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Niosomes as carriers for tretinoin. I. Preparation and properties

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Abstract

Tretinoin-loaded niosomes were prepared from polyoxyethylene (**4**) lauryl ether, sorbitan esters and a commercial mixture of octyl/decyl polyglucosides, in the presence of cholesterol and dicetyl phosphate. Liposomes made of hydrogenated and non-hydrogenated phosphatidylcholine were also prepared as a comparison reference. A study was made of the influence of vesicle composition and preparation method on the vesicle structure (MLV, LUV, SUV), size distribution, entrapment efficiency and in vitro release of incorporated tretinoin. Results showed that in the presence of cholesterol all the amphiphiles used were able to form stable vesicle dispersions with or without tretinoin. Vesicle sizes were dependent on the preparation method, bilayer composition and drug load. Multilamellar (MLV) vesicles were larger than extruded (LUV) and sonicated (SUV) vesicles while drug-loaded vesicles were generally smaller than empty ones. Entrapment efficiencies of tretinoin were always very high especially for multilamellar (91–99%) and extruded (88–98%) vesicles. The in vitro release of tretinoin from the prepared vesicular formulations was studied using the vertical Franz diffusion cells. The rate of drug release through a Silastic membrane from a liposomal and niosomal tretinoin dispersion was generally faster than from a tretinoin solution. Release data showed that tretinoin delivery is mainly affected by the vesicular structure and that tretinoin delivery increased from MLVs to LUVs to SUVs.

Keywords: Niosomes; Non-ionic surfactant vesicles; Liposomes; Tretinoin; In vitro release

1. Introduction

Liposomal formulations have been extensively studied to enhance the efficiency of the delivery of drugs via several routes of administration (Gregoriadis, 1976, 1988). In the last 20 years, many studies have reported that topical delivery of liposomally encapsulated drugs may offer several advantages over conventional formulations, such as decreased side effects and the control of the rate and extent of drug release into defined skin strata (Mezei, 1988, 1993; Schreier and Bouwstra, 1994). Non-ionic Surfactant Vesicles (NSVs or niosomes) have also been studied because they offer

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several advantages over liposomes: higher chemical stability, intrinsic skin penetration enhancing properties and lower costs (Handjani-Vila et al., 1979; Baillie et al., 1985; Uchegbu and Florence, 1995; Hofland et al., 1994; Van Hal et al., 1996; Uchegbu and Vyas, 1998).

Tretinoin (TRA) is effective in the topical treatment of different skin diseases such as acne vulgaris, icthyosis and psoriasis (Peck, 1984; Peinni and Vigolti, 1991; Layton and Cunlife, 1992). Unfortunately, oral use of TRA is unacceptable due to the severe side effects. Topical administration of tretinoin provides effective treatment of dermatologic disorders while decreasing systemic exposure and toxicity (Kligman et al., 1969; Layton and Cunlife, 1992). However, its topical application is limited by several drawbacks, such as skin irritation, very low water solubility and photolability (Elbaum, 1988; Lehman et al., 1988). The low solubility may limit the incorporation of TRA into a suitable vehicle, while its photolability may render the topically applied drug ineffective. In order to overcome all these disadvantages, tretinoin liposomal formulations have been studied (Foong et al., 1990; Masini et al., 1993; Imbert et al., 1994). It has been found that liposomes can decrease TRA toxicity, modify its pharmacokinetics and bioavailability (Masini et al., 1993) and improve its photostability (Thoma and Joachan, 1992; Yung and Gregoriadis, 1996; Manconi et al., 1999). Furthermore, topically applied liposomes can increase the residence time of drugs in the stratum corneum and the epidermis while reducing the systemic absorption of the drug (Masini et al., 1990; Montenegro et al., 1996). Although many authors have proposed the use of tretinoin liposomal formulations, little work has been carried out on tretinoin niosomal formulations (Wang et al., 1995; Manconi et al., 1999). As niosomes seem to be a very promising vehicle in dermal and transdermal drug delivery (Junginger et al., 1991; Ozer et al., 1991; Hofland et al., 1994), we have started to research tretinoin niosomal formulations with the aim of developing new topical application forms suitable for TRA. The incorporation of TRA in niosomes could give the same benefits reported above for liposomes. More precisely the presence of non-ionic surfactants could improve its skin penetration and increase its accumulation in the superficial skin strata. In this paper, we present our findings on the relationship between the method of preparation, the nature of the bilayer components, and size distribution, entrapment efficiency and vesicular structure. The work described is part of a larger study, which is concerned with the photostability and the skin penetration of niosomal tretinoin. Consequently, we prepared several vesicle formulations with different techniques (i.e. the film method, sonication and extrusion) and using different types of surfactants. In particular we studied the capability of non-ionic surfactants with estereal (Sorbitan esters) or ethereal linkage such as polyoxyethylene (**4**) lauryl ether (Brij® 30) to form stable tretinoin niosomal formulations. In addition to non-ionic surfactant monomers already used in pharmaceutical niosomal formulations (i.e. Span® 40 and 60, Brij® 30), we also evaluated the capability of a commercial sugar ether surfactant: octyl/decyl polyglucoside (Triton® CG 110, a hydrosoluble detergent, largely employed in the cosmetic field) to form stable TRA loaded vesicles. In order to carry out an appropriate comparison, tretinoin liposomal formulations made from hydrogenated and non-hydrogenated soya phosphatidylcholine (P90H and P90) were also prepared and characterised. All liposomal and niosomal formulations contained cholesterol while dicetyl phosphate (DCP) was also incorporated into the bilayer to prevent aggregation of the vesicles. The vesicle suspensions, whose composition is reported in Table 1, were characterised by transmission electron microscopy (TEM) for vesicle formation and morphology; dynamic laser light scattering for mean size and polidispersivity index; HPLC for incorporation efficiency; and in vitro drug release through a synthetic membrane both to check stability and as a prerequisite to the investigation of the topical application.

