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Liposomes with tretinoin: a physical and chemical evaluation

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Abstract

The comedolytic activity of tretinoin, incorporated in liposomes, is five to ten times higher compared to the conventional preparations and also the local tolerability is much better. This implies the big interest of a study on tretinoin in liposomes. First, the encapsulation capacity of tretinoin in the liposomes was determined. Therefore, a series of liposomes was prepared with different concentrations of tretinoin (1–6 mg/ml buffer) and lipids (100–300 mg/ml buffer) (egg phosphatidyl choline/cholesterol) with buffers pH = 5 and 7. These series of liposomes were evaluated microscopically on the presence of tretinoin crystals outside the liposomes. The highest incorporation capacity was obtained using 2 mg of tretinoin and 300 mg of lipids per milliliter of buffer pH = 5. The chemical stability of tretinoin in the liposomes, evaluated during 1 year, revealed no remarkable loss in tretinoin content, even when stored at 25 °C. The photo-degradation of tretinoin in the liposomes was about two times slower than in castor oil, but tretinoin degraded to ≈25% of its initial content. The chemical evaluation of the lipid fraction showed no oxidative degradation of the polyunsaturated fatty acids in EPC because the determined concentration of conjugated dienes and hydroperoxides, two oxidative degradation products, was < 1%, which is negligible. Finally, the in-vitro release of tretinoin from the liposomes, evaluated with a dialysis technique, was very low, but this is not a problem for topical use. © 2001 Published by Elsevier Science B.V.

Keywords: Tretinoin; Liposomes; Incorporation capacity; Chemical and photochemical stability; Release

1. Introduction

Tretinoin, also called retinoic acid or vitamin A acid, is a widely used drug in the topical treatment of acne, photo-aged skin, psoriasis and other skin disorders.

The main effect of tretinoin in the treatment of acne is to reduce the size and the number of comedones and therefore, tretinoin is commonly used in a concentration of 0.05% (w/w), incorporated in a lotion, a hydrogel or an o/w cream. Unfortunately, if used in this concentration, unpleasant side effects often appear in the form of scaling, erythema, burning and stinging [1].

A previous study, performed by Meybeck [2], revealed that the maximum comedolytic activity

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of the drug is reached at a concentration of five to ten times lower when tretinoin is incorporated in liposomes, compared to the conventional alcoholic gels. If the concentration of the drug could be decreased, the topical side effects would also decrease [3]. Moreover, the same study revealed that the percutaneous penetration to the blood vessels, which is not desired because of important systemic side effects, is two times lower.

Tretinoin is also very unstable to radiation: an irradiated tretinoin lotion only contains 30% of the initial tretinoin concentration after 10 min of irradiation [4]. On the other hand, it was found in the literature that the incorporation of tretinoin in liposomes can protect the drug against photodegradation [5].

All these advantages of tretinoin liposomes suggested the importance of a study about this subject. However, in the earlier studies, nothing was mentioned about the maximal incorporation capacity. Moreover, no data are available on the physical and chemical stability as a function of storage time for the active compound or for the liposomal ingredients.

So, the first aim of the study was to see if it was possible to prepare tretinoin liposomes with our simple equipment, using a liposomal composition, proved to produce stable liposomes in earlier studies (personal communication). Determination of the incorporation capacity of the prepared liposomes for tretinoin was another aim of this work. Would it be possible to reach the normally used concentration of 0.05% (w/w) in a dermatological preparation containing liposomes?

Secondly, the chemical and the photochemical stability of tretinoin in the liposomes was evaluated, since we know that tretinoin is not a very stable product, especially not in the presence of a light source.

The chemical stability of the lipids, the main components of the liposomes, has also been evaluated. The lipids contain polyunsaturated fatty acids, which are very susceptible to oxidation. One of the first steps in the oxidation process is the formation of conjugated dienes, which are easily detectable by UV-spectrophotometry. Later in the oxidation process, hydroperoxides can be formed which can also be measured by spectrophotometry after derivatization. It is important to evaluate the oxidation of the liposomes because this can influence the stability of tretinoin, which is also susceptible to oxidation.

Finally, the release of tretinoin from the liposomes was studied.

