the EE% and relative deformability of Transfersomes, different edge activators (in four different PC:EA ratios) with different physicochemical properties and structures were utilized, namely, sodium cholate, sodium deoxycholate, tween 80, span 80 and span 85.

3.2. Effect of vesicle composition on EE% and relative deformability of Transfersomes

3.2.1. Effect of PC:EA ratio

Initially, the EE% increased non-significantly with increasing edge activator concentration from 2 to 5% (w/w) (i.e. from 98:2 to 95:5). Further increase in edge activator concentration to 15 then 25% (w/w) showed a decrease in EE% (Table 1). The ratio 95:5% (w/w) showed optimum EE%. Upon incorporation of edge activator in low concentration, growth in vesicle size occurred (Van den Bergh et al., 2001), whereas, further increase in the content of edge activator may have led to pore formation in the bilayers. When edge activator concentration exceeded 15%, mixed micelles coexisted with the Transfersomes, with the consequence of lower drug entrapment due to the rigidity and smaller size of mixed micelles (Jain et al., 2003).

The degree of deformability is a crucial and unique parameter of Transfersomes as it differentiates them from other lipid vesicles that are unable to cross intact skin. Whenever required, Transfersomes can get more deformable than any other lipid vesicles by up to 5 orders of magnitude (Cevc et al., 1995). This membrane deformability is achieved by combining at least 2 lipophilic/amphiphilic components (PC + EA), with sufficiently different packing characteristics, into a single bilayer. Transfersomes, consequently, undergo shape changes, whenever deformations are enforced by surrounding stress or space confinements, which minimizes the risk of vesicle rupture upon penetrating the skin pores. However, Transfersomal deformability is composition dependent. In this study, the optimum deformability PC:EA ratio was 85:15% (w/w) (Table 1). Ratios 98:2 and 95:5% (w/w) were less deformable, probably due to the insufficient amount edge activator in the bilayers. Likewise, the ratio 75:25% (w/w) was also less deformable, but in this case, due to the formation of rigid of mixed micelles.

3.2.2. Effect of edge activator type

Table 1 demonstrates the effect of edge activator types on the EE% of different Transfersomal formulations. Upon comparing the ratio of maximum EE% (95:5%, w/w), SP85-2 showed the highest EE% (61.92%) followed by SP80-2 (55.19%), SC-2 (53.63%), SDC-2 (52.70%) and finally TW80-2 (50.84%). One way to explain these findings is to consider the HLB of these edge activators. They are 1.8, 4.3, 15, 16.7 and 16.7 for span 85, span 80, tween 80 and sodium cholate and sodium deoxycholate, respectively. Based on these HLB values, the affinity for lipids was expected to be in the order of SP85 > SP80 > TW80 > SC and SDC. This explanation verifies the higher EE% encountered with SP85-2 and SP80-2 compared to TW80-2, SC-2 and SDC-2. Interestingly and unexpectedly, TW80-2 did not show a higher EE% than SC-2 and SDC-2. Finally, SC-2 and SDC-2 Transfersomes, showed a non-significant difference in EE% despite the difference in their structures (Sodium cholate has three OH groups while sodium deoxycholate has only two) (Subuddhi and Mishra, 2007).

Table 1 shows that the deformability of Transfersomes is also affected by the type of edge activator used, which could be interpreted by the differences in their chemical structures. By comparing the optimum deformability ratio (85:15%, w/w), it was found that, TW80-3 showed the highest deformability (4 s). This could be attributed to the highly flexible and nonbulky hydrocarbon chains of tween 80. SDC-3 and SC-3 had lower deformability than TW80-3 (13 and 13.3 s, respectively) due to their steroid-like structures which are bulkier than the hydrocarbon chains of tween 80. The difference in deformability between SC-3 and SDC-3 was non-significant. Finally, SP80-3 and SP85-3 showed least deformability (55 and 90 s, respectively). This could be a result of their high hydrophobicity, which reduced the formation of transient hydrophilic holes, hence, minimizing the amphiphilic property of the bilayers responsible for membrane fluidity. Furthermore, span 85 being a sorbitan triester while span 80 is a sorbitan monoester, caused span 85 to be bulkier and offer less flexible membranes, hence SP85-3 was less deformable than SP80-3.

3.2.3. Effect of charge-inducing agent

Being positively charged, stearylamine would be expected to increase EE% of the negatively charged DS. Hence, stearylamine was added (in a molar ratio of 7:1 mixed lipid:stearylamine) to impart a positive charge to selected Transfersomal formulations in terms of optimum EE% and deformability (Table 2). Strikingly, the opposite occurred, and stearylamine markedly decreased the EE% ($p < 0.001$) especially in Transfersomes prepared using the ratio 95:5% (w/w). This behaviour is in agreement with previous findings. Fang et al. (2001) explained that stearylamine induced repulsion within the lipid double layers and Mokhtar et al. (2008) reported an electrostatic induced chain tilt, subsequently causing changes in the lateral packing of the bilayers.