2. Experimental methods

2.1. Materials

Enriched soya phosphatidylcholine (Phospholi-

pon® 90, P90), and hydrogenated soya phosphatidylcholine (Phospholipon® 90H, P90H) were kindly obtained from Natterman Phospholipids, Gmb. Triton® CG 110 (TrCG110) was obtained from Sinerga (Milan, Italy). Span® 40 (Sp40), Span® 60 (Sp60), Brij® 30 (Br30) and all the other products were of analytical grade and were purchased from Aldrich. Perthèse® (thickness 0.125 mm) was purchased from G.F., Electromedics (Florence, Italy).

2.2. Vesicle preparation

Compositions of the tested samples are reported in Table 1. Multilamellar vesicles (MLVs) were prepared according to the film hydration method described by Bangham et al. (1965). The phospholipids or surfactants, cholesterol, DCP and tretinoin (4 mg/ml) were dissolved in chloroform. The organic solvent was vacuum evaporated and the resulting lipid film was dried under a stream of nitrogen for 30 min. The obtained lipid film was then hydrated under mechanical stirring with distilled water (pH 5). Large unilamellar vesicles (LUVs) were prepared by the extrusion technique. The MLV dispersion was transferred into an extrusion device Liposofast® (Avestin) and LUVs were generated by forcing the preparation (21 times for each preparation) through a polycarbonate filter of definite pore size (Nucleopore®, 400 nm). Sonicated unilamellar vesicles (SUVs) were prepared by sonication (ten times for 1 min each) of the MLV dispersion using a Soniprep 150 (MSE, Crowley) probe sonicator. In order to prepare vesicles at a temperature above the gel-liquid transition temperature (T_c) of the amphiphiles used, we worked at 80 °C (P90H) or 60 °C (sorbitan esters) or at room temperature (Triton CG 110, $T_c < 2$ °C). Final pH of the prepared formulations ranged between 5.3 and 5.8. All suspensions were prepared under yellow light and kept in the dark at all times.

2.3. Vesicle purification

The tretinoin-entrapped vesicles were separated from the unentrapped material by gel chromatography on Sephadex G50 or G75. All purified samples were diluted with distilled water in order to achieve the same tretinoin concentration (i.e. 0.2 mg/ml). The purification of Triton CG 110 niosomes was also carried out by exhaustive dialysis in order to verify if the alkyl polyglucoside monomers could be removed during this long procedure, as previously described by Kiwada et al. (1985) for alkyl glycosides shorter than C_{14}. The vesicles were transferred into a Visking tubing (36/32 S.I.C.) and dialysed against distilled water for 24 h.

Table 1
Sample composition (molar ratio)

Components	Samples					
	1	2	3	4	5	6
P90	1	–	–	–	–	–
P90H	–	1	–	–	–	–
Span 40	–	–	1	–	–	–
Span 60	–	–	–	1	–	–
Brij 30	–	–	–	–	1	–
Triton CG 110	–	–	–	–	–	1
Cholesterol	0.1	0.1	1	1	0.50	0.70
Dicetyl phosphate	0.10	0.10	0.10	0.10	0.10	0.10

2.4. Transmission electron microscopy (TEM)

The vesicle formulations were examined by transmission electron microscopy to characterise the microstructure. A drop of vesicle dispersion was applied to a carbon film-covered copper grid. Most of the dispersion was blotted from the grid with filter paper to form a thin film specimen, which was stained with 1% phosphotungstic acid. The sample was then examined and photographed with a Zeiss EM 109 transmission electron microscope at an accelerating voltage of 80 kV.

2.5. Dynamic laser light scattering (DLLS)

Liposome size distribution was determined by dynamic light scattering (Zetasizer 3, Malvern, UK) at 25 °C. Samples were scattered (633 nm) at an angle of 90°. Data were fitted by the 'inverse Laplace transformation' and CONTIN methods. Each formulation was measured three times in triplicate during a period of 15 days.

2.6. Incorporation efficiency (E%)

Incorporation efficiencies, expressed as a percentage of the total amount of TRA used initially ($E\%$), were determined by HPLC, after disruption of purified vesicles with 0.025% Triton X-100. Tretinoin content was determined at 350 nm using a HP 1050 series liquid chromatograph (Hewlett–Packard), equipped with a variable UV detector and a computer integrating apparatus. The column was a Lichrospher 100 RP-18 (Merck). The mobile phase was a mixture of acetonitrile, water and acetic acid (84.5:15:0.5, v/v), at a flow rate of 1.2 ml/min. Retinal was used as the internal standard (I.S.).

2.7. Release studies

In vitro diffusion studies of TRA in different vesicle formulations were performed through a silicone membrane (Perthèse®) using vertical Franz diffusion cells (Rofarma, Milan). The receiver compartment had a volume of 7 cm^3 and an effective diffusion area of 0.636 cm^2. The receptor compartment was filled with a hydroalcoholic solution (ethanol:water 50:50) which was constantly stirred with a small magnetic bar and thermostated at 37 °C throughout the experiments. About 1 ml of each vesicle suspension with or without (control) TRA incorporated was placed on the silicone membrane surface and then the diffusion cells were covered with aluminium foil to prevent light exposure. A methanolic solution of TRA was also studied as a reference. Samples of the receiving solution were extracted after elapsed times of 2, 4, 6, 8 and 24 h and replaced with an equivalent volume of hydroalcholic solution to ensure sink conditions. The samples were mixed with the appropriate amount of I.S. and analysed by HPLC. At the end of the experiments, samples of the donor phase were analysed and checked for TRA content and vesicle stability. TRA recovery from the donor and receptor compartment was always more than 95–96% of the applied dose. TEM and DLLS analyses did not show any significant alteration in vesicle size and morphology.

