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Novel Elastic Nanovesicles for Cosmeceutical and Pharmaceutical Applications

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ABSTRACT

Drug delivery systems using vesicular carriers such as liposomes or niosomes, have distinct advantages over conventional dosage forms because the vesicles can act as drug containing reservoirs and the modification of the vesicular compositions or surface properties can adjust the drug release rate and/or the affinity for the target site. The optimized elastic niosomal formulations for the topical non-invasive treatment of gene therapy and local pain or inflammation have been developed. The gel containing the novel Tween 61 elastic niosomes entrapped with DCFD (diclofenac diethylammonium) did not only show physical and chemical stability for 3 months, but also high fluxes through rat skin and high *in vivo* anti-inflammatory activity in rat ear edema assay. This optimized developed gel can offer a promising formulation for DCFD in the topical non-invasive treatment of inflammation. The enhancement of transdermal absorption of luciferase plasmid (pLuc) by entrapping in non-elastic vesicular formulations together with the application of the stratum corneum (SC) stripping and iontophoresis technique as well as the entrapment in elastic nanovesicles has also demonstrated. The elastic vesicles even without any application techniques can enhance the transdermal absorption of the plasmid. The pLuc entrapped in elastic niosomes gave higher fluxes than elastic liposomes, but no significance. The superior through skin delivery of pLuc by entrapping in niosomes with the application of iontophoresis can be used as a technique to deliver genetic materials via topical administration in gene therapy. However, although pLuc entrapped in elastic nanovesicles gave lesser fluxes than by iontophoresis application on the pLuc entrapped in non-elastic vesicles, elastic vesicles appeared to be a more promising approach with more practical use for topical gene delivery than the iontophoresis technique since no additional equipment is required.

Keywords: elastic vesicles, niosomes, cosmeceuticals, pharmaceuticals.

1. INTRODUCTION

The main disadvantage of transdermal drug delivery is the poor penetration of most compounds across human skin. The main barrier of the skin is located within its uppermost layer, the stratum corneum (SC). Several approaches have been developed to weaken this skin barrier. One of the approaches for increasing the skin penetration

of drugs and many cosmetic chemicals is the use of vesicular systems, such as liposomes and niosomes. Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids, assembled into bilayers. [1]. However, liposomes have problems of variable purity and high cost of phospholipids. Niosomes or non-ionic surfactant based vesicles have been studied as an alternative to liposomes. Niosomes are formed from non-ionic surfactants in aqueous media resulting in closed bilayer structures [2]. In comparing to phospholipid vesicles (liposomes) or niosomes offer higher chemical stability, lower costs, and great availability of surfactant classes [3-6]. Drug delivery systems using vesicular systems such as liposomes [7] or niosomes [8] have advantages over conventional dosage forms because the vesicles can act as drug containing reservoirs. The modification of the vesicular compositions or surface properties can adjust the drug release rate and the affinity for the target site. Conventional liposomes and niosomes are usually not efficient to transdermally delivery across the skin, because they do not deeply penetrate the skin. But, they rather remain on the upper layer of SC. Several researchers have developed novel elastic nanovesicles in order to deeply and easily penetrate through the skin [9-11]. Ethanol, bile salts and many surfactants have been used to prepare these elastic vesicles. The high flexibility of vesicular membranes permits the elastic vesicles to squeeze themselves through the pores which are much smaller than their diameters [12, 13]. Thus, elastic nanovesicles could overcome the limitation of low penetration ability of the conventional liposomes and niosomes or compounds in the commercial formulations across the skin. Elastic nanovesicles have been successfully applied both in cosmeceuticals and pharmaceuticals.

2. ELASTIC NANOVESICLES

Elastic nanovesicles are novel types of liquid-state vesicles which have been developed in the early 1990s. Elastic nanovesicles, which composed of phospholipids, ethanol and water, could better penetrate the intact skin comparing to the conventional vesicles since they can squeeze through small pores in SC which are smaller than their vesicular sizes and can also deliver the drugs or compounds at low and high molecular weight. Furthermore, they can prolong the release and demonstrate a better biological activity in comparing to the conventional nanovesicles. Elastic nanovesicles are classified into phospholipid and detergent based types.