For further interpretation of this behaviour, DSC was performed for all formulations prepared using PC:EA (95:5%, w/w). Upon comparing the transition temperatures (T_m) of vesicles with and without stearylamine, its effect could be verified. Lipid vesicles undergo distinct structural changes at the phase transition temperature. Below the pre-transition temperature, the bilayer lipids are in highly ordered gel state, in the tilted one-dimensional arrangement. At the pre-transition temperature, lipids change from tilted one-dimensional arrangements to two-dimensional arrangements

Table 2
Entrapment efficiencies of Diclofenac sodium Transfersomal formulations after the addition of the positive charge inducer, stearylamine.

Transfersomal formulation code[a,b]	Entrapment efficiency (EE%) (% ± SD)[c]
SC-2/SA	35.45 ± 0.42
SC-3/SA	46.89 ± 0.78
SDC-2/SA	38.28 ± 0.092
SDC-3/SA	43.60 ± 0.70
TW80-2/SA	39.99 ± 0.56
TW80-3/SA	34.97 ± 0.32
SP80-2/SA	39.82 ± 0.24
SP80-3/SA	21.40 ± 0.85
SP85-2/SA	59.57 ± 0.24
SP85-3/SA	46.50 ± 0.19

[a] SC: sodium cholate, SDC: sodium deoxycholate, TW80: Tween 80, SP80: Span 80, SP85: Span 85, SA: stearylamine.
[b] Mixed lipid:stearylamine ratio = 7:1 molar ratio.
[c] Average of three determinations ± standard deviation (SD).

with periodic undulations. Above T_m, rotational isomerisation occurs which decreases the thickness of the bilayer and the system reverts to one-dimensional arrangements, thus, the lipids become more fluid (El Maghraby et al., 2000b).

Since all EA-containing Transfersomes had similar trends, the chart for SP80-Transfersomes was chosen as a representative (Fig. 3). The DSC thermograms produced T_m values of 92.77, 88.15, 87.88, 82.91 and 81.55 °C for TW80-2, SP80-2, SC-2, SP85-2 and SDC-2 formulae, respectively. Upon the addition of stearylamine, the T_m values were significantly reduced ($p < 0.01$) to 80.76, 81.00, 79.95, 82.20 and 79.96 °C, respectively. This indicated that stearylamine interacted with the lipid vesicles, by fitting its lipophilic portions between the hydrocarbon chains of the lipid bilayers, thus perturbing the packing characteristics and fluidizing the lipid bilayers. Consequently, EE% decreased in all formulations and so deformability studies were not carried out.

3.3. In vitro drug release/flux studies

In vitro studies for the release of DS from Transfersomes containing sodium cholate, sodium deoxycholate, tween 80, span 80 and span 85 (with different PC:EA ratios) were performed. Fluxes from Transfersomes at 24 h first increased with increasing edge activator concentration (from 2 to 15%, w/w) in the formulation and then decreased, a common phenomenon seen with all edge activators (Table 1). These results suggested that a too low or high concentration of edge activator was not beneficial for the improved DS delivery from deformable vesicles. A possible explanation for lower drug release at low edge activator concentrations may be that the lipid membranes were more ordered and less leaky, which impeded drug release. Similarly, at high edge activator concentrations, drug delivery was low due to the loss of vesicular structure and formation of rigid mixed micelles (as previously mentioned)

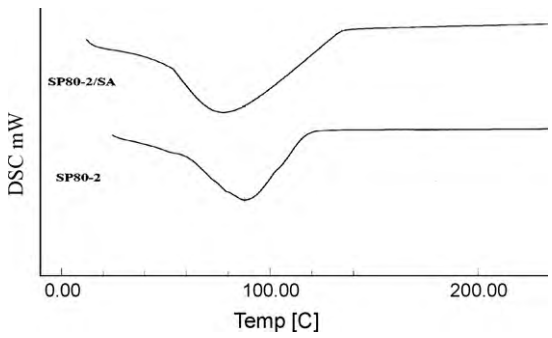

Fig. 3. DSC thermogram of Span 80 Transfersomes with and without stearylamine.