2. Materials and methods

2.1. Preparation of liposomes [6]

Tretinoin (BASF, Ludwigshafen, Germany) is used as the active compound.

The basic composition of the lipid fraction of the liposomal suspensions is always 72% (w/w) egg phosphatidyl choline (EPC) (Lipoid GmbH, Ludwigshafen, Germany) and 28% (w/w) cholesterol (extra pure, Merck, Darmstadt, Germany) known as the best composition to produce stable membranes [7]. α-Tocopherol (Merck) is used as an anti-oxidant to protect the lipids and tretinoin against oxidation.

Different series of liposomes were prepared, altering the concentration of the lipids, the concentration of tretinoin or the pH of the buffer used to hydrate the lipid film. The composition of the different liposomal suspensions is given in Table 1.

The easiest and most correct way to prepare the liposomes is using stock solutions of the different components (cholesterol, tretinoin and α-tocopherol; EPC) in chloroform (HPLC grade, Merck). Then, the appropriate volumes of the stock solutions are mixed and eventually diluted with chloroform in order to obtain the same volume of mixture.

The lipids and the drug were then deposited as a thin film in a round bottom flask by rotatory evaporation under nitrogen using a Rock'n Roller (Snijders Analysers, Tilburg, The Netherlands). This apparatus enables us to evaporate different flasks at the same time and to adjust the position of the flasks from vertical to horizontal as the volume of the solvent in the flasks decreases so that the surface of the film could be enhanced. The obtained lipid films were stored in vacuum in a lyophilizer (Vanderheyden, Brussels, Belgium)

overnight to facilitate the removal of any residual solvent. The films were then hydrated by the addition of an appropriate volume of buffer and homogenized in an overhead shaker (Heidolph, Swabach, Germany).

A 0.05 M isotonic buffer solution pH = 5 was prepared by mixing 2.952 g $Na_2HPO_4.12H_2O$, 0.816 g citric acid H_2O, 0.1055 g NaCl and water to 100 ml. To prepare a 0.05 M isotonic buffer pH = 7, 0.42 g $NaH_2PO_4.2H_2O$, 1.43 g $Na_2HPO_4.12H_2O$, 0.4495 g NaCl were used together with water up to 100 ml. All the reagents were of pro analysis grade and were purchased from Merck and the water was Milli-Q water.

2.2. Microscopic evaluation of the liposomal suspensions

Microscopic evaluation was performed to determine the presence of tretinoin crystals at the outside of the liposomes. The amount of crystals was counted within 20 squares of a Bürker counting apparatus (W. Schreck, Hofheim, Germany), each square having a side of 0.0025 mm and a depth of 0.1 mm, using a Standard Junior 2 monocular micrometer (Carl-Zeiss, Oberkochen, Germany).

Table 1
Composition of the liposomal suspensions

	Lipids (mg)	EPC (mg)	Cholesterol (mg)	Tretinoin (mg)	α-Tocopherol (mg)	pH buffer (1 ml)	Total volume (ml)
Series A							
1	100	72	28	2	–	7	1.1
2	100	72	28	4	–	7	1.1
3	100	72	28	6	–	7	1.1
4	200	144	56	2	–	7	1.2
5	200	144	56	4	–	7	1.2
6	200	144	56	6	–	7	1.2
7	300	216	84	2	–	7	1.3
8	300	216	84	4	–	7	1.3
9	300	216	84	6	–	7	1.3
Series B							
1	100	72	28	1	–	7	1.1
2	100	72	28	1.5	–	7	1.1
3	100	72	28	2	–	7	1.1
4	200	144	56	1	–	7	1.2
5	200	144	56	1.5	–	7	1.2
6	200	144	56	2	–	7	1.2
7	300	216	84	1	–	7	1.3
8	300	216	84	1.5	–	7	1.3
9	300	216	84	2	–	7	1.3
Series C							
1	100	72	28	1	–	5	1.1
2	100	72	28	1.5	–	5	1.1
3	100	72	28	2	–	5	1.1
4	200	144	56	1	–	5	1.2
5	200	144	56	1.5	–	5	1.2
6	200	144	56	2	–	5	1.2
7	300	216	84	1	–	5	1.3
8	300	216	84	1.5	–	5	1.3
9	300	216	84	2	–	5	1.3
Series D	300	216	84	1.5	2.25	5	1.3

2.3. Chemical stability of tretinoin in liposomes

The concentration of tretinoin, encapsulated in the liposomes was evaluated as a function of time.

