Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 334 (2007) 56–61
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Investigation on the photostability of tretinoin in creams

M. Brisaert [a], J.A. Plaizier-Vercammen [b,*]

[a] *Erasmushogeschool Brussel, Health Care Department, Laarbeeklaan 121, B-1090 Brussels, Belgium*
[b] *Vrije Universiteit Brussel, Department of Pharmaceutical Technology and Physical Pharmacy, Laarbeeklaan 103, B-1090 Brussels, Belgium*

Received 17 May 2006; received in revised form 10 October 2006; accepted 13 October 2006
Available online 21 October 2006

Abstract

In this investigation, the photodegradation of some tretinoin cream formulations was evaluated. Several oils were selected to prepare the cream formulations: olive oil, maize oil, castor oil, isopropyl myristate and Miglyol 812.

A solubility study showed that tretinoin is best soluble in castor oil (0.60 g/100 ml), followed by isopropyl myristate, maize oil, Miglyol 812 and olive oil, respectively, 0.35, 0.30, 0.29 and 0.22 g/100 ml.

The photostability of tretinoin in oils is comparable with the photostability of a tretinoin lotion (ethanol/propylene glycol 50/50), castor oil and olive oil giving slightly better results than the other oils.

Investigation of the photodegradation of tretinoin in o/w creams, prepared with the same oils as mentioned above, revealed that tretinoin is far more stable in the cream formulations than in the respective oils, however it is not clear whether this is due to the formulation or due to a different irradiation technique. Tretinoin seemed to be most stable in the olive oil cream, followed by the castor oil cream. However microscopic investigation revealed the presence of tretinoin crystals in the olive oil cream, while the other creams were free of it. As a conclusion, one can say that the cream prepared with castor oil seems to be the most suitable one, in terms of solubility of tretinoin and in terms of photostability.
© 2006 Elsevier B.V. All rights reserved.

Keywords: Tretinoin; Photostability; Cream formulations

1. Introduction

Tretinoin is the most effective topical comedolytic agent having its highest activity when it is formulated in a lotion (ethanol/propylene glycol, 50/50) and decreasing activities over gel and (o/w) cream to fatty (w/o) cream (Plewig et al., 1971). Unfortunately, the lotion formulation has been shown to degrade very fast under irradiation with a xenon lamp resulting in an equilibrium, which is called the photostationary state. At this photostationary state, 80% of the initial tretinoin concentration is isomerized. Although the most important isomer is isotretinoin, which is also therapeutically effective, about 40% tretinoin is isomerized to inactive isomers (Brisaert and Plaizier-Vercammen, 2000).

In the clinical practice, tretinoin cream formulations are often prescribed as magistral preparations, although a commercial cream formulation (Retinova®, Johnson and Johnson) exists. A cream formulation causes far less irritation than an alcoholic lotion or a gel formulation, thus helping to increase compliance during therapy (Galvin et al., 1998).

The photostability of tretinoin in various formulations has been investigated by several other researchers in the past years (Martin et al., 1998; Lin et al., 2000; Nyirady et al., 2002; Manconi et al., 2003; Ioele et al., 2005; Nighland et al., 2006), however, none of these investigations deals with a tretinoin cream formulation, nor are the photostability data of the commercial product available in the literature. The evaluation of the photodegradation of tretinoin in cream formulations, seemed, for that reason an interesting study.

For the composition of the cream formulation, Pemulen TR-1®, a high molecular copolymer of acrylic acid and alkyl metacrylate, was selected as emulsifying agent as it was proved to have excellent emulsifying properties resulting in very stable emulsions, both with low and high oil concentrations of apolar or more polar oil phases (Brisaert and Plaizier-Vercammen, 1997). The selection of an appropriate oil phase was a very important issue. Most of the creams, commonly used for the preparations of