3. Results and discussion

3.1. Vesicle formation

In the presence of cholesterol, all amphiphiles used during this study were able to form stable vesicle dispersions with or without tretinoin. As niosomal vesicles strongly aggregated in the absence of a charge inducer, dicetylphosphate was added to all the formulations. During this study we also prepared niosomes made of Span 80, but the vesicular dispersions showed a very low stability and a high TRA leakage (data not shown). The vesicle-forming ability of all the studied surfactants was investigated using TEM, which allowed the visualisation of vesicle formation, their morphological evaluation and the formation of drug crystals. Fig. 1 shows a few photomicrographs of the prepared liposomes and niosomes in which clear evidence of the external multi- or single-layered membrane is shown. As may be noted (Fig. 1B), LUV structures obtained according to the experimental section are often bi- or multilamellar.

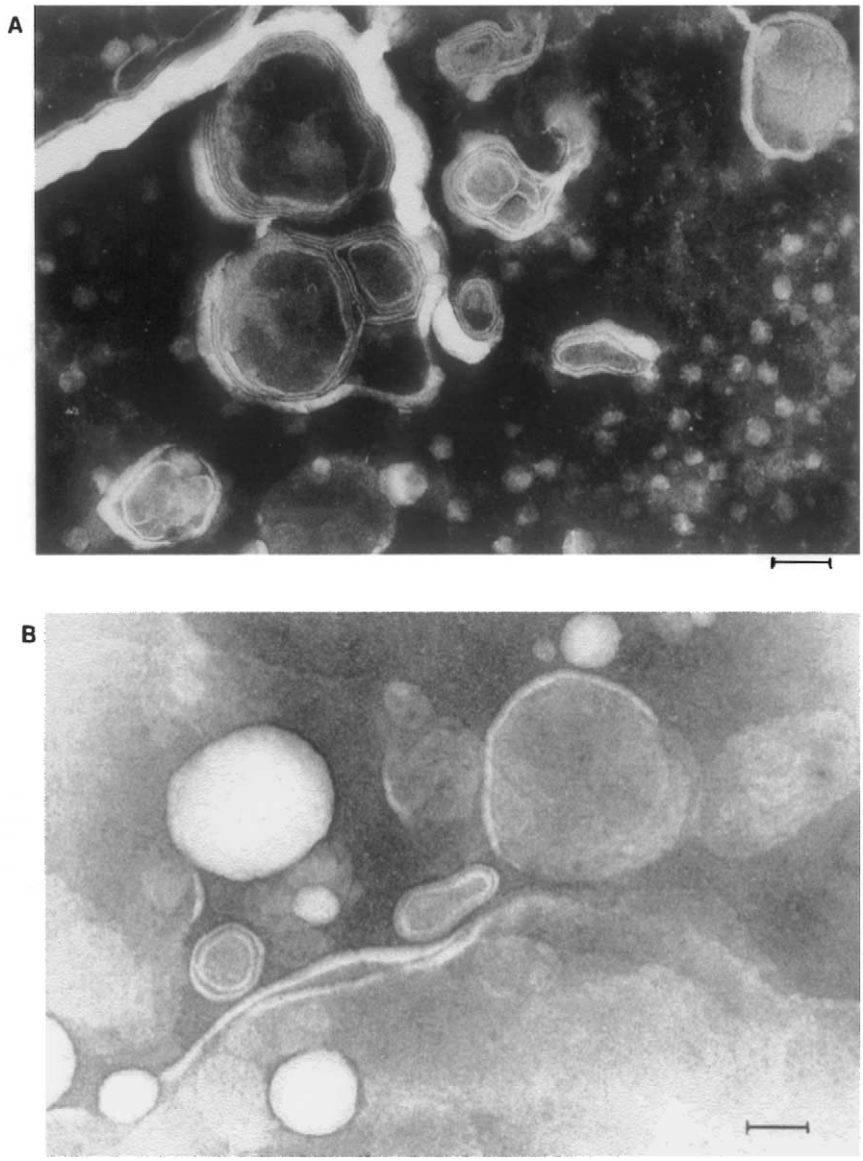

Fig. 1. Negative stain electron micrographs of tretinoin-loaded vesicles prepared from Triton® CG 110 (A, MLVs; B, LUVs; C, SUVs), P90H (D, SUVs), Span® 60 (E, LUVs) and Brij 30 (F, MLVs); bars, 200 nm.

3.2. Vesicle sizes

In order to study the influence of the loaded drug on the vesicle size, empty and drug loaded vesicles were prepared. The size of the prepared liposomes and niosomes detected by light scattering experiments are reported in Fig. 2.

Results indicate that vesicle sizes are dependent on both the method of vesicle preparation and the composition of the bilayer and drug load. In fact, vesicles prepared by the film method (MLVs) were always larger than those prepared by extrusion through policarbonate membranes (400 nm, pore size) (LUVs) or by sonication (SUVs). As known, sonication generally gives small unilamellar vesicles (SUVs, size less than 100 nm) whose size

depends on experimental conditions (i.e. time of sonication) and bilayer composition. In this study, all sonicated vesicles ranged from about 200 to 500 nm. These larger vesicles were produced using exactly the same method of preparation in order to obtain an appropriate comparison with the other formulations. We still call them SUVs because they are unilamellar and are prepared by sonication. Empty multilamellar niosomes were smaller than the corresponding empty liposomes. Hydrogenated soya phosphatidylcholine (P90H) liposomes were always larger than the non-hydrogenated phospholipidic (P90) liposomes and differences in size are evident especially in empty liposomes. On the other hand, empty niosomes did not show an important change in size related to the composition of the bilayer, especially for MLVs.