In the first set of experiments, the concentration of tretinoin in the whole liposomal suspension and in the supernatant, after centrifugation, was determined. These experiments revealed that the concentration of tretinoin in the supernatant was negligible in comparison with the concentration in the whole suspension, on condition that no crystals were determined in the suspension. Therefore, only the whole liposomal suspensions were analyzed in the following experiments. The suspension was first diluted appropriately (100–500 ×) in a mixture of methanol (HPLC grade, Biosolve, Valkenswaard, The Netherlands): chloroform (HPLC grade, Merck) 2:1 (v/v) and was then analyzed with this HPLC method: a Lichrocart RP 18 column (250 × 4) filled with Licrospher 100 (5 μm) particles (Merck), a mobile phase consisting of 80% (v/v) acetonitrile (HPLC grade, Biosolve, Valkenswaard), 20% (v/v) water and 1% (v/v) acetic acid 98%(w/w) (Merck), a flow set at 1 ml/min, a detection wavelength of 350 nm and an injection volume of 20 μl. The HPLC apparatus consisted of a Merck Hitachi L 6200 pump, a Merck Hitachi L 4000 UV-detector, a Merck Hitachi D 2500 chromato-integrator and a Rheodyne 20 μl loop (Cotati, CA).

The concentration of tretinoin in the suspensions was calculated from a calibration line, which was analyzed every day of the experiments.

2.4. Photo-degradation of tretinoin liposomes

The liposomal suspensions were irradiated during different times, using the irradiation equipment described in another study [4] This equipment consisted of a XM-450 H/V xenon lamp (ORC Lighting Products, Azusa, CA) and a Siemens apparatus (Munich, Germany), to adjust the current at 14 A and the voltage at 22 V. A sample holder house with a quartz cell was placed at a distance of 28 cm from the xenon lamp. To ensure that the radiation was penetrating in the whole liposomal suspension, the conventional 1-cm pathlength cell was replaced by a cell with detachable windows and a pathlength of 0.01 cm (Hellma, Müllheim, Germany). Between the two windows, ≈35 μl of suspension was homogeneously spread taking care not to include air bubbles and the exact amount of suspension was weighed. After irradiation for a certain time, the two cell windows were opened and both put in a recipient containing 5 ml of methanol. Then, the recipient was agitated gently to dissolve the liposomal suspension in the methanol. The two windows were withdrawn and the solution was analyzed with the HPLC method, as described previously.

2.5. Chemical stability of the lipids

2.5.1. SPE extraction procedure

The extraction of the liposomal suspension is necessary to separate tretinoin, cholesterol and α-tocopherol from EPC to avoid interference on the determination of conjugated dienes or hydroperoxides. The SPE extraction technique, discussed by Gabriëls [8], is used with a minor modification. Instead of 0.1 ml liposomal suspension, 0.2 ml is used and the 20 ml of methanol, obtained after the extraction, is divided in 5 and 15 ml: 5 ml for the determination of conjugated dienes and 15 ml for the determination of hydroperoxides.

2.5.2. Determination of conjugated dienes [6]

Five milliliters of the methanol extract were evaporated and the residue was dissolved in ethanol and analyzed spectrophotometrically at 233 nm. The concentration of dienes in the sample was deduced from a calibration line. For the calibration line, concentrations of linoleic acid (Sigma, St. Louis) between 0 and 50 μM in 0.2 M borate buffer at pH = 9 was derivated to conjugated dienes using the enzyme lipoxidase (Sigma) and the absorption at 233 nm was measured [9]. The borate buffer was prepared by dissolving 12.37 g boric acid in 1 l of water and using NaOH to adjust the pH.

2.5.3. Determination of hydroperoxides

For the determination of hydroperoxides, the modified IDF method described in detail by

Gabriëls [8] was used. Fifteen milliliters of the methanol extract were evaporated and the residue was dissolved in 2.5 ml of a methanol/chloroform (2:1 v/v) mixture. A total of 2 ml of this solution was withdrawn, diluted with 4 ml of the methanol/chloroform mixture and 3 ml of this solution was than derived with the modified IDF method to measure its absorption at 478 nm. The concentration of hydroperoxides in the sample was deduced from a calibration line, where the absorption at 478 nm was plotted as a function of the concentration of cumene hydroperoxide (Fluka, Bucks, Switzerland).