* Corresponding author. Tel.: +32 2 477 45 98; fax: +32 2 477 47 35.
E-mail addresses: myriam.brisaert@docent.ehb.be (M. Brisaert), jplaizie@vub.ac.be (J.A. Plaizier-Vercammen).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.10.023

magistral formulations, contain liquid paraffin or vaselin, which are possibly contra-indicated in the treatment of acne because of their occlusive properties. Moreover, the oil phase can influence the photostability of the tretinoin cream, because tretinoin will presumably dissolve in this phase, since it is practically insoluble in water. Therefore, several oils were selected basically on criteria as polarity (castor oil), and hence on dissolving properties for tretinoin, non-comedogeneity (olive oil and maize oil) and penetration enhancing properties (Miglyol 812 and isopropyl myristate). Since the activity of tretinoin is mediated by interactions with cytosolic proteins and with two classes of nuclear retinoid receptors (RAR and RXR), which are present in the human skin, predominantly in epidermal keratinocytes and dermal fibroblasts (Levin et al., 1992), tretinoin should penetrate through the epidermis to reach the dermis. On the other hand, percutaneous absorption of the drug into the blood should be avoided because of the side effects of tretinoin.

As there is very little information in literature about the solubility of tretinoin in the selected oil phases, the first experiment that had to be performed was a solubility test. Depending on the results of this test, an appropriate percentage of oil phase in the cream formulation could be determined and the creams could be formulated.

It seemed also interesting to evaluate the photostability of tretinoin in the pure oils so that we could compare it with its photostability in cream formulations containing the same oil. About the instrumentation, little or no literature is available and it was a challenge to develop a method for irradiating cream samples with our equipment which was only used for irradiating solutions until now.

2. Materials and methods

2.1. Determination of the solubility of tretinoin in oils

Tretinoin (Alpha Pharma, Zwevegem, Belgium) is used as active compound. Maize oil (Vandemoortele, Izegem, Belgium), olive oil (Federa, Brussels, Belgium), castor oil (Federa), isopropyl myristate (Henkel, Düsseldorf, Germany), and Miglyol 812 (Federa) were the oil phases in which the solubility of tretinoin was determined.

An excessive amount of tretinoin was added to 10 ml of each oil, to be sure that the oil solutions were oversaturated. The oversaturated oil solutions were than placed in a horizontally shaker (Gerhardt, Bonn, Germany) during several days at 25 °C in a dark room to exclude photodegradation.

After 5 days of shaking, the oil solutions were centrifugated at 3000 rpm (1512 × *g*) during 15 min with a Mistral 400 refrigerated centrifuge (MSE Scientific Instruments, West Sussex, England) to remove the excess of tretinoin. A sample of clear oil solution was taken and after appropriate dilution with dichloromethane (Merck, Darmstadt, Germany), the sample was analyzed spectrophotometrically at 367 nm. Dichloromethane was chosen as diluting solvent because it was miscible with all the oils. The concentration of tretinoin, dissolved in the oil sample was determined from a calibration line of tretinoin in dichloromethane (0–1 × 10^{-3}%, w/v), using an identically diluted solution of the corresponding oil in dichloromethane as a blank.

After the first sample was taken, the oversaturated oil solutions were placed on the shaker again and the whole procedure was repeated after 6 and 10 days of shaking.

2.2. Photostability of tretinoin in oils

The apparatus, used to irradiate the samples was previously used to irradiate the tretinoin lotion (Brisaert and Plaizier-Vercammen, 2000) and consisted of a XM-450 H/V xenon lamp (ORC Lighting Products, Azusa, California) and a Siemens (Münich, Germany) VX 501 r-5b apparatus to adjust the current at 14A and the voltage at 22 V. A black, closed sample holder house contained a disposable, plastic cell of 1 cm pathlength containing the oil solution, which was placed at a distance of 28 cm from the xenon lamp. A heat filter, containing water was placed between the xenon lamp and the sample to avoid overheating of the sample.

The sample, containing a concentration of 0.05% (w/v) tretinoin in each of the five selected oils, was irradiated during 5–8 min and every minute a sample was taken and analyzed with HPLC. HPLC was chosen as analytical method instead of UV-spectrophotometry in order to separate tretinoin from its isomers.