2.1 Phospholipid-based Type

Phospholipid based elastic nanovescicles are the first generation of elastic vesicles, consisting of phospholipids and edge activators which are single chain surfactants such as cholate, Span 80 and Tween 80. Transfersome® and Ethosome are the examples of phospholipid based elastic nanovescicles.

2.1.1 Transfersome®: Phospholipid-based elastic nanovesicles are called deformable liposomes (Transfersomes®). They are the first generation of elastic nanovesicles introduced by Cevc et al. [11]. Transfersomes® composed of phospholipids as their main ingredients with 10-25% of the edge activators (such as sodium cholate). They were reported to penetrate intact skin and carry the drugs, but only when applied under non-occlusive conditions.

2.1.2 Ethosomes: They are phospholipid-based elastic nanovesicles containing high content of ethanol (20-45%). Ethanol is known as an efficient permeation enhancer and has been added in the vesicular systems to prepare the elastic nanovesicles. It can interact with the polar head group region of

the lipid molecules, resulting in the reduction of the melting point of the SC lipid, thereby increasing lipid fluidity, and cell membrane permeability. The high flexibility of vesicular membranes from the added ethanol permits the elastic vesicles to squeeze themselves through the pores which are much smaller than their diameters [9, 12]. Ethosomal systems are much more efficient in delivering substances to the skin in the terms of quantity and depth, than either conventional liposomes or hydroalcoholic solution [9]. However, ethosomes may have problems of variable purity and high cost of the phospholipids.

2.2 Detergent-based Type

Detergent-based elastic nanovescicles are the second generation of the deformable or elastic vesicles. These vesicles which mainly consist of non-ionic surfactants are self-forming bilayer vesicles in an aqueous solution and have high elasticity resulting from the solubility property of the surfactant(s). The advantages of the detergent-based elastic nanovescicles in comparing to the phospolipid-based elastic nanovescicles are low cost, ease of storage and slightly more elasticity. The first detergent-based elastic nanovescicles developed by Van den Bergh et al. [12], consist of bilayer-forming surfactant L-595 (sucrose laurate ester) and micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester). Manosroi et al. [13] have developed the novel elastic niosomes composing of non-ionic surfactant, cholesterol, ethanol and water.

3. APPLICATIONS OF ELASTIC NANOVESICLES IN PHARMACEUTICALS

3.1 A Depot Formulation for Many Pharmaceuticals

Twinkal et al. [14] have developed the topical dosage form of an anti-migraine agent, rizatriptan, which can selectively deliver the drug to carnial nerves in the brain and ear. The *in vitro* skin permeation across rat skin of rizatriptan entrapped in elastic liposomal formulations was proximately 8-19 times higher than the drug in solution. The amount of drug deposited and the biological activity of the drug in an optimized elastic liposomal formulation was 10 folds and 3 folds higher than the drug in solution, respectively. This result has indicated that elastic liposomal formulation can provide a sustained action of the drugs due to the depot effect in the deeper layer of the skin.

3.2 Improvement of Transdermal Delivery of the Drugs

Elsayed et al. [15] have demonstrated that the drugs outside, and both inside and outside the Transfersomes® showed significant improvement in cumulative amount permeated and skin deposited after 24 h over the drug in an aqueous solution. They have suggested that both the penetration enhancing effect and the intact vesicle permeation into the SC might play an important role in improving skin delivery of the drugs by Transfersomes® under the non-occlusive conditions. Transfersome® can also be applied to transcutaneous immunization which is a novel needle-free immunization method developed for the delivery of vaccines [14, 15].

Dinesh et al. [16] have investigated the transdermal potential of ethosomes bearing methotraxate (MTX), an anti-psoriatic and anti-neoplastic which is highly hydrosoluble with limited transdermal permeation. The formulation containing 3% phospholipid and 45% ethanol showed the highest entrapment, optimal nanometric size range, very low aggregation and growth in vesicular size after 120 days of storage. MTX loaded ethosomal carriers also provided an enhanced transdermal flux of $52.7 \pm 4.34\ \mu g/cm^2/h$ and decreased lag time of 0.9 h across human cadaver skin. This formulation has also demonstrated an enhanced permeation of

Rhodamine Red to the deeper layers of the skin (170 μm) and retained its penetration power after storage.