2.6. Release of tretinoin from liposomes

Evaluation of the release of tretinoin from the liposomes was performed with a dialysis technique using a Kontron-Diapack model 4000 apparatus (Kontron Instruments, Zürich, Switzerland). The donor and the acceptor compartment were separated with a Teflon T35 membrane filter, 0.2 μm, ∅50 mm (Schleicher and Schüll, Dassel, Germany) previously impregnated with dodecanol (Merck). The donor compartment was filled with 2 ml of the liposomal suspension containing tretinoin and the acceptor compartment with 2 ml of empty liposomal suspension. The dialysis experiment was performed during 48 h at least and the suspension in the acceptor and in the donor compartment was then analyzed with the HPLC method, previously described after appropriate dilution with methanol.

3. Results and discussion

3.1. Determination of the incorporation capacity for tretinoin in the liposomes

In order to determine the incorporation capacity of tretinoin in liposomes, the presence of tretinoin crystals in the liposomal suspensions was evaluated. Because tretinoin is insoluble in water, even in buffers, the fraction of tretinoin not entrapped in the liposomes will precipitate in the suspension and will be microscopically perceptible as crystals. Liposome suspensions with different tretinoin concentration were prepared, altering the lipid concentration and the pH. Theoretically, the entrapment efficiency should increase with increasing lipid concentration because tretinoin is a lipid soluble drug. Moreover, since the pK_a of tretinoin is 6, we wanted to examine if there was a difference in incorporation capacity at a pH which was higher (pH = 7) or lower (pH = 5) than the pK_a of the drug.

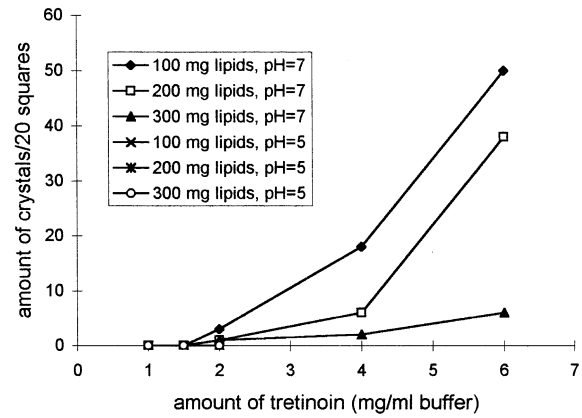

Fig. 1. Crystallization of tretinoin as a function of the amount of tretinoin used to prepare the liposomes.

The composition of the different liposomal suspensions is given in Table 1. In the first series, the concentration of tretinoin varied between 2 and 6 mg/ml buffer added. This means that the final concentration of tretinoin was 2, 4 or 6 mg per 1.1 ml suspension when 100 mg of lipids was used, per 1.2 ml of suspension when 200 mg of lipids was used and per 1.3 ml of suspension when 300 mg of lipids was used. The density of the lipid fraction equaled 1, as determined previously, because the lipid fraction consisted of cholesterol, which has a high density and EPC, which has a density lower than 1.

To simplify things, the concentration will always be expressed in milligram tretinoin per milliliter of buffer added to prepare the liposomal suspension and it will be mentioned whether we used 100, 200 or 300 mg lipids per milliliter buffer added, to prepare the liposomes.

The results are shown in Fig. 1, in which the amount of detected crystals per 20 squares was plotted as a function of the tretinoin amount per

milliliter of buffer. The figure shows that in the first series of liposomal suspensions, 2–6 mg tretinoin, crystals were seen in all the suspensions. So, one can conclude that the concentrations of tretinoin were too high to prepare good liposomal suspensions. The amount of crystals increased sharply with increasing tretinoin concentration when 100 or 200 mg lipids was used and less with 300 mg lipids. These data show also that precipitation of tretinoin declined with increasing lipid concentration, as expected. The second and third series of liposomal suspensions were prepared with lower tretinoin concentrations, between 1 and 2 mg tretinoin per milliliter buffer and at two pH's. The results, given in Fig. 1, show that no crystals were detected with 1 or 1.5 mg tretinoin and few crystals with 2 mg tretinoin and buffer pH 7. On the other hand, in the liposomal suspensions prepared with buffer pH = 5, no crystals were detected at all, not even with 2 mg tretinoin. This can be explained by the fact that tretinoin has a carboxyl function in its structure with a $pK_a = 6$ and so, at pH = 5, much more acidic molecules of tretinoin could be found than at pH = 7, which are lipophilic and are preferably entrapped in the lipid fraction.