Dynamic light scattering analyses pointed out that vesicle sizes were affected by the inclusion of the drug. In fact, tretinoin-incorporated MLVs were always smaller than the corresponding

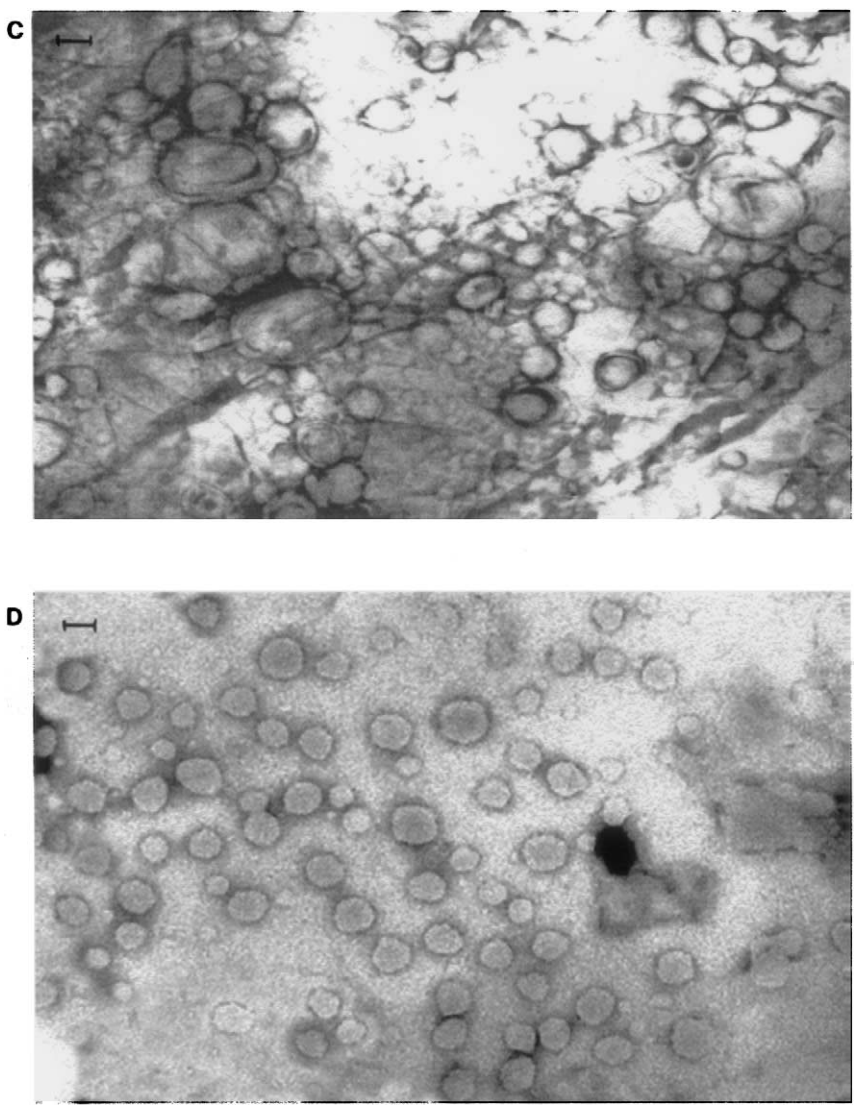

Fig. 1. (*Continued*)

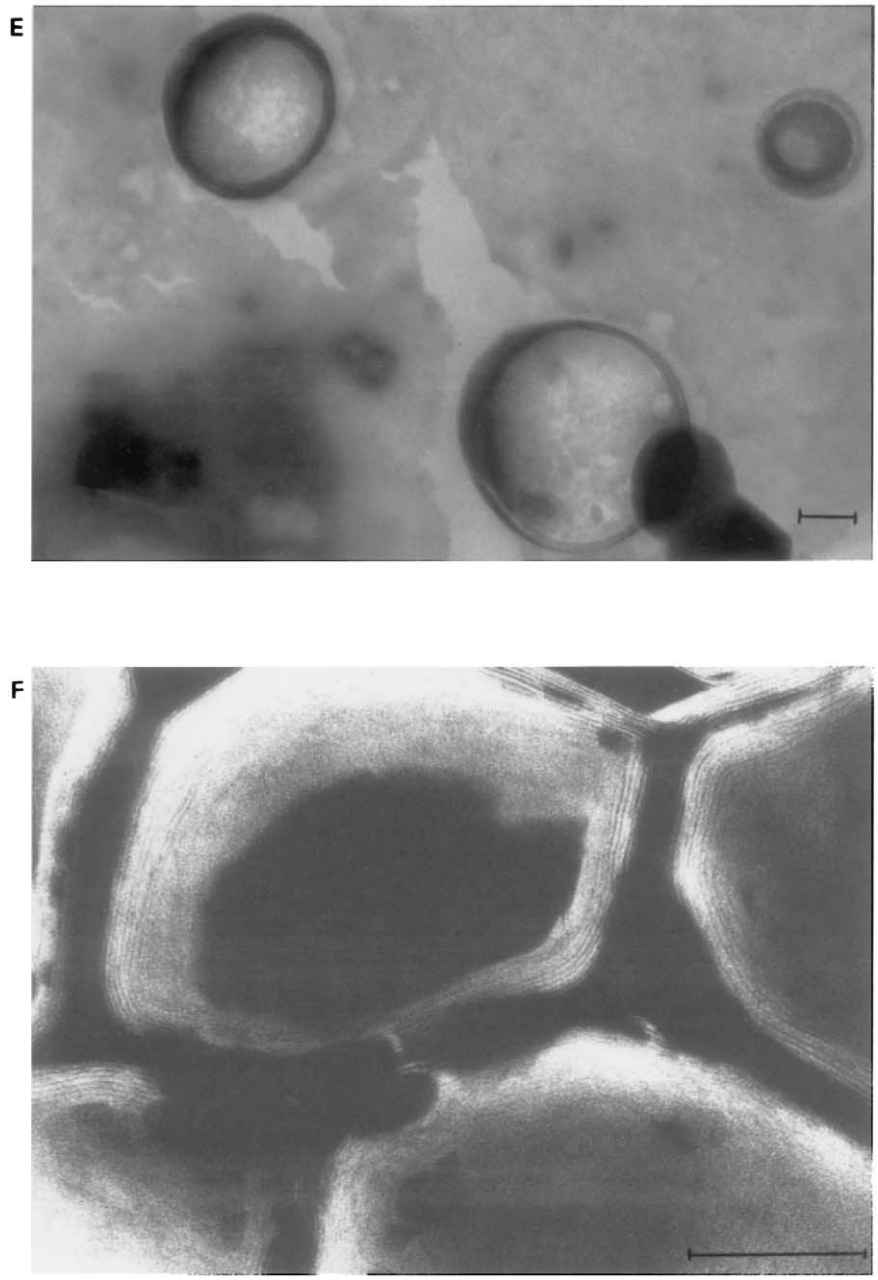

Fig. 1. (*Continued*)