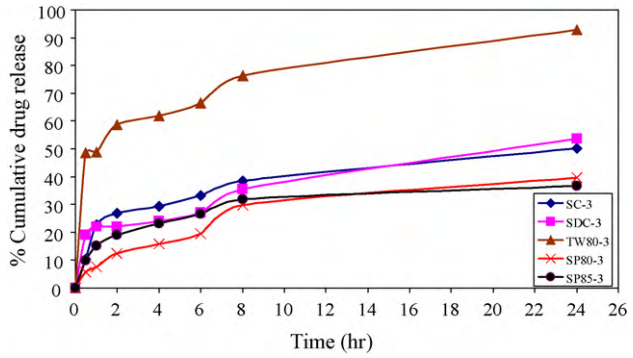

Fig. 4. Comparison between the release profiles of different Transfersomal formulations prepared with the ratio 85:15% (w/w) (PC:EA).

which were much less sensitive to a water-activity gradient than Transfersomes. These findings are in agreement with published data (Cevc et al., 1995, 1997; El Maghraby et al., 2000b; Jain et al., 2003; Hiruta et al., 2006). This hypothesis is also supported by the report of Cevc et al. (1996), who compared the penetration ability of Transfersomes, liposomes and mixed micelles by confocal laser scanning microscopy (CLSM) and observed that the least deformable mixed micelles were restricted to the top most part of the stratum corneum while Transfersomes penetrated to a deeper skin layer.

The release profiles of DS from different Transfersomes (results not shown) were apparently biphasic release processes, where rapid release of the surface-adsorbed drug was observed during the initial phase (first 2 h), followed by a sustained release profile for up to 24 h. The formulation prepared using the ratio with optimum release (85:15%, w/w) was selected to compare the release patterns from different edge activator-containing vesicles (Fig. 4). The %cumulative drug release after 24 h were 92.99, 53.76, 50.18, 39.78 and 36.65% for TW80-3, SDC-3, SC-3, SP80-3 and SP85-3 Transfersomes, respectively. These significant variations ($p < 0.05$) in drug release could be explained by variations in molecular ordering and deformability caused by particular edge activators i.e. the edge activator with highest deformability, showed highest drug release and vice versa. Our results disagree with Jain et al. who reported that the release of dexamethasone from Transfersomes was in the order: SP80 > SDC > TW80 (Jain et al., 2003). But this observation maybe argued in terms of the difference in drug nature, dexamethasone being a lipophilic drug.

It was necessary to further prove this relation between drug release and relative deformability results. The calculated correlation coefficients (r) between cumulative amounts of drug release and relative deformability for all formulations proved significant correlation (Table 3).

Linear regression analysis of the release data revealed that DS was released from Transfersomes by a diffusion controlled mechanism. These results are in agreement with many research workers (Arica et al., 1995; Hathout et al., 2007; Nasr et al., 2008).

Table 3
Correlation coefficient (r) between %cumulative drug released after 24 h and relative deformability for Transfersomes prepared from different edge activators.

Transfersomal formulations (TF)	r
SC-TF	0.985
SDC-TF	0.997
TW80-TF	0.971
SP80-TF	0.985
SP85-TF	0.997

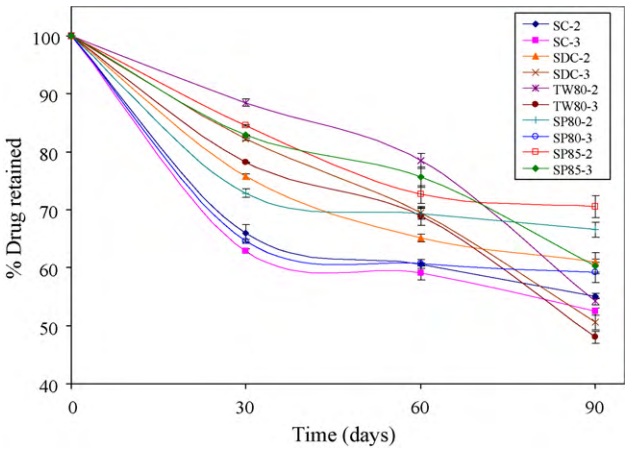

Fig. 5. Effect of storage on the amount of DS retained in selected Transfersomal formulations.