Elastic niosomes can be applied to entrap high mucosal irritation and low skin penetration drugs such as diclofenac, a wildly used anti-inflammatory drug. Manosroi et al. [13] have developed the gel containing elastic niosomes which composed of Tween61 and cholesterol at 3:7 molar ratios with 25% ethanol and entrapped with 1% diclofenac diethylammonium (DCFD). The gel containing DCFD elastic niosomes exhibited the highest amount in SC, viable dermis and epidermis (VED) and the receiving solution in comparing to Emulgel, a commercial formulation and gel containing the unentrapped DCFD and gel containing DCFD entrapped in conventional niosomes (Figure 1). It gave the DCFD amounts after 6 h in SC, VED and the receiving solution of 1069.39±28.14, 94.81±8.90 and 21.01±7.92 μg/cm^2 which were 3.14, 2.31 and 25.94 times higher than Emulgel which gave 340.18±53.52, 40.96±5.84 and 0.81±0.12 μg/cm^2, respectively (Table 1). Gel containing DCFD entrapped in elastic niosomes and conventional niosomes showed a 2.45 and 1.77 times higher fluxes in VED, respectively as compared to the gel containing the unentrapped DCFD. The DCFD entrapped in conventional niosomes was not found in the receiving solution. This result has indicated that conventional niosomes are usually not efficient to transdermally delivery across the skin, because they do not deeply penetrate the skin, but rather remain on the upper layer of SC. A synergistic mechanism has been suggested for ethanol, vesicles and the skin lipids [9]. Ethanol may provide the vesicles with soft flexible characteristics which allow them to more easily penetrate into the deeper layers of the skin. It was also proposed that phospholipid vesicles with ethanol may penetrate into the skin and influence the bilayer structure of the SC [17], leading to an enhancement of drug penetration.

Elastic niosomes can be modified to have cationic characteristics by adding cationic lipids, such as dimethyl dioctadecyl ammonium bromide (DDAB), in the vesicular compositions. Elastic cationic niosomes are useful for delivery genetic materials in gene therapy. The positive charge on the vesicular surface can interact with the negative charge of DNA or any genetic materials by electrostatic interaction. Manosroi et al. [18] have demonstrated that elastic cationic niosomes can

Table 1. The cumulative amounts (μg/cm^2) and fluxes (μg/cm^2 /h) in SC (stratum corneum), VED (viable epidermis and dermis) and receiver chamber following transdermal absorption across excised rat skin by vertical Franz diffusion cells from various gel formulations. Each value represents the mean ± SD (n = 3) [13].

Formulation	amount of DCFD (μg/cm^2)			Flux (μg/cm^2 /h)		
	SC	VED	receiver chamber	SC	VED	receiver chamber
Commercial Emulgel	340.18±53.52	40.96±5.84	0.81±0.12	60.84±13.63	7.33±1.7	0.14±0.01
Gel containing the unentrapped DCFD	333.62±31.37	38.24±4.54	0	59.67±14.32	6.84±1.46	0
Gel containing the entrapped DCFD in conventional niosomes	711.22±55.35	67.84±7.86	0	127.21±13.75	12.13±1.21	0
Gel containing the entrapped DCFD in elastic niosomes	1069.39±28.14	94.81±8.90	21.01±7.92	191.27±9.52	16.96±2.77	3.76±0.54

