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Niosomes as carriers for tretinoin
II. Influence of vesicular incorporation on tretinoin photostability

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Abstract

In this work, we compared the chemical stability of tretinoin (TRA) in methanol and in vesicular suspensions exposed both to UV and artificial daylight conditions with the aim of evaluating the potential of niosomes as topical carriers capable of improving the stability of photosensitive drugs. Tretinoin-loaded niosomes were prepared from polyoxyethylene (4) lauryl ether (Brij® 30), sorbitan esters (Span® 40 and Span® 60) and a commercial mixture of octyl/decyl polyglucosides (Triton® CG110). Liposomes made from hydrogenated (P90H) and non-hydrogenated (P90) soy phosphatidylcholines were also prepared and studied. In order to evaluate the influence of vesicle structure on the photostability of tretinoin, TRA-loaded vesicles were prepared by the film hydration method, extrusion technique and sonication. After UV irradiation, TRA dissolved in methanol degraded very quickly while the incorporation in vesicles always led to a reduction of the photodegradation process. The photoprotection offered by vesicles varied depending on the vesicle structure and composition. After fluorescent light irradiation for 21 days, not all the studied vesicular formulations improved TRA stability when compared with the free drug in methanol. Tretinoin incorporated in P90 or Span vesicles presented a half-life shorter or very close to that of the free drug. However, the inclusion of TRA in P90H liposomes and Brij® 30 or Triton® CG110 niosomes retarded the drug photodegradation.

Keywords: Tretinoin; Niosomes; Non-ionic surfactant vesicles; Liposomes; Photostability

1. Introduction

Tretinoin or all-*trans* retinoic acid (TRA), a metabolite of Vitamin A, has been the subject of growing interest during recent years because of its ability to regulate epithelial cell growth and differentiation, sebum production and collagen synthesis. These qualities have led to its use in the treatment of various dermatological disorders, such as acne, psoriasis, photodamage and skin cancer (Peinni and Vigolti, 1991; Orfanos et al., 1997).

Due to the severe side effects when systematically administered, TRA is almost exclusively topically employed, resulting in little if any tretinoin being absorbed systemically (Lucek and Colburn, 1985; Layton and Cunlife, 1992). However, even its topical use is limited by several drawbacks, such as irritation of the treated area, very low solubility and high instability in the presence of air, light and heat (Elbaum, 1988; Lehman et al., 1988). In fact, tretinoin is sensitive to light, readily oxidised and easily isomerised. The photostability of tretinoin in the solid state, in

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solution and in several delivery systems has been investigated by several authors. Motto et al. (1989) have shown that the irradiation of a TRA solution leads to the formation of nine different isomers, the most abundant of which is isotretinoin, which is less active than the TRA. Oyler et al. (1989) characterised the major autoxidation products of TRA under gold fluorescent light. Tan et al. (1992, 1993) determined the degradation of *cis*- and *trans*-retinoic acid both in the solid state and in solution, under light protection conditions at different temperatures. In their work, a new method was also developed to calculate the shelf-lives and half-lives of TRA and isotretinoin. Mehta and Calvert (1987) studied the stability of retinoic acid eye-drops under different temperatures and light conditions over an 8-week period: samples unprotected from light showed immediate deterioration. Brisaert et al. (1995) investigated the chemical stability of tretinoin in different dermatological preparations, under different temperature and light conditions. In a recent article, the same authors found that the photodegradation of a TRA lotion placed in front of a xenon lamp is very fast and neither inclusion in β-cyclodextrin or solubilisation in micelles affected its stability (Brisaert and Plaizier-Vercammen, 2000). Lin et al. (2000) reported that the inclusion of TRA into 2-hydroxypropyl-β-cyclodextrin did improve the drug photostability.

Over the past 20 years, it has been suggested that liposomes and niosomes provide topical delivery benefits for both systemically and locally active drugs. Vesicular structures have also been proposed as systems capable of improving the stability of photosensitive drugs, although contradictory results are often reported. In fact, while riboflavin (Habib and Asker, 1991) and doxorubicin (Uchegbu and Florence, 1995) were found to be more stable in the liposomes and niosomes, respectively, than in the solution, liposomal retinol showed an increased decomposition rate than the free drug in methanol (Young and Gregoriadis, 1996). It was also reported that the tretinoin had a higher stability in liposomes than in the methanol (Thoma and Joachan, 1992) or in castor oil (Brisaert et al., 2001).

In a previous paper, we reported that tretinoin can be incorporated in high yields in niosome structures giving rise to very stable formulations from which the in vitro drug release can be modulated by varying the structure and/or bilayer composition (Manconi et al., 2002). In the present work, we have studied the degradation of TRA in methanol and in vesicular suspensions exposed both to UV and artificial daylight conditions. This research focused on how the association of TRA to vesicular bilayers can affect its stability in order to evaluate the influence of vesicle structure (MLV, LUV, SUV) and bilayer composition on the photostability of tretinoin. Hence, we have studied and compared the photodegradation of TRA in methanol and in vesicular dispersions, each with different vesicle structures and membrane compositions. As reported in the previous paper, TRA-loaded niosomes were prepared from polyoxyethylene (4) lauryl ether (Brij® 30), sorbitan esters (Span® 40 and Span® 60) and a commercial mixture of octyl/decyl polyglucosides (Triton® CG110), in the presence of cholesterol (Chol) and dicetyl phosphate (DCP). Liposomes made of hydrogenated and non-hydrogenated phosphatidylcholines were also prepared and studied.