The HPLC method consisted, as in the previous study (Brisaert and Plaizier-Vercammen, 2000), of a Lichrocart (250, 4 mm) column (Merck, Darmstadt, Germany) filled with Lichrospher 100 RP-18 particles of 5 μm as stationary phase. The mobile phase was 80% acetonitrile (HPLC-S gradient grade, Biosolve, Valkenswaard, The Netherlands), 20% water (Milli-Q), 1% (v/v) acetic acid (glacial 100%, Merck, Darmstadt, Germany). To dilute the samples before injection, an appropriate solvent had to be selected which on one hand had to resemble the mobile phase as close as possible to obtain optimal resolution of the peaks, while, on the other hand, it had to dissolve the different oils. A mixture of 50% dichloromethane and 50% (v/v) acetonitrile seemed to be the most suitable one. A calibration line was performed in a concentration range of 0–1 × 10^{-3}% (w/v) tretinoin, dissolved in the same solvent.

The HPLC equipment was a Merck Hitachi (Hitachi, Tokyo, Japan) L-6000 pump, pumping at a flow of 1 ml/min, a Merck Hitachi L-4000 UV detector, set at a wavelength of 350 nm and a Merck Hitachi D-2500 Chromato-integrator.

2.3. Preparation of the creams

Five creams were prepared, respectively, using maize oil, olive oil, castor oil, isopropyl myristate and Mygliol 812 as oil phases. Each cream consisted of 50 mg tretinoin, 0.8 g Pemulen TR-1® (BFGoodrich, Leidschendam, The Netherlands), 30 ml oil phase, 0.25 g Tween 80 (Alpha Pharma, Zwevegem, Belgium) and water (Milli-Q) up to 100 ml. Neutrol TE (BASF, Brussels, Belgium) was used to adjust the pH of the creams to pH 5. This pH was chosen to be sure that tretinoin was non-ionized ($pK_a = 6$) while Pemulen TR-1® retained its emulsifying properties.

To ensure that the pH was identical in all the creams, a double concentrated stock solution was prepared, using 1.6% (w/v) Pemulen TR-1® in water adjusted at pH 5 with Neutrol TE.

For the preparation of the creams, 50 mg tretinoin was first dissolved in 30 ml of the appropriate oil phase using ultrasonication for 10 min. This solution was then mixed with 50 g Pemulen TR-1® stock solution, whereafter Tween 80 was added and the cream was brought to volume with water. The obtained cream was then homogenized during two minutes using a low shear mixer (Bamix, ESGE, Metten, Switzerland). Finally the cream was passed through an ointment mill (Exakt, Norderstedt, Germany) to pulverize tretinoin particles that may be precipitated upon mixing the oil phase with the other ingredients.

2.4. Irradiation of cream samples

In previous studies performed in our laboratory, the photostability of tretinoin in solutions or liquid liposome suspensions was studied. For the irradiation of the solutions, we could use a simple cell. For the irradiation of the creams, another technique was needed because it was not possible to fill a cell homogeneously with cream. Moreover, we had to be aware that the light beam could penetrate the whole depth of the sample for homogeneous irradiation.

The use of a cell with detachable windows and a pathlength of 0.01 cm (Hellma, Müllheim, Germany), used in a previous study for irradiation of liposome suspensions (Brisaert et al., 2001), was considered but this system had two major disadvantages. First, the capacity of the cell was very small so that the amount of cream sample (<50 μg) was too small to analyze analytically with our method. After irradiation tretinoin had to be extracted from the cream for analysis and since the sample was so small, the whole sample was needed. Therefore, the cell was opened and the two windows were placed in a plastic tube containing the extraction solvent; this tube was then shaken during a certain time to extract the cream. So the second disadvantage of this system was the fragility and the high cost of this quartz cell.

Since the principle of this "sandwich system" was good, we developed our own technique using microscope slides, as shown in Fig. 1, taking care of eliminating the disadvantages mentioned above. Two small pieces of a microscope slide were sticked at both ends of another slide, in order to become a cavity in the middle with a depth equal to the thickness of the slides (=1 mm). The dimensions of the cavity were chosen in a way that about the whole cavity's surface could be irradiated. An amount of cream was brought in the cavity (1) and the whole was covered with a second slide, so that the excessive amount of cream was coming out of the system at both sides of the cavity. In that way, it was possible to use approximately the same amount of cream (≈0.6 g) anytime, which was weighed analytically. This irradiating system was then placed in a holder so that the cream sample was protected from air during irradiation and it was prevented from drying up.