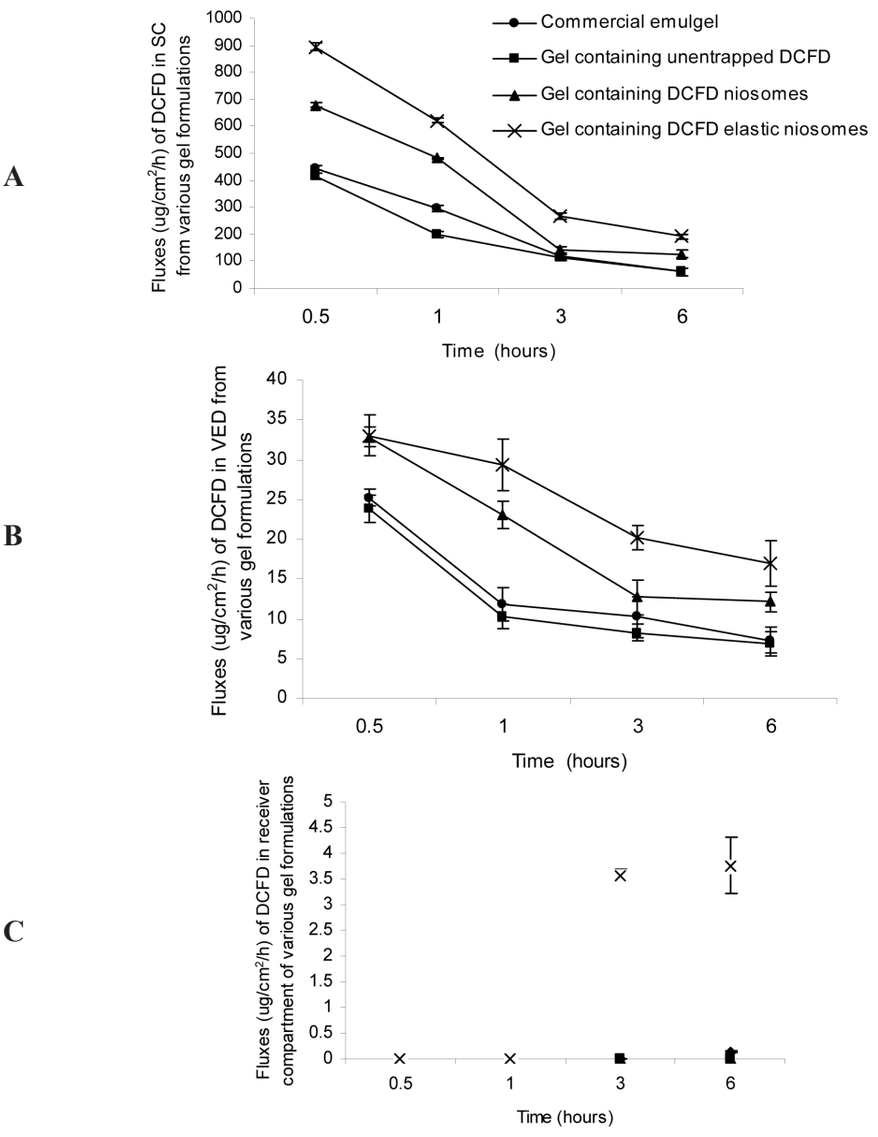

Figure 1. The fluxes (μg/cm^2 /h) of DCFD in SC (stratum corneum) (A), VED (viable epidermis and dermis) (B) and receiver chamber (C) versus times (hours) following transdermal absorption across excised rat skin by Franz diffusion cells from various gel formulations. Each value represents the mean ± SD (n = 3) [13]

entrap the luciferase plasmid at 100% entrapment efficiency with higher pLuc stability than the non-elastic cationic niosomes. For transdermal absorption, elastic niosomes exhibited the average flux of pLuc in viable epidermis and receiving solution at 6 h of 2.84±0.04 and 1.96±0.21, respectively (Table 2), whereas no pLuc was found in receiving solution for the unentrapped and entrapped pLuc in non-elastic niosomes (Figure 2). This is certainly the effects of ethanol existing in the vesicles. Various

Table 2. The cumulative amounts (μg/cm^2) and fluxes (μg/cm^2/h) of pLuc in SC (stratum corneum), VED (whole skin of viable epidermis and dermis) and receiver chamber following transdermal absorption across excised rat skin by vertical Franz diffusion cells at 6 h [18].