The aim of this study was to evaluate the potential of niosomal formulations as topical delivery systems capable of improving the stability of photosensitive drugs, such as tretinoin. Although topical formulations are protected during storage by suitable packaging, photosensitive drugs can undergo decomposition even after their administration. Tretinoin degradation was reported to occur within 1–2 h after application (Elbaum, 1988). Light-sensitive drugs can be affected either by sunlight or artificial light sources (i.e. fluorescent light). In order to evaluate the total photosensitivity of TRA for the development of vesicular formulations as topical drug carriers, the photostability testing was carried out both in forced degradation conditions and under direct fluorescent light exposition as a confirmatory test. In the first case, the study was performed by irradiating TRA-loaded vesicular formulations with UV light set at 366 nm. As a reference the degradation of free tretinoin dissolved in methanol was studied.

2. Experimental methods

2.1. Materials

Enriched soy phosphatidylcholine (Phospholipon® 90, P90) and hydrogenated soy phosphatidylcholine

Table 1
Sample composition (molar ratio)

Component	Sample					
	1	2	3	4	5	6
P90	1	–	–	–	–	–
P90H	–	1	–	–	–	–
Span® 40	–	–	1	–	–	–
Span® 60	–	–	–	1	–	–
Brij® 30	–	–	–	–	1	–
TrCG110	–	–	–	–	–	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

(Phospholipon® 90H, P90H) were kindly obtained from Natterman Phospholipids, Gmb. Triton® CG110 (TrCG110) was obtained from Sinerga (Milan, Italy). Cholesterol, DCP, polyoxyethylene (4) lauryl ether (Brij® 30) and sorbitan esters (Span® 40 and Span® 60) were of analytical grade and were purchased from Aldrich.

2.2. Methods

2.2.1. Vesicle preparation

Table 1 lists the composition of the formulations tested in this study. Multilamellar vesicles (MLVs) were prepared according to the film hydration method as previously reported (Manconi et al., 2002). 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 nitrogen stream for 30 min. The obtained lipid film was then hydrated under mechanical stirring with distilled water (pH 5). The final pH of the prepared formulations ranged between 5.3 and 5.8. Large unilamellar vesicles (LUVs) were prepared by the extrusion technique. The MLV dispersion was transferred into a Liposofast® (Avestin) extrusion device 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 (10 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® CG110, $T_c < 2$ °C). All suspensions were prepared under yellow light and then stored in the dark at all times.

Vesicle dispersions were purified by gel chromatography on a Sephadex G50 or G75 column. All formulations were diluted with distilled water in order to achieve the same TRA concentration (i.e. 0.2 or 0.4 mg/ml).

2.2.2. Vesicle characterisation

Vesicle dispersions were characterised by transmission electron microscopy (TEM) for vesicle formation and morphology; dynamic laser light scattering (DLLS) for mean size and polydispersivity index; HPLC for incorporation efficiency as previously reported (Manconi et al., 2002).

2.2.3. HPLC method

Tretinoin and isotretinoin were determined at 350 nm using an HP series liquid chromatograph (Hewlett-Packard), equipped with a variable UV detector and a computer integrating apparatus. The column was a Lichrospher 100 RP-18 (5 μm, 250 mm × 4 mm, Merck). The mobile phase was a mixture of acetonitrile, water and acetic acid (90:9.68:0.32 v/v/v), at a flow rate of 1.2 ml/min. Retinal was used as the internal standard (I.S.). The quantitative determination of tretinoin in the tested samples was obtained from the calibration curve, which gave good linearity ($R^2 > 0.999$; $n = 5$) and reproducibility.

2.3. Photodegradation studies

2.3.1. UV irradiation

The degradation of tretinoin was studied using a UV lamp set at 366 nm (Min UVIS, Desaga, GmbH, Germany). The TRA methanolic solution or vesicle suspension (2 ml in a glass flask) was maintained at room temperature and exposed to UV radiation from a 30 W lamp (366 nm) for 1 h at a fixed distance of 10 cm. At regular time intervals, samples were first stirred and then 100 μl of the dispersion was removed and diluted with methanol in order to quantify the TRA concentration. Experiments were carried out on samples with different initial TRA concentrations (i.e. 0.4 and 0.2 mg/ml). Samples were taken every 10 min for 1 h. For the formulations with

an initial TRA concentration of 0.2 mg/ml, photolysis experiments were also carried out in which the irradiation was extended to 8 h and samples were taken every 30 min. In order to measure TRA half-life experimentally, experiments were carried out in which TRA concentration was measured by taking samples every 5 min when the left tretinoin approached to 50% (i.e. TRA concentration between 60 and 40%). All experiments were carried out at 25 ± 1 °C.

2.3.2. Fluorescent light radiation

Stability testing in which the formulations were exposed to artificial light was also carried out. Confirmatory tests were carried out maintaining samples (2 ml in a glass flask) at room temperature and irradiating them with fluorescent light for 21 days. Six new and continuously illuminated fluorescent tubes (58 W) were used. The overall light source intensity was 460 lx. At regular time intervals (every 24 h), samples were stirred and then 100 μl of the dispersions were removed and diluted with methanol in order to quantify the TRA concentration.

2.3.3. Statistics

Data were analysed statistically by the one-way ANOVA test and by the Student's *t*-test ($P < 0.05$).