3.4. Size analysis

Formulae SC-3, SDC-3, TW80-3, SP80-3 and SP85-3 were used in this study. The investigation revealed that they exhibited a mean particle size of 171.5, 168.9, 206.9, 165.4 and 122.8 nm, respectively. These variations in particle size were highly significant ($p < 0.001$). The frequency distribution curves of particle size data were unimodal in shape. Generally, the use of edge activators with lower HLB, resulted in vesicles with smaller size. Hence, SP85-3 was the smallest in size due to its highest hydrophobicity, whereas, hydrophilic edge activators led to vesicles with larger particle size. The relationship observed between vesicle size and surfactant HLB has been attributed to the decrease in surface energy obtained with increasing hydrophobicity, thus resulting in smaller vesicles. This observation is consistent with Yoshioka et al. (1994) who compared the properties of niosomes prepared from different spans. However, TW80-3 showed a larger particle size than SC-3 and SDC-3 despite its lower HLB. Although tween 80 is a non-ionic surfactant while sodium cholate and deoxycholate are anionic surfactants, Lee et al. (2005) reported that TW80-Transfersomes exhibited a more negative zeta potential than SC and SDC-Transfersomes, leading to repulsion between the bilayers, thus, an increase in the size of Tween-containing vesicles. Therefore, we disagree with other investigators who reported that there were insignificant differences in size between the Transfersomal formulations containing different surfactants, arguing that, being prepared by a similar method and homogenized by extrusion through polycarbonate membranes, they should have similar sizes (El Maghraby et al., 2000a; Jain et al., 2003). We disagree with this hypothesis because, Transfersomes being flexible, should be able to pass through the polycarbonate membranes without being affected in size.

3.5. Stability studies

Formulae SC-2, SC-3, SDC-2, SDC-3, TW80-2, TW80-3, SP80-2, Sp80-3, SP85-2 and SP85-3 were considered in this study as they represent optimum formulae with the highest EE% and deformability.

Drug leakage from the vesicles was evaluated after 30, 60 and 90 days. The results are illustrated in Fig. 5 in terms of % drug retained in the Transfersomes. Tw80-3 showed the lowest % of drug retained after 90 days (48.01%) while SP85-2 showed highest % drug retained (70.53%). This variation can mainly be attributed to the difference in bilayer ordering caused by different surfactants. Ordered bilayers confer more structural stability than non-ordered ones, thus, span 85 formed much more stable vesicles than tween 80.

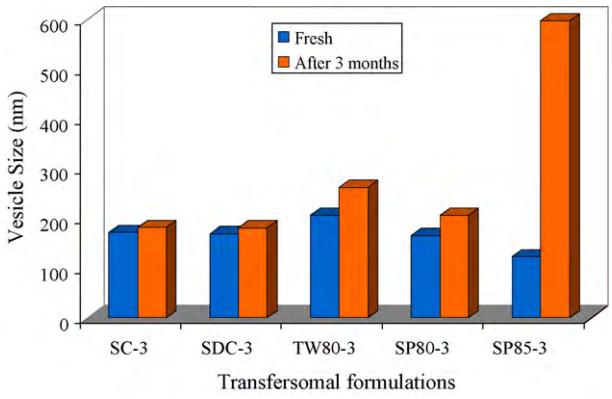

Fig. 6. Effect of storage on the vesicle size of selected Transfersomal formulations.

This physical instability problem of Transfersomes can be circumvented by developing the novel, dry, precursor form of Transfersomes (Protransfersomes). These Protransfersomes appear to be a promising elegant alternative to Transfersomes in the future for topical/transdermal delivery (results are soon to be published).

The effect of storage on particle size of formulae SC-3, SDC-3, TW80-3, SP80-3 and SP85-3 is demonstrated in Fig. 6. The results showed a significant increase ($p < 0.001$) in the vesicle diameters of the stored formulations. This might be due to the fusion and aggregation of Transfersomes after storage (Fang et al., 2001; Lee et al., 2005). Moreover, SP85-3 showed the largest increase in size upon storage, which could be attributed to the higher tendency of Span 85-containing vesicles to aggregate due to the strong cohesive forces between the small hydrophobic vesicles.

3.6. Photomicroscopic analysis and electron microscopy

The photomicrograph of formula TW80-3 revealed the spherical shape and unilamellar structure of the prepared Transfersomes that exist in disperse collections (results not shown). The rotary evaporation method used in the preparation of Transfersomal formulations leads to the formation multilamellar vesicles, but