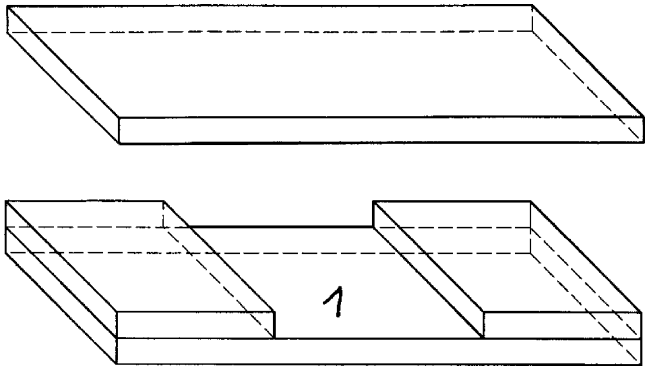

Fig. 1. Irradiation system for creams, developed in our laboratory.

2.5. Photostability of the creams

Six irradiation samples were prepared of each cream and they were irradiated, respectively, during 1, 2, 3, 4, 5 and 10 min.

After irradiation, tretinoin had to be extracted from the cream samples for HPLC analysis. Therefore, the irradiation system was opened and both microscope slides were put in a plastic tube containing 40 ml of extraction solvent, which was 80% (v/v) acetonitrile, 20% (v/v) water. Five milliliter of a 2% (w/v) sodium chloride (Merck, Darmstadt, Germany) solution in water was added to precipitate Pemulen® and the solution was shaken during 45 min using a horizontally shaker (Julabo, Seelbach, Germany). The obtained emulsion was then centrifuged during 15 min at 3000 rpm, to break the emulsion. The supernatant was analyzed after appropriate dilution. With this procedure, 100% extraction of the drug was achieved with a variation coefficient <2.5% ($n = 6$).

3. Results and discussion

3.1. Determination of the solubility of tretinoin in oils

The concentration of tretinoin, dissolved in the oils, determined 5, 6 and 10 days after preparation of the solutions is shown in Fig. 2. Maximal solubility is already reached after 5 days.

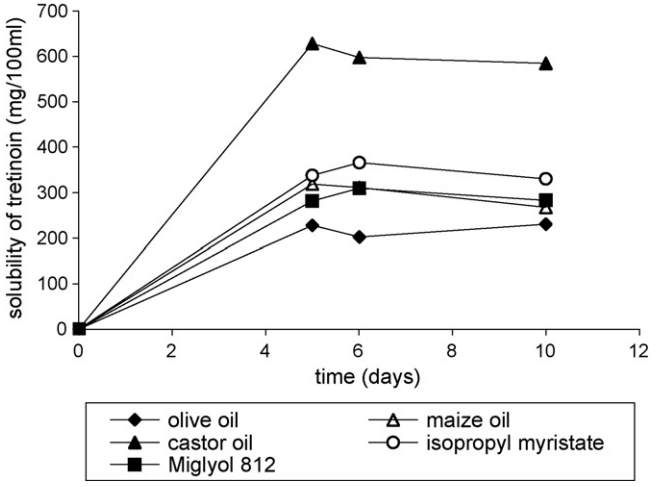

Fig. 2. Solubility curves of tretinoin in several oils as a function of time.

Table 1
Solubility of tretinoin in oils and of the respective oils in ethanol

	Average solubility of tretinoin in oil (g/100 ml) ($n=6$)	Solubility of oil in ethanol (Martindale, 1999)
Castor oil	0.604 ± 0.022	Freely soluble
Isopropyl myristate	0.345 ± 0.019	Soluble
Maize oil	0.300 ± 0.027	Slightly soluble
Miglyol 812	0.291 ± 0.016	Slightly soluble
Olive oil	0.220 ± 0.016	Very slightly soluble

Average solubilities ($n=6$) are shown in Table 1. Tretinoin is most soluble in castor oil, less soluble in isopropyl myristate, maize oil and Miglyol 812 and worst in olive oil. There is a remarkable relation between the solubility of tretinoin in an oil and the solubility of the respective oil in ethanol. The oil in which tretinoin is best soluble, is also best soluble in ethanol. One can assume that the solubility of an oil in ethanol is partly related to its polarity and that tretinoin is more soluble in oils of high polarity. Indeed, castor oil is an oil with a high amount of free hydroxyl groups, reflected in its high hydroxyl number. The solubility of tretinoin in castor oil is comparable with that in PEG 400 (0.5%, w/w) (Schlichting et al., 1973).