3. Results and discussion

3.1. Vesicle characterisation

Table 1 lists the niosomal and liposomal formulations that were used in the study. A thorough characterisation of these vesicle suspensions is described in another paper (Manconi et al., 2002), The average size of final MLV, LUV and SUV formulations is reported in Table 2.

3.2. HPLC analyses

Immediately after preparation and then at fixed time intervals, the tretinoin concentration was measured in the methanolic solution and the vesicular dispersions, using the HPLC method described in Section 2. This method gave a good separation of tretinoin and isotretinoin, its major isomer. The analytical method also allowed the separation of other isomers whose qualitative identification was not included in the aim of this work (Fig. 1).

Table 2
Average size of liposomal and niosomal vesicles as determined by PCS analyses

Formulation	Main component	Mean average size		
		MLV	LUV	SUV
1	P90	536 ± 49	385 ± 25	293 ± 53
2	P90H	993 ± 122	454 ± 27	135 ± 56
3	Span® 40	580 ± 42	504 ± 24	284 ± 9
4	Span® 60	620 ± 68	529 ± 1	230 ± 32
5	Brij® 30	608 ± 39	355 ± 14	277 ± 30
6	TrCG110	548 ± 29	425 ± 20	317 ± 32

3.3. Degradation of tretinoin under UV irradiation

The HPLC analyses showed that the photodegradation of TRA in methanol is very fast and after only 10 min of UV exposure several isomers are formed with isotretinoin being the most important (Fig. 1a). After 1 h of UV light irradiation, only about 40% of the initial concentration was still present. When the photodegradation study was carried out by irradiating the TRA solution for 8 h, the reaction reached a photostationary state after 300–330 min of irradiation. The TRA left intact was 10% and it remained constant after 8 h of study.

In previous literature, the photodegradation of TRA in methanol was reported to follow a first order kinetics (Tan et al., 1993; Brisaert and Plaizier-Vercammen, 2000). However, in this study a linear correlation between the TRA concentration and time was observed during the first hour of irradiation. The photodegradation of SUV-incorporated TRA (0.2 mg/ml) after 1 h of UV irradiation is reported in Fig. 2a. As shown, the plotting of intact TRA concentration as a function of time gave good linearity. The same linearity was obtained studying the photodegradation of the drug in similar formulations containing 0.4 mg/ml tretinoin (Fig. 2b). In Table 3, rate constants (K) and half-life ($t_{1/2}$) for free and vesicle-incorporated tretinoin are reported. As can be seen, half-life was proportional to the initial TRA concentration and the more concentrated formulations showed greater stability. Hence, the degradation behaviour throughout the experimental range can be treated as a zero order model.