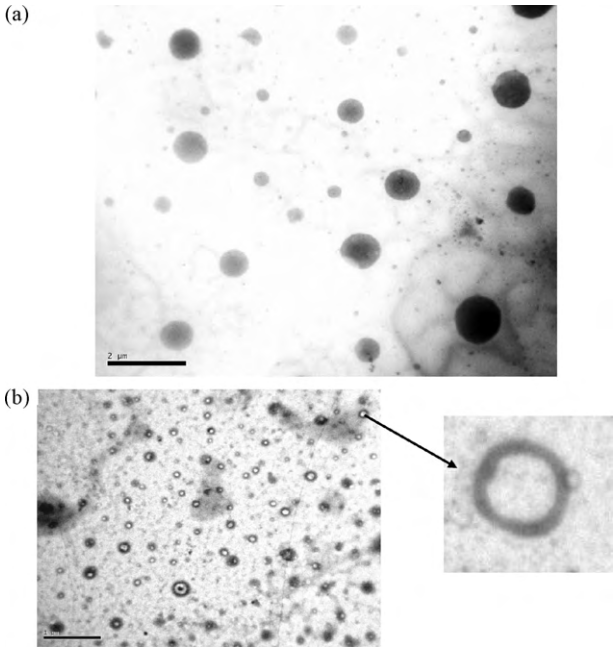

Fig. 7. The electron micrographs of TW80-3 Transfersomes consisting of PC:TW80 (85:15%, w/w) (a) without sonication and (b) after sonication.

Table 4
Amount of DS permeated through and deposited in the skin after 24 h, maximum flux (J) and permeability coefficient (k_p) of the tested formulations.

Tested formulation	Amount of drug permeated[a] (μg ± SD)	Amount of drug deposited[a] (μg ± SD)	J max[a] (μg/cm^2/hr ± SD)	k_p[a] (cm^2/h ± SD)
TW80-3	886.9 ± 62.6	107.3 ± 6.92	60.41 ± 5.2	$0.0604 \pm 5.2 \times 10^{-3}$
Olfen® gel	434 ± 64.4	21.6 ± 3.21	24.67 ± 4.42	$0.0247 \pm 4.42 \times 10^{-3}$

[a] Average of three determinations ± standard deviation (SD).

upon sonication, these vesicles were converted to unilamellar vesicles.

The electron micrographs of TW80-3 are shown in Fig. 7. They show the outline and core of the well-identified spherical vesicles, displaying the retention of sealed vesicular structure. Fig. 7a displays the vesicles before sonication, they appear to be multilamellar with a heterogenous particle size distribution. Whereas, Fig. 7b shows the vesicles after sonication, they are smaller unilamellar vesicles with a more homogenous size distribution. The magnification of one of the vesicles reveals the bilayers structure of the vesicle's membrane.

3.7. *In vitro skin permeation and skin deposition studies*

In this study we compare the efficacy of ultradeformable vesicles for the enhanced delivery of DS through skin to a marketed product (Olfen® gel). TW80-3 was chosen for this study due to its acceptably high EE% and maximum deformability amongst all other formulae. Based on the amount of drug permeated and deposited in skin, maximum flux (J max) and permeability coefficient (k_p), TW80-3 was found superior to Olfen® gel.

Table 4 shows that Transfersomes delivered 886.9 μg of DS, which was twice the amount delivered by Olfen® gel (434 μg) ($p = 0.0009$). Also, the residual amount of drug remaining in the skin was nearly 5 times more in case of Transfersomes. Moreover, the maximum flux (J max) was significantly higher ($p < 0.001$) in Transfersomes, with an ER of 2.45.

Fig. 8 demonstrates the %cumulative amount of DS transferred from Transfersomes and Olfen® gel over 24 h. Through out the experiment period, Transfersomes showed a larger amount of drug permeation, except at 4 and 6 h, Olfen® gel was higher. This could be due to the fact that Transfersomes, unlike gels, act as a depot, hence, offer a mean for sustained release (Jain et al., 2003).

Two mechanisms have been proposed for the improved skin delivery by deformable vesicles (Honeywell-Nguyen and Bouwstra, 2003; Honeywell-Nguyen et al., 2003). The first mechanism proposes that vesicles can act as drug carrier systems, whereby intact vesicles enter the stratum corneum carrying vesicle-bound drug molecules into the skin, under the influence of the naturally occurring *in vivo* transcutaneous hydration gradient (Cevc and Blume, 2001). This mechanism can explain why Transfersomes were able to deposit much larger amounts of DS in the skin, compared to the gel. The second mechanism proposes that vesicles can act as penetration enhancers, whereby vesicle bilayers enter the stratum corneum and subsequently modify its intercellular lipids, hence, raising its fluidity and weakness. Thus, drugs can further penetrate solitary (El Maghraby et al., 2001; Verma et al., 2003). Moreover, phospholipids have a high affinity for biological membranes, thus, the mixing of vesicle-phospholipid bilayers with the intercellular lipid layers of the skin may also contribute to permeability enhancement of Transfersomes. This mechanism justifies the higher skin permeation of Transfersomes over the gel.