We can conclude that the best set of liposomal suspensions, where all the tretinoin is entrapped in the lipid fraction, consisted of 300 mg lipids and 2 mg tretinoin per milliliter of buffer at pH = 5. Therefore, the incorporation capacity can be defined as 2 mg tretinoin per 300 mg lipids or 0.68% (w/w). This capacity is thus more than ten times higher than the normally used concentration of tretinoin in dermatological formulations (0.05%), so that the liposomal dispersion could be diluted at least ten times for the formulation of topical preparations. In order to compare this capacity with the entrapment efficiency determined by Bonté [10], we should express it as the number of moles of tretinoin per mole of phosphorus, present in EPC. As it was found in the analytical data provided by the manufacturer [11], the content of phosphorus in EPC was 3.93% (w/w). So, the entrapment efficiency was calculated at 24.5 millimoles tretinoin per mole of phosphorus. This is about four times higher than that calculated by Bonté, which equals 6.10. Meybeck [12] prepared a tretinoin liposomal suspension and formulated it in a Carbopol® gel with a final concentration of 10^{-4}–10^{-2}% tretinoin and 2% lipids (lecithine). This suggests that the entrapment capacity is between 0.5 and 0.005 g tretinoin per 100 g of lipids, of which the upper limit is approximately two times lower than the limit we achieved (0.9 g/100 g). Cortesi [13], on the other hand, stated that the final molar ratio phospholipids/tretinoin was 25/1. Keeping in mind the molecular weight of 300 for tretinoin and 775 for EPC, the ratio phospholipids/tretinoin equals 65/1 (w/w), which means that the incorporation capacity is ≈ 1.5 times higher, as we found (108/1).

3.2. Chemical stability of tretinoin in liposomes

The authors dealing with tretinoin liposomes did not mention anything about chemical stability of tretinoin liposomes, but it is known that the drug is susceptible to oxidation [14], although this is not always proven [15]. Therefore, it seemed interesting to follow the stability of the prepared tretinoin liposomes during a certain time. Liposomal suspensions with and without an anti-oxidant (AO) were prepared, (see series C and D in Table 1), all were stored at 25 °C, while the suspension with AO was also stored at 4 °C to evaluate the influence of the storage temperature. The results are shown in Fig. 2, in which the remaining amount of tretinoin (expressed in percentage) compared with the initial concentration, is plotted as a function of time.

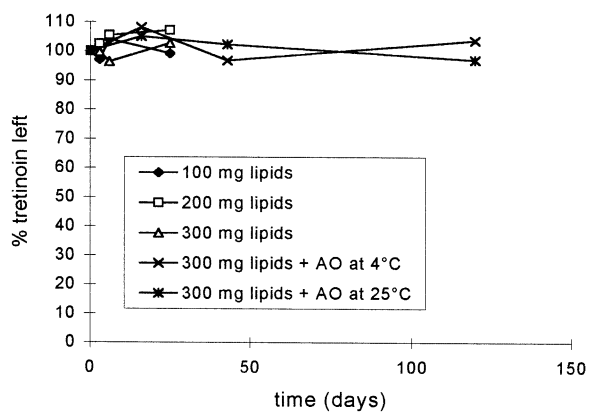

Fig. 2. Chemical stability of tretinoin in liposomes.