empty vesicles and they showed a more irregular size distribution than LUVs and SUVs. Reductions in the size of drug-loaded vesicles are especially evident in liposomal formulations. We think that this fact may be due to the intercalation of the amphipathic drug tretinoin into the lipid bilayer, made from the double chain surfactant and cholesterol. The cohesion among the apolar portions of the membrane increases, therefore, causing a reduction in the vesicle diameter. On the

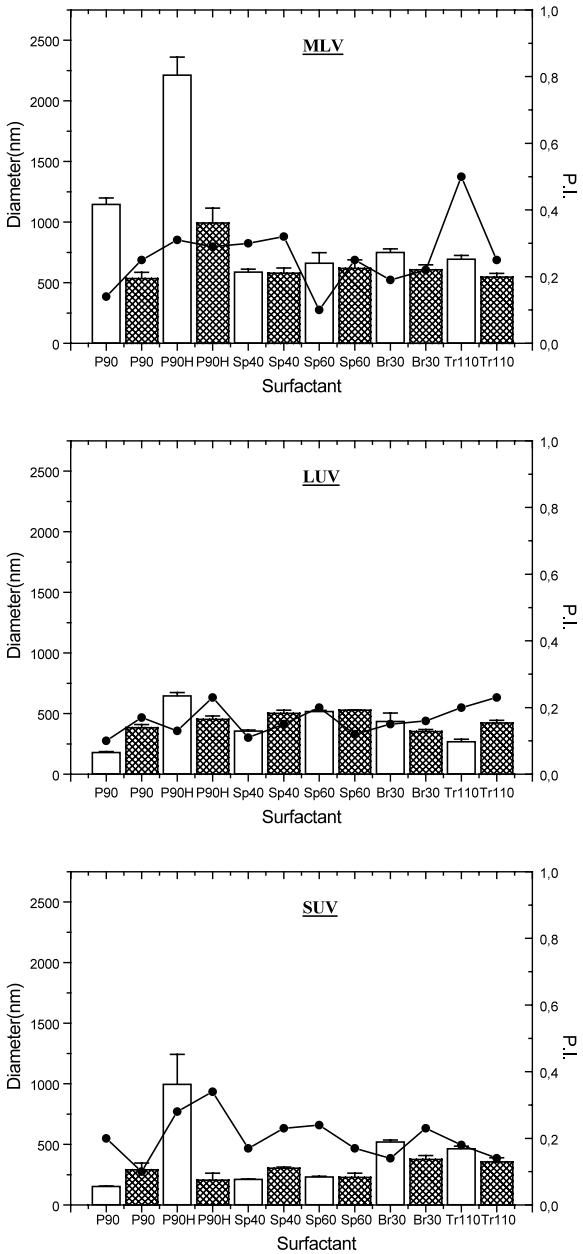

Fig. 2. The mean size and polydispersity index (P.I.) of empty and tretinoin-loaded liposome and niosome formulations, whose composition is reported in Table 1. For each formulation, the main lipid component is reported. Diameters and polydispersity indices were determined by DLLS and the data represent the mean ± S.D. ($n = 3$). (A) Multilamellar vesicles (MLVs); (B) extruded vesicles (LUVs); (C) sonicated vesicles (SUVs); solid bars, empty vesicles; hatched bars, tretinoin-loaded vesicles; (—●—) polidispersity index.

contrary, the cohesion of the membrane of niosomal vesicles is due to the combination of the single chain surfactant with a higher amount of cholesterol than in liposomes (Table 1). Indeed, as the chol/surfactant molar ratio decreases in the niosomal formulations, the reduction in size between empty and drug-loaded vesicles is more evident (Fig. 2). However, techniques used in LUV and SUV preparations yielded smaller vesicles, which seemed to increase in size in the presence of tretinoin when empty vesicles had a diameter smaller than a limit value of 200 nm.

Some interesting results were obtained for sorbitan ester niosomes. None of the TRA loaded vesicles made of sorbitan monostearate (Span 60, C_{18}) showed any appreciable difference in size with respect to the empty ones. Moreover, sorbitan monostearate vesicles were generally larger than sorbitan monopalmitate (Span 40, C_{16}) niosomes, though the difference was very low. These results are contrary to those found in the literature for unsonicated doxorubicin sorbitan monoester vesicles, where it seems that vesicle size is directly proportional to the surfactant monomer hydrophilicity (Uchegbu, 1994). In addition, the size of the TRA-incorporated sorbitan ester vesicles were smaller than those reported in the literature for hydrosoluble molecules (Yoshioka et al., 1994; Uchegbu, 1994). These results confirm the strong influence of the nature of the drug incorporated in the niosomes on vesicle sizes.

3.3. Incorporation efficiencies

TRA incorporation efficiencies of all liposomal and niosomal formulations are reported in Table 2, where it is evident that the incorporation capability of all the studied formulations is very high. Incorporation efficiencies of MLV and LUV liposomes and niosomes were in agreement with those reported in the literature for similar phospholipid vesicles (Nastruzzi et al., 1990; Montenegro et al., 1996). SUVs generally showed lower drug loading efficiency ($E\%$) than multilamellar and extrused vesicles and these results seem to be correlated to the smaller size of these aggregates.