We therefore support that both, the penetrating enhancing effect and the intact vesicle permeation into the skin, played a role in the enhanced skin delivery of DS by Transfersomes under non-occlusive conditions. This conclusion is in accordance with El Maghraby et al. (2006) and Elsayed et al. (2006) who reported that one of the 2 mechanisms might predominate according to the vesicle composition and characteristics, as well as, the physicochemical properties of the drug.

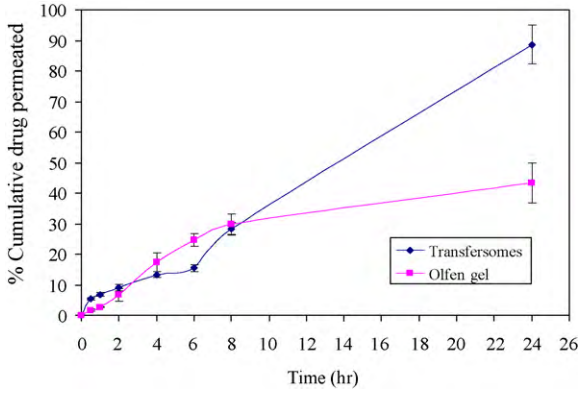

Fig. 8. %Cumulative drug permeated through skin from a selected Transfersomal formulation (TW80-3) and Olfen® gel.

4. Conclusion

Transfersomes are specially optimized ultraflexible lipid vesicles which can respond to an external stress by rapid shape deformation, which enables them to pass through skin pores which are much smaller in size than the vesicles. Specific types and concentrations of edge activators are required for providing the maximum deformability to vesicle membranes. Tween 80 is more effective as compared to bile salts and spans, as it provides maximum deformability to the vesicle membrane.

Transfersomes can significantly improve the *in vitro* skin delivery of DS compared to the marketed product (Olfen® gel) when applied non-occlusively. This enhancement is attributed to the synergistic ability of Transfersomes to act as drug carriers, as well as, permeation enhancers. Hence, Transfersomes create a window of opportunity for the well-controlled skin delivery of drugs that cause side effects upon administration by other routes.

References

Arica, B., Ozer, A.Y., Ercan, M.T., Hincal, A.A., 1995. Characterization, in vitro and in vivo studies on primaquine diphosphate liposomes. J. Microencapsul. 12, 469–485.
Bahia, A.P., Azevedo, E.G., Ferreira, L.A.M., Frθzard, F., 2010. New insights into the mode of action of ultradeformable vesicles using calcein as hydrophilic fluorescent marker. Eur. J. Pharm. Sci. 39, 90–96.
Bartolomei, M., Bertocchi, P., Antoniella, E., Rodomonte, A., 2006. Physico-chemical characterisation and intrinsic dissolution studies of a new hydrate form of Diclofenac sodium: comparison with anhydrous form. J. Pharm. Biomed. Anal. 40, 1105–1113.
Boinpally, R.R., Zhou, S.L., Poondru, S., Devraj, G., Jasti, B.R., 2003. Lecithin vesicles for topical delivery of Diclofenac. Eur. J. Pharm. Biopharm. 56, 389–392.
Cevc, G., 2003. Transdermal drug delivery of insulin with ultradeformable carriers. Clin. Pharmacokinet. 42, 461–474.
Cevc, G., Blume, G., 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim. Biophys. Acta 1104, 226–232.
Cevc, G., Vierl, U., Mazgareanu, S., 2008. Functional characterisation of novel analgesic product based on self-regulating drug carriers. Int. J. Pharm. 360, 18–28.
Cevc, G., Blume, G., 2001. New, highly efficient formulation of Diclofenac for the topical, transdermal administration in ultradeformable drug carriers, Transfersomes. Biochim. Biophys. Acta 1514, 191–205.