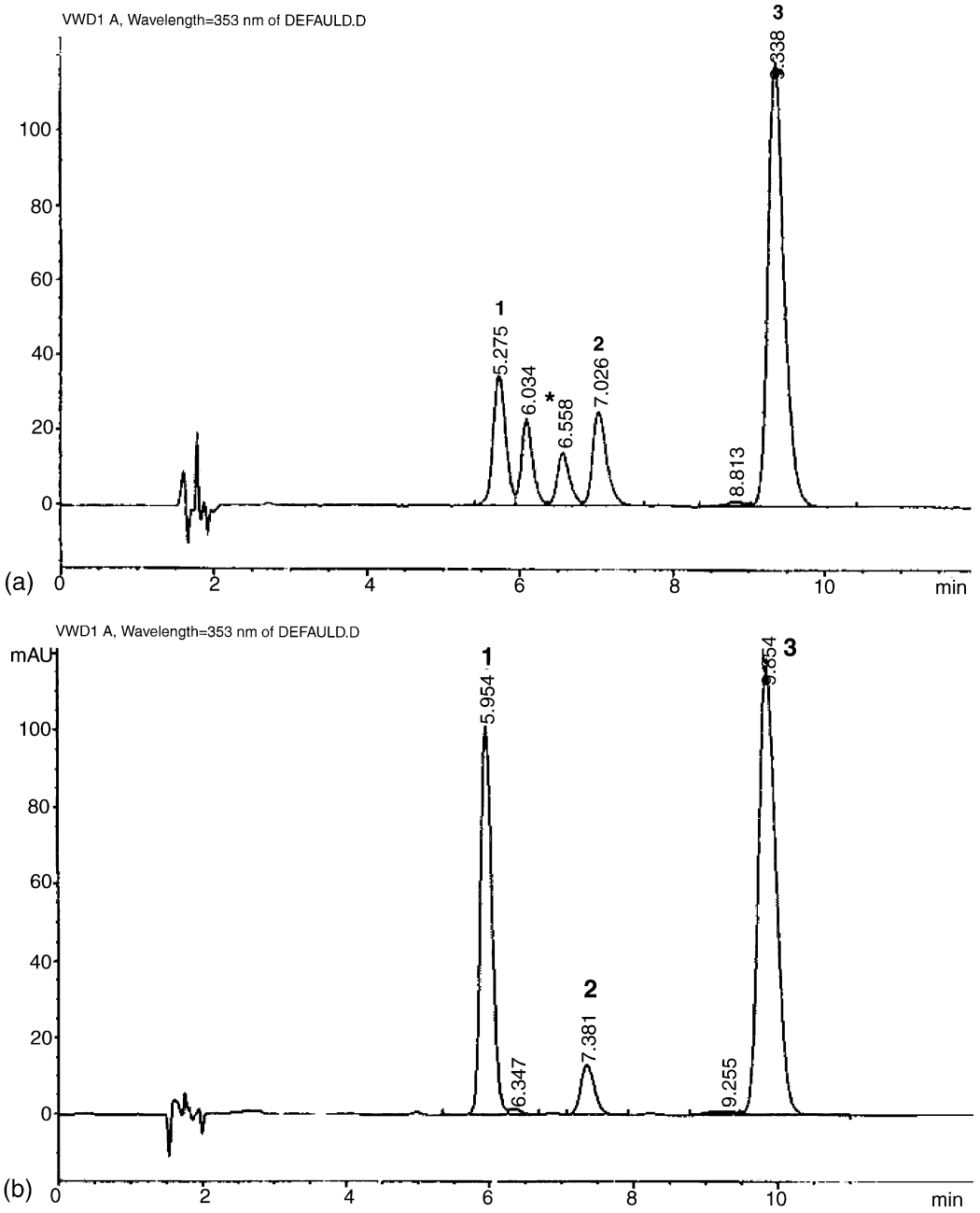

Fig. 1. Chromatograms of tretinoin in methanol (a) and in Span® 40 SUVs (b) after 8 h of UV irradiation. 1: tretinoin; 2: isotretinoin; 3: retinal (I.S.); (∗) tretinoin isomers.

Data in Table 3 show that the incorporation of TRA in vesicles always led to a reduction of the photodegradation process of this drug and the photoprotection offered by the vesicles varied as a function of vesicle structure and composition. However, the half-lives of vesicle-incorporated TRA were all greater than 1 h. Therefore, we decided to experimentally measure tretinoin half-life. The experimental determination of

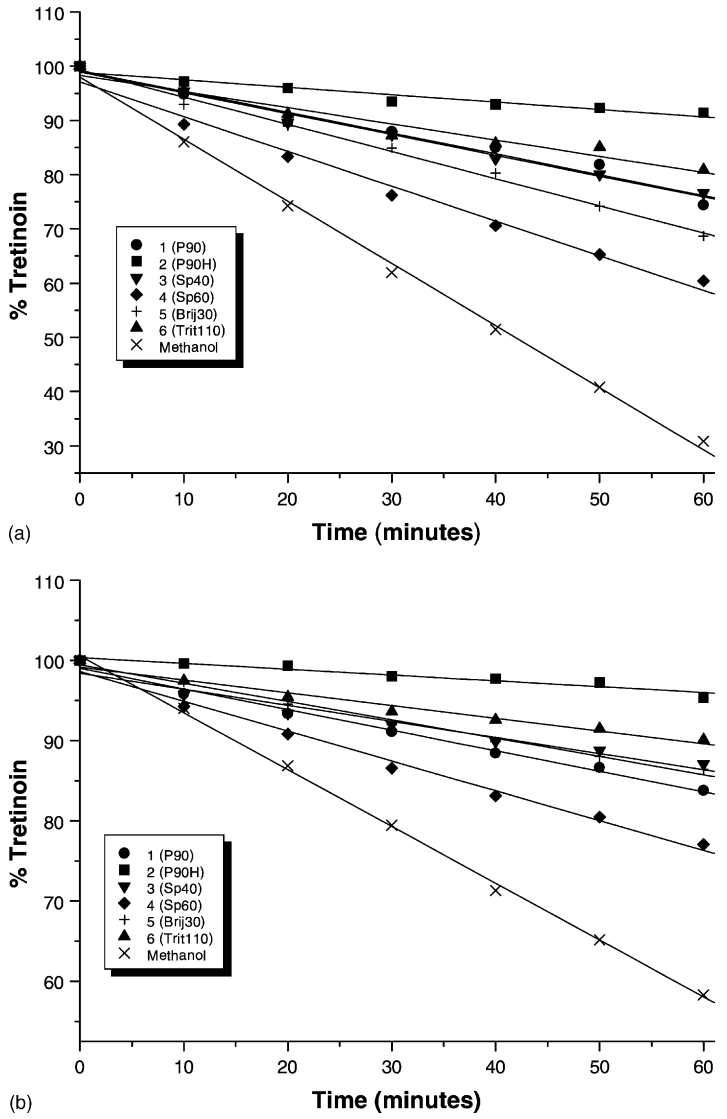

Fig. 2. Photodegradation of tretinoin in methanol and in SUV formulations after 1 h of UV irradiation ($\lambda = 366$ nm). (a) Samples with 0.2 mg/ml initial TRA concentration; (b) samples with 0.4 mg/ml initial TRA concentration. For each formulation the main lipid component is reported.