To prepare creams containing 0.05% (w/v) tretinoin, dissolved in the oil phase, one should need 8 ml castor oil, 14 ml isopropyl myristate, 17 ml maize oil and Miglyol 812 and 23 ml olive oil. In order to have the same composition for all the creams, we used 30 ml oil ensuring a complete dissolution of tretinoin in all the creams.

3.2. Photostability of tretinoin in oils

The results of the photodegradation of tretinoin in oils are summarized in Figs. 3 and 4. Respectively, six and three replicates were performed for olive oil and for all the other oils, resulting in an average reproducibility of 3.25% (variation coefficient). The photodegradation of tretinoin in the oils follows first order kinetics, as we can see in the semi-logarithmic plot, and is comparable with the degradation in a 50/50 ethanol/propylene glycol lotion (Brisaert and Plaizier-Vercammen, 2000). From the figures, it is not very clear if there is a significant difference between the five oils, and a statistical approach is needed. In order to prove if there is a significant difference between two photodegradation curves, the slopes of the respective semi-logarithmic plots in Fig. 4 are compared, using the method described by Massart et al. (1989), which is based on a Student's t-test. In this method, a Fisher F-test is used to check the homogeneity of the residual variances in the calibration lines to compare. If the difference between variances is not significant, the usual t-test is used to compare the similarity of the slopes. However, if a significant difference between the residual variances is found, another approach, described in the article, is used. The results of this statistical analysis are shown in Table 2.

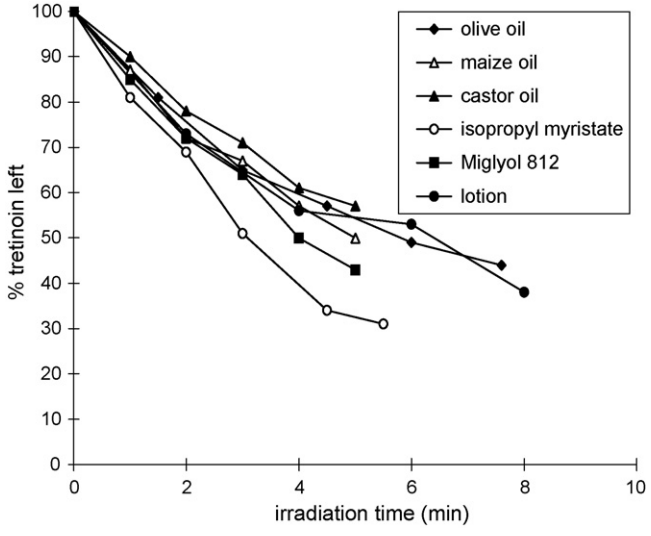

Fig. 3. Photodegradation curves of tretinoin in several oils as a function of irradiation time.

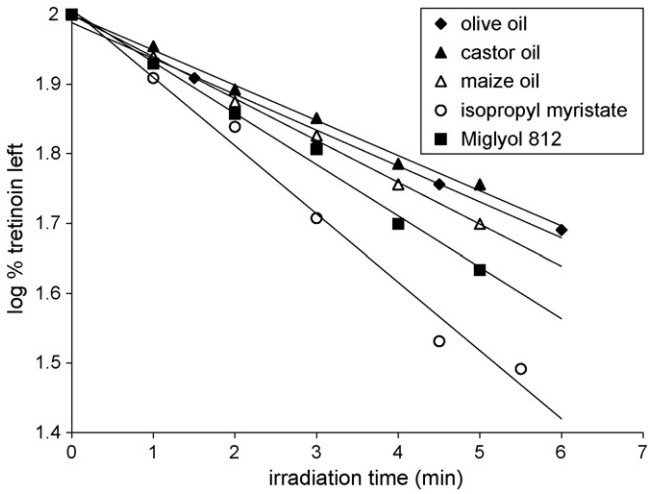

Fig. 4. Semi-logarithmic plot of the photodegradation of tretinoin in several oils as a function of irradiation time.