Methods	Cumulative amounts of pLuc (μg/cm^2)			Fluxes (μg/cm^2/h)		
	SC	VED	Receiver chamber	SC	VED	Receiver chamber
Free pLuc	0	0	0	0	0	0
Free pLuc (SC stripping)	0	0	0	0	0	0
Free pLuc (iontophoresis)	0	0	0	0	0	0
Non-elastic liposomes	0	0	0	0	0	0
Non-elastic liposomes (SC stripping)	0	4.37±0.74	0	0	2.73±0.46	0
Non-elastic liposomes (iontophoresis)	0	2.80±0.49	2.80±0.49	0	7.01±1.22	6.710.31
Non-elastic niosomes	0	0	0	0	0	0
Non-elastic niosomes (SC stripping)	0	2.70±0.52	0	0	3.83±0.73	0
Non-elastic niosomes (iontophoresis)	0	3.84±0.53	3.84±0.53	0	9.60±1.31	8.820.28
Elastic liposomes	0	3.72±0.11	2.04±0.06	0	2.79±0.09	1.92±0.10
Elastic niosomes	0	3.79±0.05	2.07±0.03	0	2.84±0.04	1.96±0.21

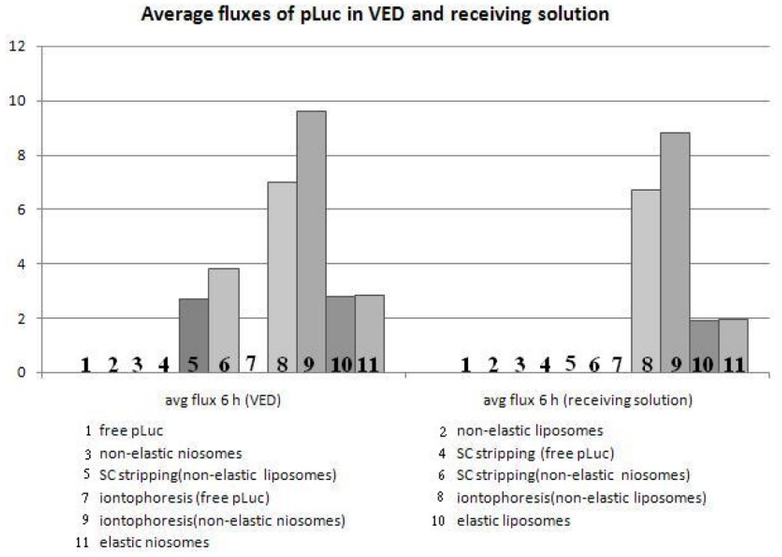

Figure 2. Average fluxes of pLuc in VED and receiving solution from various vesicular formulations at 6 h of the *in vitro* transdermal absorption through rat skin by vertical Franz diffusion cells [18].

mechanisms have been reported for the skin permeation enhancement effect of ethanol, for examples, by increasing the diffusion of the drugs through the lipid pathway of the skin [19], reduction of lipid polar head interactions or disordering liquid-crystalline phases within the membrane [20], and increasing the drug solubility in the SC [21]. Furthermore, the surfactant compositions in the niosomal formulation can also change the structure of the SC resulting from their solubilization property.

3.3 Enhancement of Biological Activities

Gupta et al. [22] have demonstrated that Transfersomes® containing soya phosphatidylcholine (SPC)/sodium deoxycholate (SDC) at 85:15% w/w showed the highest entrapment efficiency of tetanus toxoid (72.7±3.4) and deformability index (124±4.2) with optimum vesicular size (196±10.2 nm) in comparing to the conventional liposomes and niosomes. For topical immunization in albino rats, the maximum response of Transfersomes® entrapped with tetanus toxoid was observed after 42 days. After secondary immunization on day 28, Transfersomes® elicited the maximum immune response again on day 42. The response was significantly comparable to that elicited by intramuscular injection of the same dose of alum-adsorbed tetanus toxoid. In comparison to Transfersomes®, liposomes and niosomes elicited weaker immune response. Thus, Transfersomes® are promising effective non-invasive topical delivery systems for antigens.