TRA concentration at the time corresponding to the calculated TRA half-life, shown in Table 3, led us to study the photodegradation of tretinoin for further 7 h. Measured and calculated data are listed in Table 4. As can be seen, the half-life values experimentally determined are similar to those calculated after 8 h of UV light exposure. Results obtained during these 8-h UV radiation experiments clearly indicate that the photodegradation rate of vesicle-incorporated TRA is different in the first hour of irradiation. In particular, the degradation rate is higher during the first hour of the experiments, while it decreases in the next 7 h leading to strong differences in the slope of the curves obtained in the two different sets of experiments. Constant rate and half-life in Table 5 are obtained by treating the data from 1 to 8 h according

Table 3
Photodegradation rate constants (K_0) and half-lives ($t_{1/2}$) of free and vesicle-incorporated tretinoin exposed to UV light for 1 h

Sample	Vesicle structure	Main component	TRA (0.2 mg/ml)		TRA (0.4 mg/ml)	
			$-K_0$[a]	$t_{1/2}$[b] (h)	$-K_0$[a]	$t_{1/2}$[b] (h)
Methanol	–	–	69.07	0.70	42.51	1.19
1	MLV	P90	30.99	1.59	16.31	3.08
2	MLV	P90H	6.91	7.17	3.50	14.31
3	MLV	Span® 40	30.35	1.50	19.78	3.08
4	MLV	Span® 60	42.60	1.15	21.48	2.15
5	MLV	Brij® 30	20.81	2.13	12.10	4.14
6	MLV	TrCG110	23.95	1.88	13.70	3.65
1	LUV	P90	18.62	2.58	9.52	5.23
2	LUV	P90H	12.98	3.90	6.87	7.31
3	LUV	Span® 40	29.01	1.61	15.88	3.17
4	LUV	Span® 60	45.19	1.05	24.62	2.01
5	LUV	Brij® 30	20.67	2.24	11.09	4.43
6	LUV	TrCG110	19.40	2.42	9.96	4.74
1	SUV	P90	25.92	1.75	13.03	3.81
2	SUV	P90H	7.53	6.42	4.01	12.50
3	SUV	Span® 40	21.31	2.23	10.99	4.37
4	SUV	Span® 60	35.27	1.27	20.48	2.51
5	SUV	Brij® 30	27.53	1.72	12.66	3.84
6	SUV	TrCG110	16.88	2.85	8.79	5.63

[a] Photodegradation rate constants (K_0) were determined from HPLC analyses of TRA and were calculated by means of the following equation: $K_0 = (y - b)/t$, where y = % TRA concentration; $b = 100 \pm 2.1$.
[b] $t_{1/2}$ calculated according to the regression line determined by considering the first hour of the experiments.

to a zero order process: the half-life calculated in this way matches quite well with the experimental $t_{1/2}$ (compare with Table 4).

Tretinoin stability is a rather complex issue in which several degradation processes are involved. Differences in $t_{1/2}$ values of SUVs (0.2 mg/ml) obtained from 1- to 8-h UV irradiation seem to point out that the $t_{1/2}$ values depend on the duration of the experiments. This hypothesis is supported by the fact that several authors have studied the TRA degradation process, but different results have often been reported as a consequence of different experimental conditions, starting TRA concentration and duration of the experiments (ranging from 10 min to several days). Here,

Table 4
Half-life values measured and calculated for tretinoin (0.2 mg/ml) in methanolic solution and SUV formulations after 8-h UV irradiation

Formulation	Composition	$t_{1/2}$	
		Measured[a] (h)	Calculated[b] (h)
Free TRA	Methanol	0.70 ± 0.12	0.85 ± 0.23
1	P90/Chol/DCP	6.90 ± 0.43	7.09 ± 0.23
2	P90H/Chol/DCP	15.20 ± 0.45	14.83 ± 0.35
3	Span® 40/Chol/DCP	4.00 ± 0.21	4.20 ± 0.15
4	Span® 60/Chol/DCP	4.10 ± 0.12	4.35 ± 0.07
5	Brij® 30/Chol/DCP	5.70 ± 0.19	5.62 ± 0.13
6	TrCG110/Chol/DCP	6.12 ± 0.21	5.94 ± 0.11

[a] Measured $t_{1/2}$ was determined by analysing the concentration of intact TRA in the formulations exposed to UV light, every 30 min. Samples were taken every 5 min when intact tretinoin approached to 50% (i.e. TRA concentration between 60 and 40%).
[b] Half-lives of vesicle-incorporated TRA were calculated according to the regression line determined by considering time between 1 and 8 h of the experiments.

Table 5
Photodegradation rate constants (K_0), half-life ($t_{1/2}$), correlation coefficient (R^2) and percent left drug from free and vesicle-incorporated tretinoin after 8 h of UV irradiation

Formulation	Vesicle structure	Main bilayer component	$-K_0$[a]	$t_{1/2}$ (h)	R^2	% TRA left
Methanol	–	–	4.44[b]	0.85[b]	0.901	9.80 ± 0.65
1	MLV	P90	4.97	9.76	0.979	59.77 ± 1.33
2	MLV	P90H	2.38	21.30	0.992	79.81 ± 2.76
3	MLV	Span® 40	11.95	3.40	0.952	6.86 ± 0.21
4	MLV	Span® 60	11.02	3.12	0.960	7.81 ± 0.06
5	MLV	Brij® 30	6.48	6.33	0.993	4.40 ± 0.17
6	MLV	TrCG110	12.80	4.08	0.998	44.14 ± 1.15
1	LUV	P90	4.41	11.02	0.994	63.23 ± 2.37
2	LUV	P90H	4.07	12.50	0.989	67.09 ± 1.11
3	LUV	Span® 40	11.73	2.71	0.923	4.90 ± 0.21
4	LUV	Span® 60	11.04	2.77	0.975	5.53 ± 0.18
5	LUV	Brij® 30	8.72	4.39	0.995	23.34 ± 0.86
6	LUV	TrCG110	9.81	5.11	0.984	16.73 ± 0.54
1	SUV	P90	6.16	7.09	0.994	45.24 ± 1.72
2	SUV	P90H	3.34	14.83	0.986	72.40 ± 0.37
3	SUV	Span® 40	9.64	4.20	0.993	13.82 ± 0.29
4	SUV	Span® 60	11.69	4.35	0.990	36.18 ± 0.63
5	SUV	Brij® 30	8.50	5.62	0.993	33.41 ± 0.77
6	SUV	TrCG110	7.78	5.94	0.998	5.09 ± 0.22