Cevc, G., Blume, G., 2003. Biological activity and characteristics of triamcinolone-acetonide formulated with the self-regulating drug carriers, Transfersomes. Biochim. Biophys. Acta 1614, 156–164.
Cevc, G., Blume, G., 2004. Hydrocortisone and dexamethasone in very deformable drug carriers have increased biological potency, prolonged effect, and reduced therapeutic dosage. Biochim. Biophys. Acta 1663, 61–73.
Cevc, G., Blume, G., SchAtzlein, A., 1997. Transfersomes-mediated transepidermal delivery improves the regio-specificity and biological activity of corticosteroids in vivo. J. Control. Release 45, 211–226.
Cevc, G., Blume, G., SchAtzlein, A., Gebauer, D., Paul, A., 1996. The skin: a pathway for systemic treatment with patches and lipid-based agent carriers. Adv. Drug Deliv. Rev. 18, 349–378.
Cevc, G., Gebauer, D., 2003. Hydration-driven transport of deformable lipid vesicles through fine pores and the skin barrier. Biophys. J. 84, 1010–1024.
Cevc, G., Gebauer, D., Stieber, J., SchAtzlein, A., Blume, G., 1998. Ultraflexible vesicles. Transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochim. Biophys. Acta 1368, 201–215.
Cevc, G., SchAtzlein, A., Blume, G., 1995. Transdermal drug carriers: basic properties, optimization and transfer efficiency in the case of epicutaneously applied peptides. J. Control. Release 36, 3–16.
Dragicevic-Curic, N., GrAfe, S., Gitter, B., Winter, S., Fahr, A., 2010. Surface charged temoporfin-loaded flexible vesicles: In vitro skin penetration studies and stability. Int. J. Pharm. 384, 100–108.
El Maghraby, G.M., Barry, B.W., Williams, A.C., 2008. Liposomes and skin: from drug delivery to model membranes. Eur. J. Pharm. Sci. 34, 203–222.
El Maghraby, G.M., Williams, A.C., Barry, B.W., 2001. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in-vitro. J. Pharm. Pharmacol. 53, 1069–1077.
El Maghraby, G.M., Williams, A.C., Barry, B.W., 2006. Can drug-bearing liposomes penetrate intact skin? J. Pharm. Pharmacol. 58, 415–429.
El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000a. Skin delivery of oestradiol from lipid vesicles: importance of liposome structure. Int. J. Pharm. 204, 159–169.
El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2000b. Oestradiol skin delivery from ultradeformable liposomes: refinement of surfactant concentration. Int. J. Pharm. 196, 63–74.
Elsayed, M.M.A., Abdallah, O.Y., Naggar, V.F., Khalafallah, N.M., 2006. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. Int. J. Pharm. 322, 60–66.
Elsayed, M.M.A., Abdallah, O.Y., Naggar, V.F., Khalafallah, N.M., 2007. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int. J. Pharm. 332, 1–16.
Escribano, E., Calpena, A.C., Queralt, J., Obach, R., Domθnech, J., 2003. Assessment of Diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. Eur. J. Pharm. Sci. 19, 203–210.
Fang, J.Y., Hong, C.T., Chiu, W.T., Wang, Y.Y., 2001. Effect of liposomes and niosomes on skin permeation of enoxacin. Int. J. Pharm. 219, 61–72.
Guinedi, A.S., Mortada, N.D., Mansour, S., Hathout, R.M., 2005. Preparation and evaluation of reverse-phase evaporation and multilamellar niosomes as ophthalmic carriers of acetazolamide. Int. J. Pharm. 306, 71–82.
Gupta, P.N., Mishra, V., Rawat, A., Dubey, P., Mahor, S., Jain, S., Chatterji, D.P., Vyas, S.P., 2005. Non-invasive vaccine delivery in Transfersomes, niosomes and liposomes: a comparative study. Int. J. Pharm. 293, 73–82.
Hathout, R.M., Mansour, S., Mortada, N.D., Guinedi, A.S., 2007. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. AAPS Pharm. Sci. Tech. 8, 1.
Hiruta, Y., Hattori, Y., Kawano, K., Obata, Y., Maitani, Y., 2006. Novel ultra-deformable vesicles entrapped with bleomycin and enhanced to penetrate rat skin. J. Control. Release 113, 146–154.
Hofer, C., van Randenborgh, H., Lehmer, A., Hartung, R., Breul, J.r., 2004. Transcutaneous IL-2 uptake mediated by Transfersomes« depends on concentration and fractionated application. Cytokine 25, 141–146.
Honeywell-Nguyen, P.L., Arenja, S., Bouwstra, J.A., 2003. Skin penetration and mechanisms of action in the delivery of the D2-agonist rotigotine from surfactant-based elastic vesicle formulations. Pharm. Res. 20, 1619–1625.
Honeywell-Nguyen, P.L., Bouwstra, J.A., 2005. Vesicles as a tool for transdermal and dermal delivery. Drug Discov. Today: Technol. 2, 67–74.