Triterpene saponins, such as ammonium glycyrrhizinate, present an anti-inflammatory activity. The application of this compound as a potential topical anti-inflammatory drug can be further improved by using certain drug delivery systems such as ethosomes. Paolino et al. [23] have demonstrated that ethosomes composed of ethanol 45% (v/v) and lecithin 2% (w/v) elicited an increase *in vitro* percutaneous permeation of ammonium glycyrrhizinate (63.2% of the applied dose). Ethosomes showed good skin tolerability in human volunteers when applied for a long period (48 h) and are able to significantly enhance the anti-inflammatory activity and the sustained release of ammonium glycyrrhizinate in comparing to an ethanolic or aqueous solution.

Gel containing DCFD entrapped in elastic niosomes developed by Manosroi et al. [13] showed the percentages of inhibition of rat ear edema after 1 h of application higher than the commercial Emulgel, gel containing DCFD entrapped in conventional niosomes and gel containing the unentrapped drug of 16.22, 10.81 and 24.33%, respectively, but lower than phenylbutazone of 5.4% (Figure 3). This has indicated that the *in vivo*

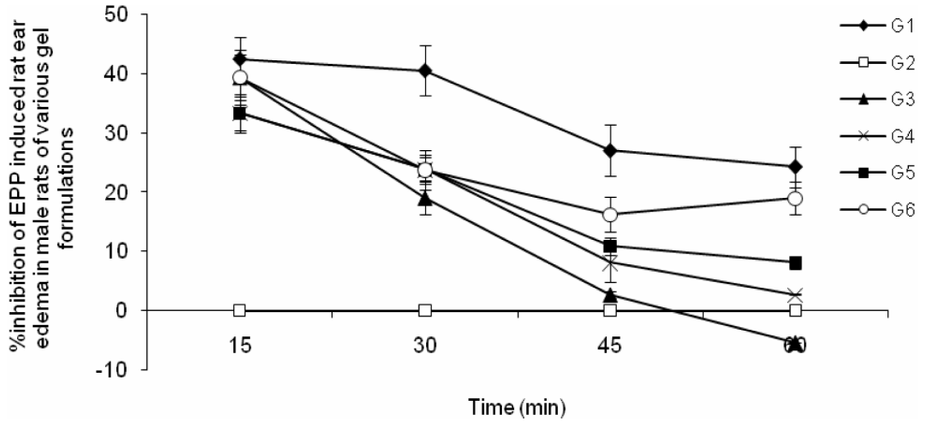

Figure 3. The plot of the %inhibition of EPP induced rat ear edema of phenylbutazone (G1), gel base (G2), gel containing the unentrapped DCFD (G3), commercial Emulgel (G4), gel containing conventional niosomal vesicles entrapped with DCFD (G5) and gel containing elastic niosomal vesicles entrapped with DCFD (G6) [13].

anti-inflammatory activity of DCFD can be enhanced when entrapped in niosomes, especially the elastic niosomes. The commercial Emulgel gave a slight higher percentage of ear edema inhibition than the gel containing the unentrapped drug, since the commercial Emulgel did not contain any bilayer vesicles, but rather may contain some transdermal absorption enhancers.

4. APPLICATIONS OF ELASTIC NANOVESICLES IN COSMECEUTICALS

The advantages of applying nanovesicles in cosmeceuticals are not only to increase the stability of the cosmetic chemicals and decrease of the skin irritation for those irritating cosmetic chemicals, but also the transdermal permeation enhancement, especially in the elastic forms. However, the compositions and sizes of the vesicles are the main factors to be considered to obtain these advantages of the elastic vesicles for cosmeceutical applications. Topical administration of many antioxidants is one of several approaches to diminish oxidative injury in the skin for cosmetic and cosmeceutical applications. But, antioxidants are usually not stable and can be degraded by exposing to light. These antioxidants include vitamin E, vitamin C, and flavonoids. Vitamin E is one of the major exogenous lipophilic antioxidants which is usually found in tissues. Its topical application can enhance the skin protection from exogenous oxidants. When vitamin E is added to cosmetic and many dermatological products, it is found to decrease the production of lipid peroxides in the epidermis as well as to protect against UV exposure [24, 25] and those destructive chemicals and physical agents [26]. In order to deliver vitamin E into the deeper layer of SC, Marina et al. [27] have formulated several deformable liposomes by using hydrogenated soya lecithin (HPC) and sodium cholate (SC), polysorbate 80 (T80), dipotassium glycyrrhizinate (DPG), or saccharose monopalmitate (SMP). The lipid to surfactant in w/w ratio which is necessary to obtain elastic vesicles depends on the O/W surfactant and ranges from 4: 1 to 20: 1. Deformability of the elastic liposomes was confirmed by filtration through the microporous filters and differential scanning calorimetry measurements. For *in vitro* permeation studies, all systems showed negligible fluxes, below the UV-HPLC detection limit (Table 3). This has suggested