[a] Photodegradation rate constants (K_0) were determined from HPLC analyses of TRA and were calculated by means of the following equation: $K_0 = (y - b)/(t-1)$, where y = % TRA concentration and b is the residual percent at time $t = 1$ h.
[b] Photodegradation constant for free TRA in methanol was determined from HPLC analyses of % TRA and was calculated according to a first order kinetics as follows: $K_1 = (y - b)/t$, where y = ln % TRA concentration; $b = 4.6 \pm 0.8$.

the differences amongst TRA half-lives could be just due to the incorporation into the vesicles which is able to prevent the tretinoin oxidation process. In fact, it is evident that free TRA half-life did not change appreciably in both experiments and it is also very close to the $t_{1/2}$ value experimentally measured.

Results obtained after studying the photodegradation of TRA for 8 h (Table 5) confirmed that the vesicular incorporation of this drug always extended the stability of tretinoin, whose half-life increased up from 3- to 25-fold. The HPLC analyses of the vesicle-incorporated TRA showed that during the 8 h of UV light exposure the only detectable degradation product was isotretinoin, which is still therapeutically active (Fig. 1b). Moreover, the photoprotection offered by the vesicles was dependent both on the bilayer composition and vesicular structure. In fact, liposomes were always more efficient than the niosomes in the photoprotection of TRA, and liposomes made from hydrogenated soy phosphatidylcholine (P90H) were capable of greatly improving the TRA stability. MLV-incorporated TRA showed a half-life 25 times longer than the "free" tretinoin. The high photoprotection offered by P90H liposomes is the result of the high stability of these vesicles whose main component has a high transition temperature ($T_c = 80\,^{\circ}C$). A great improvement of TRA stability was also obtained with liposomes made from P90, a mixture of pure soy phosphatidylcholine rich in unsaturated and polyunsaturated fatty acids and with a low T_c. However, the commercial mixture of soy phosphatidylcholine we used in this study (P90) contains 1% of α-tocopherol. Therefore, we believe that this good result depends on the presence of this antioxidant agent, which has already proven to be able to protect both the unsaturated acyl chains and retinol (Young and Gregoriadis, 1996). Consequently, the addition of α-tocopherol into the other vesicular formulations would give an improved protection effect. However, further studies are needed to verify the appropriate amount of α-tocopherol in the different vesicular formulations and to exploit the effect of this antioxidant agent addition.

Niosomal formulations slightly improved TRA stability. In fact, the half-life was only three to seven times longer than the free drug. Among the niosomal

formulations, the best results were obtained with those prepared from non-ionic surfactants whose monomers presented an ethereal linkage (i.e. Brij® 30 and Triton® CG110). Span® 40 and Span® 60 niosomes did not show statistical differences in their photoprotection capability and LUV and MLV dispersions showed very little improvement of TRA stability. The low photoprotection offered by the sorbitan ester vesicle formulations is probably due to the low stability of the bilayer in which the estereal bond of the monomers can be easily hydrolysed. Results obtained after 8 h of UV irradiation showed that liposomes and niosomes made from Brij® 30 had a higher TRA content than the methanolic solution (10%), but niosomes made from the sorbitan esters and LUVs and SUVs made from the Triton® CG110 had a very low content of intact tretinoin. This is the result of the different rate of the degradation process. As written above, TRA degradation in methanol was very fast in the first 5 h, but then it reached a photostationary state and intact tretinoin remained constant.

3.4. Tretinoin degradation under the fluorescent light

In order to verify TRA photostability in "in use" conditions, the photodegradation of both the free and vesicle-incorporated drug was also studied when exposed to fluorescent light for 21 days. It is well known that for photochemical reactions UV light is the most potentially harmful, but long exposures to direct as well as artificial daylight can also be very dangerous for photosensitive drugs. Moreover, it was reported that the wavelength of TRA instability is situated around 420 nm and not at the wavelength of its maximum absorption ($\lambda = 350$ nm) (Brisaert and Plaizier-Vercammen, 2000). Fig. 3 shows the degradation curves of tretinoin in the different studied formulations while Table 5 presents the TRA degradation constants (K_1), half-life ($t_{1/2}$) and shelf-life (t_{90}) obtained according to the linear regression analysis of a first order process. As shown in Fig. 3, the photodegradation of TRA in methanol was very fast in the first 5 days of fluorescent light exposure, then it reached a photostationary state and intact tretinoin remained constant (5.50–5.75%). HPLC analyses showed that the "free tretinoin" degraded in a mixture of products similar to those obtained under UV irradiation. On the other hand, chromatograms of vesicle-incorporated TRA always presented isotretinoin as the only quantifiable degradation product. Results obtained from the exposure of tretinoin to fluorescent light showed that not all the studied vesicular formulations were capable of improving TRA stability when compared with the free drug in methanol. In fact, the TRA incorporated in formulations made from P90 presented a half-life shorter or very close to that of the free drug. Moreover, after 7–9 days of irradiation all P90 vesicle-incorporated drug degraded completely (Fig. 3 and Table 6).