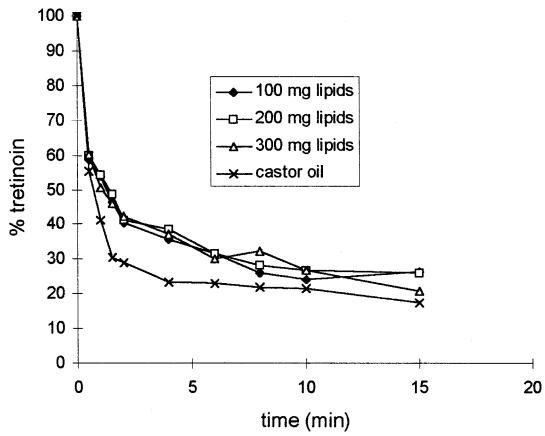

Fig. 3. Photo-chemical stability of tretinoin in liposomes and in castor oil.

Fig. 2 shows that the tretinoin liposomes are stable during at least 3 months if an anti-oxidant is added and there is no influence of storage temperature during this period. There are some variations in the tretinoin content, but they are due to variations of the analytical method. The stability of the liposomes without anti-oxidant was regularly evaluated after a shorter period, during which they were stable. This stability was also evaluated after storage time of 1 year (data not shown in the figure). After 1 year of storage, the tretinoin content was still 94, 102 and 95%, respectively for liposomes prepared with 100, 200 and 300 mg of lipids. As a result, all the liposomal dispersions remain stable during 1 year of storage.

3.3. Photo-degradation of tretinoin liposomes

Tretinoin is known to be very sensitive to light [4]. The results of the photo-degradation study, summarized in Fig. 3, show that photo-degradation is very high during the first 2 min of irradiation and decreases afterwards, reaching almost an equilibrium after 8 min. So, the liposomal preparations also degrade in the presence of light but are nevertheless more stable than when tretinoin is dissolved in castor oil. However, castor oil seemed to be the best solvent for the photo-stability of tretinoin as it was tested in an earlier study (personal communication), much better than ethanol.

The photo-degradation curves seem to follow first order kinetics between 0.5 and 2 min of irradiation and when the logarithm of the tretinoin concentration is plotted as a function of irradiation time, the photo-degradation constant is given by the slope, as shown in Fig. 4, divided by 2.303. The figure demonstrates that there is no difference in photo-degradation between the liposomal suspensions prepared with different amounts of lipids. But it is clear that liposomes do slightly protect tretinoin against photo-degradation: degradation constants in liposomes are roughly 1.8 times lower than in castor oil. A possible explanation is that the incorporated drug is less accessible to light beams because of light scattering by the vesicles. Thoma [5] came to the same conclusion in his work: the residual content of tretinoin after a 5 min exposure to sunlight in a liposome dispersion is about 1.4 times higher compared with a methanol solution.

3.4. Chemical stability of the lipids

The results of the determination of conjugated dienes and hydroperoxides, performed on the best series of tretinoin liposomes (series D), are given in Table 2. After 6 weeks of storage at 4 and 25 °C, the content of conjugated dienes in the liposome suspensions, calculated as a function of EPC content, is < 2 mol %, which is the target value of conjugated dienes for the release of a

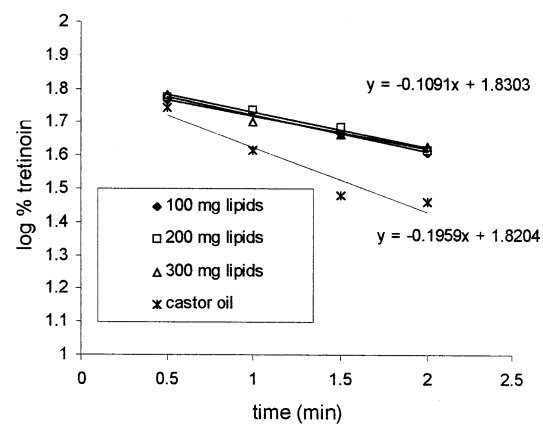

Fig. 4. Logarithmic plot of the photo-chemical stability of tretinoin in liposomes and in castor oil.

Table 2
Relative concentration of conjugated dienes and hydroperoxides compared with the EPC concentration after 6 weeks of storage

	Conjugated dienes		Hydroperoxides	
	4 °C	25 °C	4 °C	25 °C
Without tretinoin (mol %)	0.62	0.59	0	0
With tretinoin (mol %)	0.76	0.65	0	0.34

batch for clinical trials [16]. A real difference in dienes content between storage at 4 and 25 °C, or between liposomes with or without tretinoin, is not detectable at these small concentrations.