Table 3. α-Tocopherol skin permeation and skin accumulation from different liposomes containing 0.17% α-tocopherol and from a control solution. (Polysorbate 80 (T80); sodium cholate (SC); hydrogenated soya lecithin (HPC); dipotassium glycyrrhizinate (DPG) and saccharose monopalmitate (SMP)) [27].

Systems	Flux	Deformability	α-Tocopherol in the skin (μg cm $^{-2}$)
HPC	Negligible	No	3.5 (0.9)
6 : 1 HPC – DPG	Negligible	No	7.6 (0.4)
2 : 1 HPC – DPG	Negligible	No	21.6 (1.3)
6.25 : 1 HPC – DPG	Negligible	No	3.8 (0.5)
6.25 : 1 HPC – SC	Negligible	Yes	27.0 (3.8)
6.25 : 1 HPC – T80	Negligible	Yes	29.2 (2.5)
6.25 : 1 HPC – SMP	Negligible	Yes	33.3 (2.9)

Values in parentheses are standard deviations.

that although elastic and non-elastic liposomes are not beneficial for delivery of α-tocopherol through the skin, the entrapment of the vitamin either in elastic or non-elastic liposomes can increase its photo-stability under UVB irradiation (Figure 4).

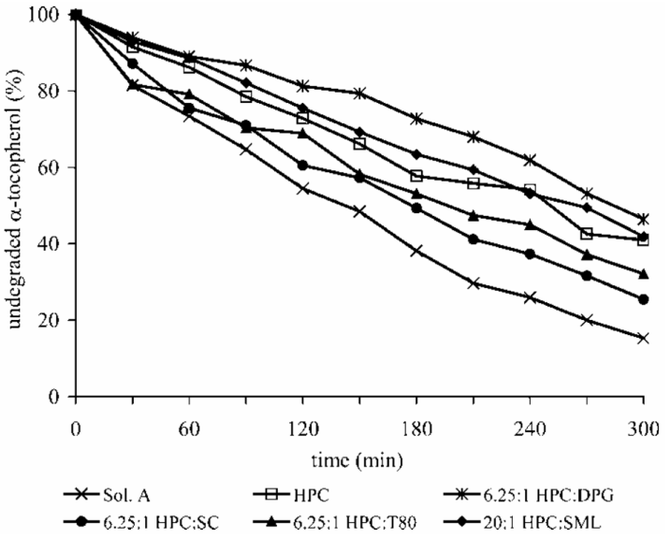

Figure 4. UVB photodegradation profiles of 0.17% w/w α-tocopherol in HPC and HPC: surfactant liposomes**.** (Polysorbate 80 (T80); sodium cholate (SC); hydrogenated soya lecithin (HPC); dipotassium glycyrrhizinate (DPG) and saccharose monopalmitate (SMP)) [27].

5. CONCLUSIONS

Nanovesicles, especially niosomes are interesting delivery systems for pharmaceuticals and cosmetics. Topically applied niosomes can increase the residence time of drugs or cosmetic chemicals in the SC and epidermis and reduce the systemic absorption of the drugs or cosmetic chemicals. However, elastic niosomes have soft and flexible vesicular characteristics. These properties allow them to penetrate easily into deeper layers of the skin and circulation. The optimized developed elastic niosomal vesicular formulations by adjusting their compositions and sizes can be promising means for not only cosmeceutical applications, but also the topical non-invasive treatment of local and systemic disorder of many pharmaceuticals as well.

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