Sorbitan monostearate (C_{18}) vesicles always showed an increased entrapment efficiency with

Table 2
Entrapment efficiency of the studied vesicle formulations

Samples	Composition	MLV E%	LUV E%	SUV E%
1	P90/Chol/DCP	97.32 ± 1.78	90.65 ± 1.78	75.98 ± 2.45
2	P90H/Chol/DCP	96.75 ± 2.22	95.00 ± 0.90	78.53 ± 2.60
3	Span 40/Chol/DCP	90.51 ± 3.63	88.54 ± 2.70	60.86 ± 2.70
4	Span 60/Chol/DCP	98.30 ± 0.65	97.35 ± 0.70	84.47 ± 3.60
5	Brij 30/Chol/DCP	95.45 ± 1.08	97.13 ± 0.78	94.00 ± 0.96
6	TrCG110/Chol/DCP	93.82 ± 1.54	93.19 ± 0.78	92.78 ± 0.98

respect to sorbitane monopalmitate (C_{16}) niosomes. These results are similar to those reported for unsonicated sorbitan monoester niosomes loaded with doxorubicin, confirming the hypothesis that E% may be correlated to the hydrophobicity of the alkyl chain of the sorbitan esters (Uchegbu and Florence, 1995). However, E% of Span 40 SUVs (60.86 ± 2.70) is much lower than that of Span 60 SUVs (84.47 ± 3.60), if compared with the differences found between MLVs and LUVs (Table 2). There are two possible explanations for this finding. First of all, while no size differences were observed among MLVs or LUVs made either from Span 60 or Span 40, Span 40 SUVs are smaller than Span 60 counterparts. The smaller vesicles are not able to entrap as much tretinoin as the larger Span 60 sonicated vesicles. Secondly, during the sonication process the vesicles can be disrupted and because of the lower affinity of tretinoin with the less hydrophobic alkyl chain (C_{16}) of Span 40, a greater amount of the drug could be lost.

Niosomes obtained from surfactant monomers with an ethereal linkage (Brij 30 and Triton CG 110) always presented a high E% which did not seem to be affected by the preparation method of vesicles, the size of which was very similar whatever the method used. Although Kiwada et al. (1985) suggested that alkyl glycosides shorter than C_{14} could not form vesicle structures and might be removed by dialysis, we observed a high E% and good stability for octyl/decyl polyglucoside vesicles. The method of vesicle purification used in this study was gel chromatography, which is faster than dialysis, and therefore, may prevent the destabilisation of our vesicles, as previously suggested for C_{12} sorbitan monoester vesicles (Uchegbu, 1994; Yoshioka et al., 1994). However, as reported in the experimental section, the purification of these vesicle dispersions was also performed by dialysis. Triton CG 110 vesicles were not found to have less stability but their incorporation efficiency was almost 100%. The amphipatic drug could play a very important role in the formation and stability of vesicle dispersions. Tretinoin, in this case, together with cholesterol may improve the ability of octyl/decyl polyglucoside to form stable vesicular aggregates because of its high hydrophobicity ($\log K_{O/W} = 6.3$) and its amphipatic structure. In fact, its molecule contains a C_9 alkyl chain which can modify the Critical Packing Parameter (CPP) and, therefore, the molecular geometry (Israelachvili, 1985) leading to very stable dispersions. All studied formulations were checked for their stability for 2 months without finding any differences in size or drug retention.

3.4. In vitro release study

The release of tretinoin from liposomes through a synthetic membrane has been studied by several authors obtaining different results (Imbert et al., 1994; Montenegro et al., 1996). These different outcomes could be due to a diverse composition and structure of the vesicles and experimental conditions (i.e. type of membrane, receiver medium, drug concentration). However, in accordance with these previous studies we have found that TRA release was an apparent first-order process. Results of the in vitro release study of TRA from the prepared niosomes and liposomes are shown in Figs. 3 and 4. The study was performed using vertical Franz diffusion cells using a mixture

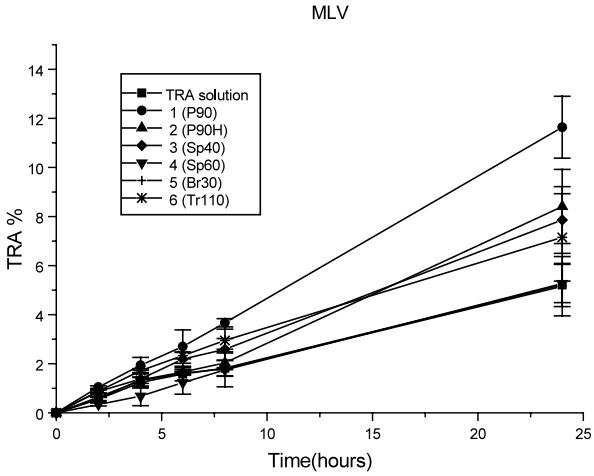

Fig. 3. The cumulative release (%) of tretinoin from a methanolic solution of free TRA and TRA-loaded multilamellar vesicular formulations 1–6. For each formulation the main lipid component is reported. The data represent the means for three replicate samples of three separate experiments.

of water–ethanol (50:50 v/v) as the receiver medium because of the very low aqueous solubility of the drug. In fact, first release experiments performed using water or phosphate buffer did not permit the quantitative determination of tretinoin. The rate of drug release through the Perthèse® membrane for the 'free' tretinoin solution was generally slower than for liposomal or niosomal tretinoin.

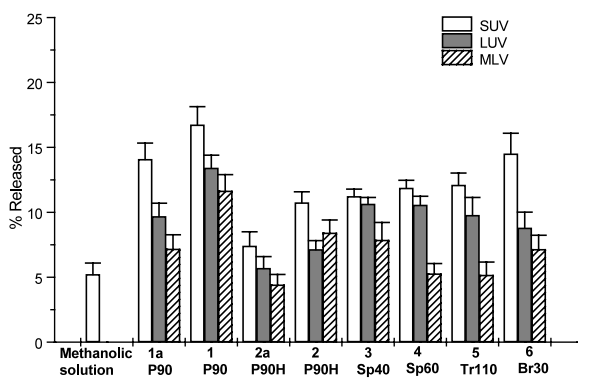

Fig. 4. Percentage of tretinoin released after 24 h through a silicon membrane from a methanolic solution of free TRA and TRA-loaded vesicular formulations 1–6. Except for the absence of DCP, formulations 1a and 2a had the same composition as samples 1 and 2. The data represent the means for three replicate samples of three separate experiments.