Honeywell-Nguyen, P.L., Bouwstra, J.A., 2003. The in vitro transport of pergolide from surfactant-based elastic vesicles through human skin: a suggested mechanism of action. J. Control. Release 86, 145–156.
Jain, S., Jain, P., Umamaheshwari, R.B., Jain, N.K., 2003. Transfersomes–a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug Dev. Ind. Pharm. 29, 1013–1026.
Kim, A., Lee, E.H., Choi, S.H., Kim, C.K., 2004. In vitro and in vivo transfection efficiency of a novel ultradeformable cationic liposome. Biomaterials 25, 305–313.
Lee, E.H., Kim, A., Oh, Y.K., Kim, C.K., 2005. Effect of edge activators on the formation and transfection efficiency of ultradeformable liposomes. Biomaterials 26, 205–210.
Li, L., Hoffman, R.M., 1997. Topical liposome delivery of molecules to hair follicles in mice. J. Dermatol. Sci. 14, 101–108.
Lopes, L.B., Scarpa, M.V., Silva, G.V.J., Rodrigues, D.C., Santilli, C.V., Oliveira, A.G., 2004. Studies on the encapsulation of Diclofenac in small unilamellar liposomes of soya phosphatidylcholine. Colloids Surf. B: Biointerfaces 39, 151–158.
Maestrelli, F., Gonzβlez-Rodrguez, M.L., Rabasco, A.M., Mura, P., 2005. Preparation and characterisation of liposomes encapsulating ketoprofen-cyclodextrin complexes for transdermal drug delivery. Int. J. Pharm. 298, 55–67.
Manconi, M., Mura, S., Sinico, C., Fadda, A.M., Vila, A.O., Molina, F., 2009. Development and characterization of liposomes containing glycols as carriers for Diclofenac. Colloids Surf. A: Physicochem. Eng. Asp. 342, 53–58.
Manconi, M., Valenti, D., Sinico, C., Lai, F., Loy, G., Fadda, A.M., 2003. Niosomes as carriers for tretinoin: II. Influence of vesicular incorporation on tretinoin photostability. Int. J. Pharm. 260, 261–272.
Manosroi, A., Jantrawut, P., Manosroi, J., 2008. Anti-inflammatory activity of gel containing novel elastic niosomes entrapped with Diclofenac diethylammonium. Int. J. Pharm. 360, 156–163.
Mokhtar, M., Sammour, O.A., Hammad, M.A., Megrab, N.A., 2008. Effect of some formulation parameters on flurbiprofen encapsulation and release rates of niosomes prepared from proniosomes. Int. J. Pharm. 361, 104–111.
Montanari, J., Roncaglia, D.I., Lado, L.A., Morilla, M.J., Romero, E.L., 2009. Avoiding failed reconstitution of ultradeformable liposomes upon dehydration. Int. J. Pharm. 372, 184–190.
Nasr, M., Mansour, S., Mortada, N.D., Elshamy, A.A., 2008. Vesicular aceclofenac systems: a comparative study between liposomes and niosomes. J. Microencapsul. 25, 499–512.
Ning, M.Y., Guo, Y.Z., Pan, H.Z., Yu, H.M., Gu, Z.W., 2005. Preparation and evaluation of proliposomes containing clotrimazole. Chem. Pharm. Bull. (Tokyo) 53, 620–624.
Paul, A., Cevc, G., Bachhawat, B.K., 1998. Transdermal immunisation with an integral membrane component, gap junction protein, by means of ultradeformable drug carriers, Transfersomes. Vaccine 16, 188–195.
Paul, A., Cevc, G., Bachhawat, B.K., 1995. Transdermal immunization with large proteins by means of ultradeformable drug carriers. Eur. J. Immunol. 25, 3521–3524.
Song, Y.K., Kim, C.K., 2006. Topical delivery of low-molecular-weight heparin with surface-charged flexible liposomes. Biomaterials 27, 271–280.
Subuddhi, U., Mishra, A.K., 2007. Micellization of bile salts in aqueous medium: a fluorescence study. Colloids Surf. B Biointerfaces 57, 102–107.
Touitou, E., Dayan, N., Bergelson, L., Godin, B., Eliaz, M., 2000. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Control. Release 65, 403–418.
Trotta, M., Peira, E., Debernardi, F., Gallarate, M., 2002. Elastic liposomes for skin delivery of dipotassium glycyrrhizinate. Int. J. Pharm. 241, 319–327.
Trotta, M., Peira, E., Carlotti, M.E., Gallarate, M., 2004. Deformable liposomes for dermal administration of methotrexate. Int. J. Pharm. 270, 119–125.
Van den Bergh, B.A.I., Wertz, P.W., Junginger, H.E., Bouwstra, J.A., 2001. Elasticity of vesicles assessed by electron spin resonance, electron microscopy and extrusion measurements. Int. J. Pharm. 217, 13–24.
Verma, D.D., Verma, S., Blume, G., Fahr, A., 2003. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur. J. Pharm. Biopharm. 55, 271–277.
Yoshioka, T., Sternberg, B., Florence, A.T., 1994. Preparation and properties of vesicles (niosomes) of sorbitan monoesters (Span 20, 40, 60 and 80) and a sorbitan triester (Span 85). Int. J. Pharm. 105, 1–6.