The content of hydroperoxides in the liposome suspensions were only detectable for the liposome suspensions containing tretinoin and stored at 25 °C, being 0.34% of the EPC content, which is again a very small amount, although it is ten times higher than the limit of detection which equals 0.037% (personal communication).

One can say that the lipid fraction of the prepared liposomes is still chemically stable after 6 weeks of storage.

3.5. In vitro release of tretinoin

Since tretinoin has a high affinity for the lipids in the liposome suspension, we wonder if it can easily be released from the liposomes. In order to determine the release of tretinoin from the liposomes, we performed a series of in vitro dialysis experiments. The first problem that occurred here, was the choice of the acceptor medium. The acceptor compartment must contain a solvent in which tretinoin is very soluble, in order to obtain SINK conditions. But, since tretinoin is insoluble in water and poorly soluble in buffers, only organic solvents are useful in order to obtain SINK conditions. On the other hand, organic solvents cannot be used because they would destroy the liposomes. Therefore, we opted for the use of an empty liposomal suspension in the acceptor compartment, having the same composition as the suspension in the donor compartment but without tretinoin. Cortesi [13] reported that the release of retinoids from liposomes was the highest when empty liposome suspension was used in comparison to pure isotonic Palitzch buffer (IPB) pH = 7.4 or IPB mixed with methanol or fetal calf serum.

The results of this experiment, summarized in Table 3, indicate that the association complex between tretinoin and liposomes is very stable: the release of tretinoin from the liposomes, after 48 h of dialysis, is very low. Only 3.6, 2.8 and 1.8% release was found, respectively, from liposomes containing 100, 200 and 300 mg of lipids per milliliter of buffer. It is remarkably that, even at these low release profiles, the release of tretinoin decreases with increasing lipid concentration, as it can be expected.

Still, the determined release is far lower in comparison to the study of Cortesi, who found a maximal release of $\approx 30\%$ within 24 h. However, the author mentioned the use of a dialysis tube with a molecular weight cut-off of 10,000–12,000, but did not specify which type of membrane was used. Although the choice of the membrane is a very important parameter in the release profile:

Table 3
Release of tretinoin from liposomes

	% Tretinoin in donor compartment	% Tretinoin in acceptor compartment
100 mg lipids	98.06	1.94
	94.98	5.02
	96.11	3.89
200 mg lipids	97.52	2.48
	97.21	2.79
	96.92	3.08
300 mg lipids	98.13	1.87
	98.24	1.76
	98.20	1.80

since tretinoin is a lipophilic drug, we chose a lipophilic membrane (Teflon). But in a study about the in vitro release of tretinoin from dermatological formulations—performed in our laboratory in the mean time (personal communication)—the release of tretinoin from a dermatological preparation was found to be higher using a cellulose nitrate membrane ($\approx$ 40%) or a regenerated cellulose membrane ($\approx$ 20%) instead of a lipophilic (Teflon) membrane ($< 5\%$).

Although despite the very small in vitro release of tretinoin from liposomes, Meybeck [2] and Masini [17] demonstrated that liposomes allowed a higher local concentration of tretinoin in the skin than a conventional alcoholic gel because the vesicular structure of the liposome seems to be lost when they penetrate the first layer of corneocytes.

4. Conclusion

We were able to prepare tretinoin liposomes with rather simple equipment using EPC and cholesterol as lipid components. Different concentrations of tretinoin and lipids and two buffers were tested and the highest incorporation capacity was obtained using 2 mg tretinoin, 300 mg lipids (216 mg EPC + 84 mg cholesterol) and 1 ml of buffer pH = 5.

Tretinoin in the liposomes seemed to be chemically stable during at least 3 months and also the photo-stability of tretinoin was better in liposomes, although there was still a decrease in content after irradiation.

Determination of conjugated dienes and hydroperoxides revealed that the lipid fraction was not yet susceptible to oxidation after 6 weeks of storage.

Only the in vitro release of tretinoin from the liposomes seemed to be very small. This is not a problem for the topical way of action, as it is considered in the treatment of acne, for the vesicular structure seems to be destroyed when liposomes are penetrating the first layers of the epidermis so that tretinoin will be released anyway.

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