Obtained results seem to indicate that the diffusion through the silicone membrane is probably rate-limiting and the differences observed in the TRA release among the various systems could be due to a different thermodynamic activity of the drug in the vesicular formulations and in the hydroalcoholic solution. However, differences found in the release experiments could be correlated to the actual structure of the vesicles or to their composition.

Indeed, TRA delivery efficiency always improved from MLVs to LUVs to SUVs, as can be seen in Fig. 4. This behaviour could be a consequence of a decreasing surface area to volume ratio from SUVs to LUVs to MLVs. Moreover, SUVs possess only a single bilayer, which could have been altered more easily than those of multilamellar vesicles because of the back diffusion of alcohol in the donor compartment. In fact, it is generally accepted that ethanol is dangerous to liposomal formulations and if used in the liposome preparation, it should then be carefully removed (Riaz et al., 1988; Perrett et al., 1991). As reported in Section 2, at the end of the experiments, vesicles were assayed to check their stability and the subsequent analyses (TEM and DLLS) did not show any noteworthy modification in vesicle size and morphology. Nevertheless, the back diffusion of a small amount of ethanol could have had a fluidising effect on the bilayers, as previously reported (Harris et al., 1987).

The actual composition of the bilayer does not seem to be as important as vesicle structure, although in some cases the TRA cumulative amounts released from the different formulations after 24 h are quite different (Fig. 4).

It may be noted that P90 liposomes are capable of delivering higher amounts of TRA with respect to both P90H liposomes and niosomes. This result could be due to a higher bilayer permeability of P90 liposomes. They are made of a mixture of pure enriched soya phosphatidylcholine (90%) with a high unsaturated and polyunsaturated fatty acid content, which can increase the bilayer fluidity (Yoshioka et al., 1994). The presence of TRA, which is supposed to be intercalated into the lipid bilayer can lead to less resistant structures. This

supposition can also explain why the prepared Span 80 (Sorbitan oleate, C_9=C_9) niosomes were not stable and showed phase separation and TRA crystal formation immediately after preparation.

Except for vesicles made of P90, whose TRA release was always much faster than that obtained from the other formulations, we did not observe a strong correlation between release rate and transition temperature (T_c) of the main component of the vesicular membrane. In fact, although SUVs prepared with surfactants with a higher T_c (i.e. P90H and Spans) showed the slowest release of TRA, the same did not happen for MLVs and LUVs. In MLV formulations, the release from P90H formulations was strangely faster than that obtained from the niosomes. The more stable bilayers appeared to be those containing Triton CG 110, which seems to be in the liquid crystal form at room temperature in the commercial solution ($T_c < 2$ °C). For LUVs the highest release was obtained from Spans' vesicles, followed by the Triton niosomes. The differences observed might be due to the presence of cholesterol, which is known to give the vesicular membrane a more or less ordered structure when above or below T_c, respectively. Moreover, the presence of DCP can contribute to the change in drug release behaviour. In fact, as can be seen in Fig. 4, the in vitro release of TRA from neutral liposomes prepared using the same phospholipid/cholesterol molar ratio of formulations 1 and 2, gave a much slower drug release if compared with the negatively charged formulations.

In conclusion, results obtained from the in vitro release study showed that the delivery of the lipophilic drug tretinoin can be modulated by varying the structure and/or bilayer composition of vesicle dispersions. Vesicles made from Triton CG 110 appear to be a very suitable carrier of TRA because they showed good stability especially when prepared by the film method. Furthermore, they are made of a mixture of octyl and decyl ether of polyalkyl ($n = 1–5$) polyglucoside with a high hydrophilicity (HLB = 16), which in the presence of cholesterol and DCP was able to give stable TRA niosomal formulations. The capability of very hydrophilic surfactants to form vesicles has already been reported (Santucci et al., 1996) and Triton CG 110 could be useful in the preparation of topically applied vesicular formulations also because of its low topical toxicity and skin irritation. We are currently examining how the incorporation of TRA in lipidic vesicular formulations could affect both tretinoin photostability and in vitro skin permeation.

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References

Baillie, A.J., Florence, A.T., Hume, L.I., Muirhead, G.T., Rogerson, A., 1985. The preparation and properties of niosomes: non-ionic surfactant vesicles. J. Pharm. Pharmacol. 37, 863–868.

Bangham, A.D., Standish, M.M., Watkins, J.C., 1965. Diffusion of univalent ions across the lamellae of swollen phospholipids. J. Mol. Biol. 13, 238–252.

Elbaum, D.J., 1988. Comparison of the stability of topical isotretinoin and topical tretinoin and their efficacy in acne. J. Am. Acad. Dermatol. 19, 486–491.

Foong, W.C., Harsanyi, B.B., Mezei, M., 1990. Biodisposition and histological evalutation of topically applied retinoic acid in liposomal, cream and gel dosage forms. In: Hamin, I., Pepeu, G. (Eds.), Phospholipids. Plenum Press, New York, pp. 279–282.

Gregoriadis, G., 1976. The carrier potential of liposomes in biology and medicine. New Engl. J. Med. 295, 704–710 765–770.

Gregoriadis, G., 1988. Liposomes as Drug Carriers: Recent Trends and Progress. Wiley, New York.

Handjani-Vila, R.M., Ribier, A., Rondot, B., Vanlerberghe, G., 1979. Dispersion of lamellar phases of non-ionic lipids in cosmetic products. Int. J. Cosmet. Sci. 1, 303–314.

Harris, R.A., Burnett, R., McQuillkin, S., Mccloud, A., Simon, F.R., 1987. Effect of ethanol on membrane order: fluorescence studies. Ann. New York Acad. Sci. 492, 125–133.