One can see that the calculated t-values are in three cases higher than the theoretical ones, which means that these photodegradation curves are significantly different from each other. Only between castor oil and olive oil, there is no significant difference. So one can say that tretinoin in most stable against radiation when it is dissolved in castor oil or olive oil and that the photodegradation increases over maize oil and Miglyol 812 to be highest in isopropyl myristate.

When we compare these photostability data with the determined solubilities, there seems to be a relation. Tretinoin is far best soluble in castor oil and also most stable in castor oil, while the solubilities and the stability in maize oil, Miglyol 812 and isopropyl myristate are comparable and lower than in castor oil. Only with olive oil there is no relation, since tretinoin is less soluble in it while it is very stable in olive oil. In a previous study performed by Halley and Nelson (1979), the rate of disappearance of tretinoin under irradiation in various solvents, caused by isomerization, increased as solvent polarity increased. This could explain the photostability of tretinoin in olive oil, but it is in contradiction with the good stability of tretinoin in the more polar castor oil.

Table 2
Statistical analysis of the regression lines from the logarithmic representation of first order kinetics photodegradation curves of tretinoin in oils using the method of Massart et al. (1989)

	Castor oil	Olive oil	Maize oil	Miglyol 812	Isopropyl myristate
Slope (s)	0.050	0.051	0.060	0.074	0.098
Variance (S^2)	7.7×10^{-5}	2.3×10^{-4}	1.9×10^{-5}	1.7×10^{-4}	6.5×10^{-4}
	$F_{calc}(=0.329) < F_{theor}(=9.12)$ $t_{calc}(=0.252) < t_{theor}(=2.24)$				
		$F_{calc}(=12.28) > F_{theor}(=6.59)$ $t_{calc}(=2.56) > t_{theor}(=2.33)$			
			$F_{calc}(=0.115) < F_{theor}(=6.39)$ $t_{calc}(=4.14) > t_{theor}(=2.13)$		
				$F_{calc}(=0.255) < F_{theor}(=6.39)$ $t_{calc}(=3.76) > t_{theor}(=2.13)$	

3.3. Photostability of tretinoin in creams

The photodegradation curves of the different tretinoin creams are shown in Fig. 5. Respectively, six and three replicates were performed for olive oil cream and for all the other creams, resulting in an average reproducibility of 5.06% (variation coefficient). This time, the olive oil cream is clearly the most stable one followed by the castor oil cream. The creams with maize oil, isopropyl myristate and Miglyol 812 are again comparable in stability, but they are far less stable than the two previous ones.

In comparison with the photodegradation of the oils, the cream with olive oil is surprisingly more stable than the one with castor oil, while there was no difference between these two oils (Fig. 3 and Table 2). However, microscopic investigation of the creams revealed the presence of tretinoin crystals in the olive oil cream while no precipitation of tretinoin was seen in the other creams. The concentration of tretinoin in olive oil (50 mg/30 ml) is approaching the saturated concentration (66 mg/30 ml), as described under Section 3.1, which can cause crystal growth, for example due to temperature changes. Since the degradation kinetics of a solid form is different than for a dissolved molecule, this can explain the remarkable stability of the olive oil cream.

As shown in Fig. 6, the courses of the degradation curves of tretinoin in the creams are somewhat different from the degradation curves of tretinoin in the oils. The degradation of tretinoin in the creams is steeper during the first 2 min, while after that period the creams seem to stabilize and they are much more stable than the oils at the end of the experiment. However, this can be due to the irradiation procedure, which is different for the creams (slides) than for the oils (cell); more specifically the lightpath and the sample's capacity are much smaller when the creams are irradiated. Also other cream ingredients or the formulation itself (emulsion) can influence the photodegradation, resulting in a different degradation kinetics than in the respective oils. As the photodegradation of tretinoin in an ethanol/propylene glycol (50/50) lotion is similar to the degradation in oils, the creams are also more stable than the lotion.