Span formulations also showed a weak effectiveness in the photoprotection of TRA, except for SUVs. In fact, the comparison of half-lives showed that TRA stability improved only up to twofold as a consequence of its incorporation in Span MLVs and LUVs. On the other hand, the inclusion of TRA in vesicular bilayers made from Brij® 30, P90H or Triton® CG110 retarded the photodegradation of the drug from 3.5 to 11 times than that of free tretinoin as shown by the comparison of TRA half-lives. In fact, TRA degradation constant is approximately 6- to 11-fold lower than in the methanol when the drug is incorporated in Brij® 30 niosomes, while the incorporation in P90H and Triton® CG110 vesicles decreased TRA degradation constant three- to fivefold than in the methanol. Recently, Brisaert and Plaizier-Vercammen (2000) reported that the inclusion of some Brij®'s in an ethanol–propylene glycol tretinoin lotion did not really affect the stability of the drug. This could be due to the high solubility of tretinoin in the ethanol–propylene glycol which prevents TRA incorporation into the micelle core. However, in our study Brij® 30 vesicles are able to improve drug stability because of the incorporation of the drug into the lipidic bilayer in which the tretinoin molecules are tightly packed closely to the mixture of the single chained surfactant, Brij® 30, and cholesterol. Therefore, results suggest that TRA photostability is strongly affected by the vesicular stability itself, which in turn depends on the vesicle bilayer composition. Vesicle formulations made from amphiphile monomers with an ethereal linkage are more efficient in TRA photoprotection as they are more stable. Probably, the prolonged exposition to water has a destabilising effect on the vesicular membrane of P90 and Span vesicles as a consequence of the estereal bond hydrolysis. In addition, P90 molecules can undergo an oxidation reaction and this can explain the

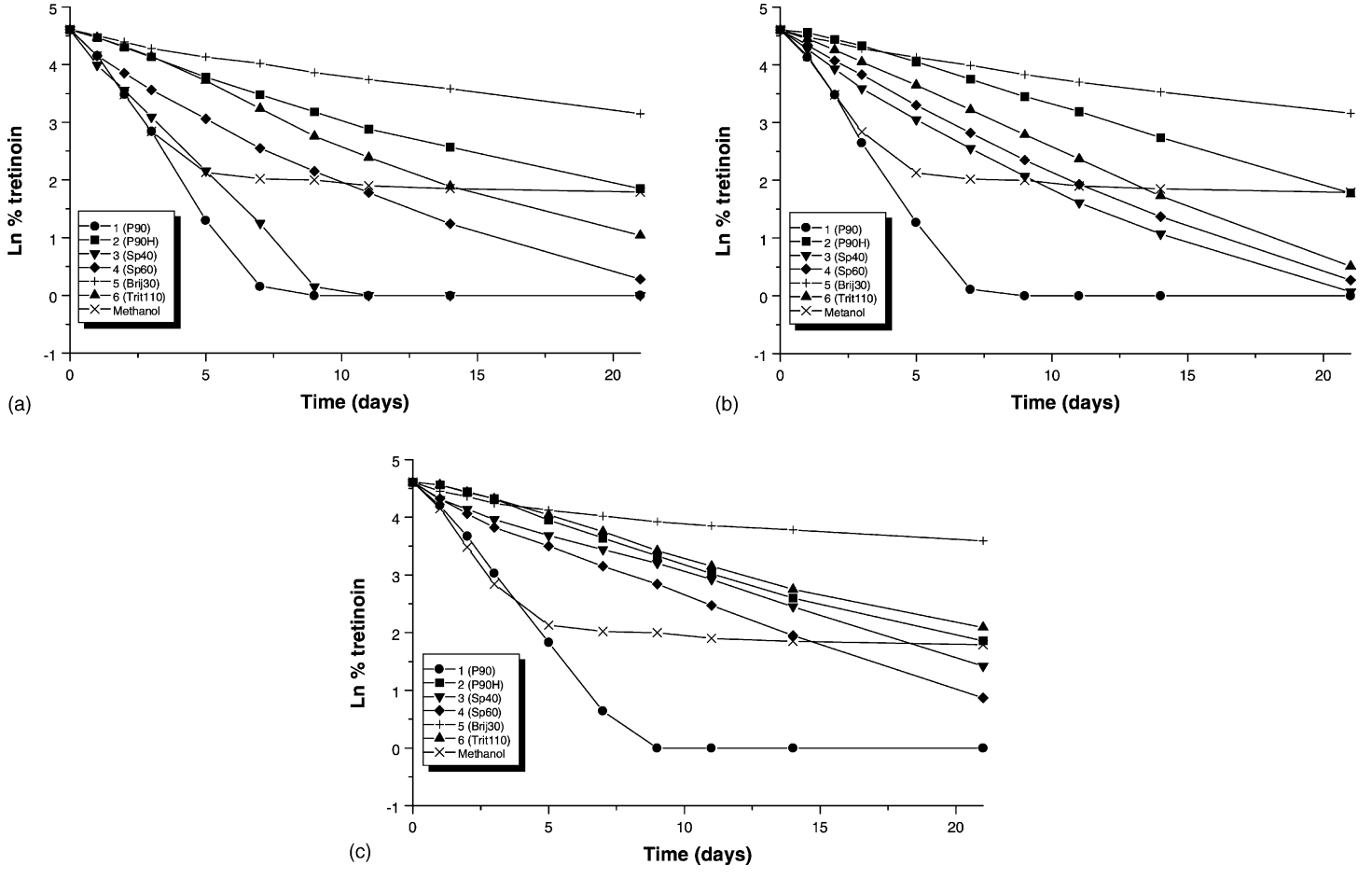

Fig. 3. First order degradation kinetics of free and vesicle-incorporated tretinoin under fluorescent light irradiation. (a) Multilamellar vesicles (MLVs); (b) extruded vesicles (LUVs); (c) sonicated vesicles (SUVs). For each formulation the main lipid component is reported.