Hofland, H.E.J., Van der Geest, R., Bodde, H.E., Junginger, H.E., Bouwstra, J.A., 1994. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum in vitro. Pharm. Res. 11, 659–664.

Imbert, D., Kasting, G.B., Wickett, R.R., 1994. Influence of liposomal encapsulation on the penetration of retinoic acid through human skin in vitro. J. Soc. Cosmet. Chem. 45, 119–134.

Israelachvili, J.N., 1985. Intermolecular and Surface Forces. Academic Press, Sydney.

Junginger, H.E., Hofland, H.E.J., Bouwstra, J.A., 1991. Liposomes and niosomes: interactions with human skin. Cosmet. Toilet. 106, 45–50.

Kiwada, H., Nimura, H., Fujisaki, Y., Yamada, S., Kato, Y., 1985. Application of syntetic alkyl glycoside vesicles as drug carriers. I. Preparation and physical properties. Chem. Pharm. Bull. 33, 753–759.

Kligman, A.M., Fulton, J.E., Plewig, G., 1969. Topical vitamin A acid in acne vulgaris. Arch. Dermatol. 99, 469–476.

Layton, A.M., Cunlife, W.J., 1992. Guidelines for optimal use of isotretinoin in acne. J. Am. Acad. Dermatol. 27, S2–S7.

Lehman, P.A., Slattery, J.T., Franz, T.J., 1988. Percutaneous absorption of retinoids: influence of vehicle, light exposure and dose. J. Invest. Dermatol. 91, 56–61.

Manconi, M., Baroli, B., Sinico, C., Valenti, D., Fadda, A.M., 1999. Liposomes and niosomes for the photoprotection of tretinoin. Proc. Int. Symp. Control. Rel. Bioact. Mater. 26, 477–478.

Masini, V., Bonté, F., Meybeck, A., Wepierre, J., 1990. In vitro percutaneous absorption and in vivo distribution of retinoic acid in liposomes and in a gel on hairless rats. Proc. Int. Symp. Control. Rel. Bioact. Mater. 17, 425–426.

Masini, V., Bonté, F., Meybeck, A., Wepierre, J., 1993. Cutaneous bioavailability in hairless rats of tretinoin in liposomes or gel. J. Pharm. Sci. 82, 17–21.

Mezei, M., 1988. Liposomes in topical application of drugs. In: Gregoriadis, G. (Ed.), Liposomes as Drug Carriers: Recent Trends and Progress. Wiley, New York, pp. 663–677.

Mezei, M., 1993. Liposomes as penetration promoters and localizers of topically applied drugs. In: Hsieh, D.S. (Ed.), Drug Permeation Enhancement. Dekker, New York, pp. 171–197.

Montenegro, L., Panico, A.M., Ventimiglia, A., Bonina, F.P., 1996. In vitro retinoic acid release and skin permeation from different liposome formulations. Int. J. Pharm. 133, 89–96.

Nastruzzi, C., Walde, P., Menegatti, E., Gambari, R., 1990. Liposome-associated retinoic acid. Anticancer Res. 259, 293–296.

Ozer, A.Y., Hincal, A.A., Bouwstra, J.A., 1991. A novel drug delivery system: non-ionic surfactant vesicles. Eur. J. Pharm. Biopharm. 37, 75–79.

Peck, G., 1984. Synthetic retinoids in dermatology. In: Sporn, M.B., Roberts, A.B., Goodman, D.S. (Eds.), The Retinoids, vol. 2. Academic press, Orlando, pp. 391–441.

Peinni, C., Vigolti, M., 1991. Drug and cosmetics in relation to the topical treatment of acne: data from a nation wide enquiry. Cosmet. Dermatol. 2, 17–26.

Perrett, S., Golding, M., Williams, P., 1991. A simple method for the preparation of liposomes for pharmaceutical applications: characterization of the liposomes. J. Pharm. Pharmacol. 43, 154–161.

Riaz, M., Weiner, N., Martin, F., 1988. Disperse systems. In: Lieberman, H.A., Rieger, M.M., Banker, G.S. (Eds.), Pharmaceutical Dosage Forms, vol. 2. Marcel Dekker Inc, New York and Basel, pp. 567–600.

Santucci, E., Carafa, M., Coviello, T., Murtas, E., Riccieri, F.M., Alhaique, F., Modesti, A., Modica, A., 1996. Vesicles from polysorbate 20 and cholesterol. A simple preparation and characterisation. STP Pharm. Sci. 6, 29–32.

Schreier, H., Bouwstra, J.A., 1994. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. J. Control. Rel. 30, 1–15.

Thoma, K., Joachan, U.E., 1992. Liposome dermatics: assessment of long-term stability. Liposome Dermatics, Griesbach Conf., Braun-Falco, O., Korting, H.C., Maibach, H.I. (Eds.), Springer, Berlin, Germany, 150–156.

Uchegbu, I.F., 1994. Some aspects of the niosomal delivery of doxorubicin. Ph.D. Thesis, University of London, England.

Uchegbu, I.F., Florence, A.T., 1995. Non-ionic surfactant vesicles (niosomes): physical and pharmaceutical chemistry. Adv. Coll. Interf. Sci. 58, 1–55.

Uchegbu, I.F., Vyas, S.P., 1998. Non-ionic surfactant based vesicles (niosomes) in drug delivery. Int. J. Pharm. 172, 33–70.

Van Hal, D.A., Bouwstra, J.A., Van Rensen, A., Jeremiasse, E., De Vringer, T., Junginger, H.E., 1996. Preparation and characterization of nonionic surfactant vesicles. J. Coll. Interf. Sci. 178, 263–273.

Wang, J.C.T., Yusuf, M., Liu, J., 1995. Skin care composition containing retinoids and liposomes. US Patent US 415975, 3 April.

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.

Yung, A.M., Gregoriadis, G., 1996. Photolysis of retinol in liposome and its protection with tocopherol and oxybenzone. Photochem. Photobiol. 63, 344–352.