Comparison of our findings with the results obtained by other authors is not easy because of the different equipment and method of irradiation, they used and the lack of photodegradation studies of tretinoin formulated in creams or oils. However, Manconi et al. (2003) revealed that tretinoin, dissolved in methanol, degraded very quickly while the incorporation in

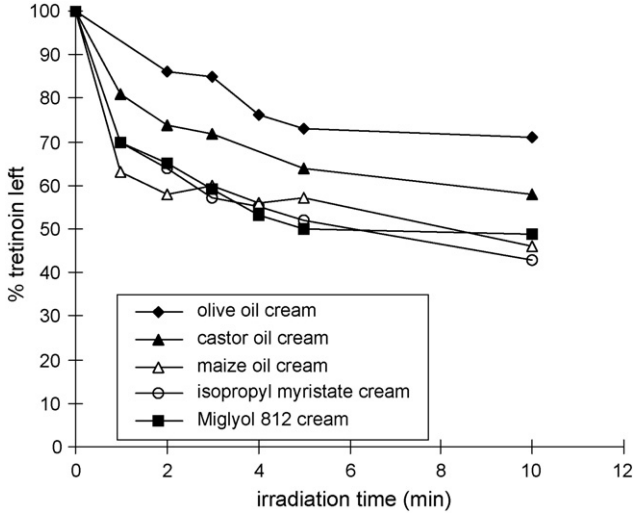

Fig. 5. Photodegradation curves of tretinoin in creams as a function of irradiation time.

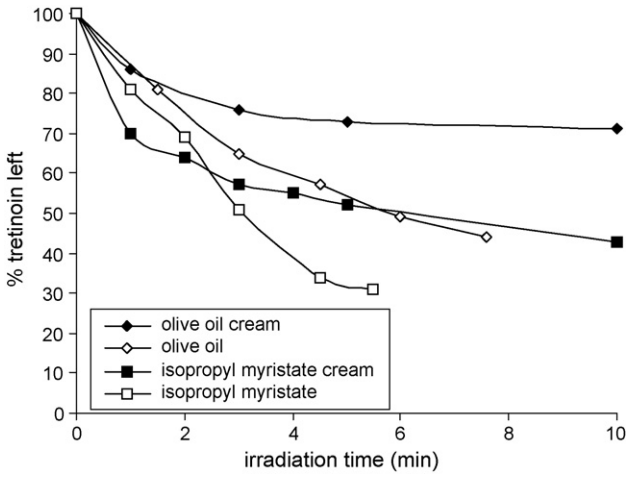

Fig. 6. Comparison between photodegradation curves of tretinoin in oils and in the respective creams.

vesicles (niosomes) led to a reduction of the photodegradation process. Also Ioele et al. (2005) concluded that tretinoin, incorporated in liposome complexes showed an increased stability in comparison to an ethanol solution. Even in another study, performed in our laboratory (Brisaert et al., 2001), it was seen that incorporation of tretinoin in liposomes resulted in two times slower photodegradation as compared to tretinoin dissolved in castor oil.

Generally, one can say that photodegradation of tretinoin seems to decrease when it is formulated in liposomes, niosomes or in the internal phase of o/w creams as compared to solutions of tretinoin.

4. Conclusion

From the five oils tested, tretinoin is best soluble in castor oil and worst in olive oil, while maize oil, isopropyl myristate and Miglyol 812 have similar, intermediate tretinoin solubilizing properties. The solubility of tretinoin in castor oil, the most polar one of the five, is comparable with its solubility in PEG 400, but it is still slightly soluble in terms of the European Pharmacopoeia.

The photostability of tretinoin in the same oils was also determined and it was comparable with the photostability of a tretinoin lotion (ethanol/propylene glycol, 50/50). Here again castor oil was the oil protecting best the drug against photodegradation, this time together with olive oil.