Table 6
Photodegradation constants (K_1), half-life ($t_{1/2}$) and shelf-life (t_{90}) of free and vesicle-incorporated tretinoin after 21 days of fluorescent light irradiation

Formulation	Vesicle structure	Main bilayer component	$-K_1$ ($\times 10^{-2}$)[a] (h^{-1})	$t_{1/2}$ (h)	t_{90} (h)	% TRA left
Methanol	–	–	2.01	24.91	5.94	5.65 ± 0.10
1	MLV	P90	2.74	29.80	3.85	–
2	MLV	P90H	0.56	106.54	18.66	5.00 ± 0.15
3	MLV	Span® 40	2.01	31.53	5.25	0.38 ± 0.04
4	MLV	Span® 60	0.86	36.12	12.32	1.33 ± 0.06
5	MLV	Brij® 30	0.28	215.25	37.19	3.01 ± 0.13
6	MLV	TrCG110	0.75	88.49	13.94	3.10 ± 0.18
1	LUV	P90	2.77	28.46	3.81	–
2	LUV	P90H	0.58	136.60	18.28	6.30 ± 0.25
3	LUV	Span® 40	0.92	41.38	11.46	1.07 ± 0.05
4	LUV	Span® 60	0.88	59.15	11.95	7.15 ± 0.16
5	LUV	Brij® 30	0.29	209.27	36.83	29.40 ± 0.68
6	LUV	TrCG110	0.83	85.49	12.63	1.66 ± 0.10
1	SUV	P90	2.41	33.98	4.37	–
2	SUV	P90H	0.58	127.04	18.16	5.55 ± 0.33
3	SUV	Span® 40	0.61	92.49	17.45	10.00 ± 0.43
4	SUV	Span® 60	0.73	71.64	14.48	2.40 ± 0.11
5	SUV	Brij® 30	0.19	269.23	54.59	36.13 ± 0.73
6	SUV	TrCG110	0.53	137.49	19.82	6.20 ± 0.12

[a] Photodegradation rate constants (K_1) were determined from HPLC analyses of TRA and were calculated according to the following equation: $K_1 = (y - b)/t$, where $y = \ln$ % TRA concentration; $b = 4.6 \pm 0.8$.

very low protection offered by formulation 1 in comparison with results obtained under UV irradiation. In contrast, good photoprotection levels were offered by the P90H formulations in which the monomer estereal functions are less reactive than those of P90 because of the higher transition temperature ($T_c = 80\,^{\circ}C$).

We have previously reported that tretinoin is released quickly from P90 liposomes as a consequence of its high bilayer fluidity, which is due to the presence of the unsaturated and polyunsaturated fatty acid content (Manconi et al., 2002). Results obtained from this photodegradation study lead us to suppose that the high TRA degradation, when it is incorporated in P90 vesicles, is the consequence of the faster exposition of the drug to daylight and air as the result of the P90 bilayer deterioration in the presence of water and light.

Vesicle formulations made from Brij® 30 and Triton® CG110, whose monomers contain an ethereal linkage, are more stable and therefore they provide a more efficient protection of tretinoin.

Finally, it has also been shown that TRA photoprotection is also dependent on vesicle structure. In fact, as under UV light tests, the highest photoprotection was offered by the monolamellar structures. A possible reason to account for this fact is that the smaller size of the sonicated vesicles (100–350 nm) prevents the incorporated TRA from being affected by the low frequency portion of the light spectrum. However, this could also be the result of a lower stability of multilamellar vesicles which progressively tend to "peel off" outermost bilayers (Yarosh, 2001). These findings seem to be in contrast with our previous results regarding TRA in vitro release which always increased from MLVs to LUVs to SUVs. However, they support the supposition that the in vitro delivery of TRA (carried out in a Franz cell apparatus using a hydroalcoholic solution as the receiver medium) was affected by the back diffusion of the alcohol in the donor compartment with a consequent easier alteration of the single bilayered vesicles (SUVs).

4. Conclusion

The results have shown that TRA stability may be improved by its incorporation in vesicular bilayers. However, the stability of the photosensitive drug in vesicular suspensions is affected by the properties of

the vesicles themselves. Therefore, the composition and structure of vesicles must be considered in liposomal and niosomal formulations of photosensitive drugs. In fact, the highest TRA stability was obtained when it was incorporated in niosomes made from non-ionic surfactant with an ethereal linkage or in liposomes made from hydrogenated phospholipids with a high transition temperature. In addition, unilamellar vesicles generally showed a higher protection of TRA than the multilamellar liposomes. In this aspect, unilamellar niosomes made from Brij® 30 were the formulations with the best protection of tretinoin.

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