Finally, the photodegradation of tretinoin in o/w creams, prepared with the same oils as mentioned above, was studied after developing a simple irradiation system for creams, using microscope slides. The photostability of tretinoin was the highest in the olive oil cream, followed by the castor oil cream. However, the olive oil cream seemed to contain a lot of tretinoin crystals, which are less susceptible to photodegradation than the dissolved form.

The degradation pattern of the creams differed slightly from the oils, but it is not clear whether this is due to the cream formulation or due to the different irradiation technique.

As a conclusion one can say that the cream prepared with castor oil as oil phase seems to be the most suitable one, in terms of solubility of tretinoin and in terms of photostability.

References

Brisaert, M., Plaizier-Vercammen, J., 1997. Investigation of the emulsifying properties of Pemulen TR-1, an acrylic acid alkyl metacrylate copolymer. S.T.P. Pharma Sci. 7, 438–444.

Brisaert, M., Plaizier-Vercammen, J., 2000. Investigation on the photostability of a tretinoin lotion and stabilization with additives. Int. J. Pharm. 199, 49–57.

Brisaert, M., Gabriels, M., Matthijs, V., Plaizier-Vercammen, J., 2001. Liposomes with tretino: a physical and chemical evaluation. J. Pharm. Biomed. Anal. 26, 909–917.

Galvin, S.A., Gilbert, R., Baker, M., Guibal, F., Tuley, M.R., 1998. Comparative tolerance of adapalene 0.1% gel and six different tretinoin formulations. Br. J. Dermatol. 139, 34–40.

Halley, B.S., Nelson, E.C., 1979. High-performance liquid chromatography and proton nuclear magnetic resonance of eleven isomers of methyl retinoate. J. Chromatogr. 175, 113–123.

Ioele, G., Cione, E., Risoli, A., Genchi, G., Ragno, G., 2005. Accelerated photostability study of tretinoin and isotretinoin in liposome formulations. Int. J. Pharm. 293, 251–260.

Levin, A.A., Sturzenbecker, L.J., Kazmer, S., Bosakowski, T., Huselton, C., Allenby, G., Speck, J., Kratzeiden, C., Rosenberger, M., Lovey, A., Grippo, J.F., 1992. 9-*Cis* retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature 355, 359–361.

Lin, H.S., Chean, C.S., Ng, Y.Y., Chan, S.Y., Ho, P.C., 2000. 2-Hydroxypropyl-beta-cyclodextrin increases aqueous solubility and photostability of all-*trans*-retinoic acid. J. Clin. Pharm. Ther. 25, 265–269.

Manconi, M., Valenti, D., Sinico, C., Lai, F., Loy, G., Fadda, A.M., 2003. Niosomes as carriers for tretinoin. II. Influence of vesicular incorporation on tretinoin photostability. Int. J. Pharm. 260, 261–272.

Martin, B., Meunier, C., Montels, D., Watts, O., 1998. Chemical stability of adapalene and tretinoin when combined with benzoyl peroxide in presence and in absence of visible light and ultraviolet radiation. Br. J. Dermatol. 139, 8–11.

Martindale, 1999. The Complete Drug Reference, 32nd ed. Pharmaceutical Press, London.

Massart, D.L., Smeyers-Verbeke, J., Rius, F.X., 1989. Method validation: software to compare the slopes of two calibration lines with different residual variance. Trends Anal. Chem. 8, 49–51.

Nighland, M., Yusuf, M., Wisniewski, S., Huddleston, K., Nyirady, J., 2006. The effect of simulated solar UV irradiation on tretinoin in tretinoin gel microsphere 0.1% and tretinoin gel 0. 025%. Cutis 77, 313–316.

Nyirady, J., Lucas, C., Yusuf, M., Mignone, P., Wisniewski, S., 2002. The stability of tretinoin in tretinoin gel microsphere 0.1%. Cutis 70, 295–298.

Plewig, G., Wolff, H.H., Braun-Falco, O., 1971. Lokalbehandlung normaler und pathologischer menschlicher Haut mit Vitamin A-Säure. Arch. Klin. Exp. Derm. 239, 390–413.

Schlichting, D.A., Wooding, W.M., Brown, M., 1973. Optimizing dermatological formulations of retinoic acid. J. Pharm. Sci. 62, 388–391.