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Investigation on the photostability of a tretinoin lotion and stabilization with additives

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Abstract

Tretinoin, a drug that is used in topical preparations for the treatment of acne vulgaris, is known to be very susceptible to degradation under daylight. The objective of this work was to investigate the degradation of a tretinoin lotion placed in front of a xenon lamp. Analysis was performed with HPLC. The tretinoin lotion was degraded to about 20% of its initial concentration within 30 min. Incorporation of tretinoin in β-cyclodextrin or in some surfactants (Brij®s) did not have any effect on the photodegradation of tretinoin. Neither could a UV-B sunscreen retard the photodegradation of tretinoin while a UV-A sunscreen had very little effect. Irradiation with selected wavelengths revealed that 420 nm seemed to be the most harmful wavelength for the degradation of tretinoin and not the wavelength of maximum absorption (350 nm) as expected. Then the addition of the yellow colourants chrysoin and fast yellow, absorbing in the region of 420 nm, was tested. These colourants did indeed retard the photo-degradation of tretinoin more or less depending on the concentration of the dye. Finally we only had to select a concentration that was still effective but that did not colour the skin.

Keywords: Tretinoin; Topical lotion; Photostability; Stabilization with yellow colourants

1. Introduction

Tretinoin or all-trans retinoic acid or vitamin-A-acid is a drug that is used widely in topical preparations for the treatment of acne vulgaris, photo-aged skin, psoriasis and other skin disorders. We are particularly interested in its use to treat acne vulgaris and for that purpose, 0.05% (w/w) tretinoin is often incorporated in a lotion, a hydrogel or an o/w cream since acne patients suffer most of the time from excessive sebum production so that more greasy vehicles are contra-indicated. It was found in literature that tretinoin was most active in a lotion containing equal parts of propyleneglycol and ethanol (Plewig et al., 1970) and therefore this lotion composition was chosen as vehicle to investigate the photostability of tretinoin.

An investigation on the photostability of tretinoin seemed to be very interesting since the

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molecule has five conjugated double bounds in its structure and it is known that conjugated polyenes, like in the vitamin A series, are very labile to photoisomerization (Liu and Asato, 1984). An earlier study revealed that irradiation of a tretinoin solution leads to the formation of nine different isomers of which tretinoin itself (= all-trans-retinoic acid) and isotretinoin (= 13-*cis*-retinoic acid) are the most important ones (Motto et al., 1989). It is also known that a large amount of locally applied tretinoin is already degraded on the skin surface within 1–2 h (Elbaum, 1988) when exposed to sunlight.

The long wavelength UV-induced photoisomerization of tretinoin in physiologic-like solvents has been investigated. Additionally, the effect of a number of physiologically relevant compounds on the course of this photoisomerization was investigated. While addition of a non-ionic detergent had no effect, a number of proteins, as well as a phospholipid, completely inhibited this process (Curley and Fowble, 1988).

The principle of photoprotection of drugs by spectral overlapping has also been described (Thoma and Klimek, 1991). They revealed that the photodegradation of nifedipine could be retarded by yellow colourants which cover very well the long-wavelength nifedipine peak, that seems to be responsible for the photolysis. Also other drugs could be photoprotected by this principle: one has to search for a substance which spectrum covers the spectrum of the drug you want to protect.

The first objective of this work was to investigate the photodegradation kinetics of a tretinoin lotion and to see to what extent tretinoin is isomerized to isotretinoin, which is also therapeutically active against acne and other skin diseases (Chalker et al., 1987; Shalita, 1991; Steijlen et al., 1991; Armstrong et al., 1992; Hughes et al., 1992).

To investigate this we had to define determined irradiation conditions since it was found in literature (Thoma and Klimek, 1991) that the degradation kinetics of a photolabile drug depends on both the intensity and the spectral distribution of the light source used. We have chosen to use the xenon lamp as light source because specially filtered xenon arc sources are specified to be useful as artificial radiation systems for simulating natural conditions outdoors as well as behind window glass (Boxhammer, 1990).

Secondly, it was tried to diminish the breakdown of tretinoin by the use of different additives.

A first group of additives were those that could protect the drug on a physical way by incorporation: surfactants, cyclodextrins and proteins. A second group of additives tried were those that absorb light themselves: sunscreen agents, both UV-A and UV-B, and other molecules like dyes. Additionally, a new polymer that will increase the Sun Protection Factor (SPF) value of sunscreens was evaluated in combination with the UV-A filter. This polymer is not an active ingredient itself, but has to be used in combination with an UV filter. The polymer enhances the chance that the radiation meets a filter molecule by scattering it (Leaflet Rohm and Haas Company).

2. Experimental methods

2.1. Materials

Tretinoin (BASF, B-Brussels) was used as active compound and denatured ethanol and propyleneglycol (Fraver, B-Kontich) as solvents to prepare the lotion. Brij® 30, 35, 78, 92, 96 and 98, used as surfactants were purchased from ICI-Surfactants (B-Everberg) and Kleptose (Roquette, F-Lestrem) was used as β-cyclodextrin. Uvinul D 50 (BASF, B-Brussels) and Parsol 1789 (Givaudan, CH- Geneve) were used respectively as UV-B and UV-A sunscreen and Acudyne™290 (Rohm and Haas Company, I-Gessate Milan) was used as formulation aid polymer that was tried to increase the effect of the sunscreen. Chrysoin and Fast yellow were used as colourants (Aldrich Chemie, B-Bornem).

Acetonitrile HPLC-S, gradient grade (Biosolve, N-Valkenswaard), Milli-Q water purified with an Millipore apparatus and acetic acid, glacial 100%, p.a. (Merck, D-Darmstadt) were used as HPLC solvents.

2.2. Composition of the solutions

The starting lotion consisted of 0.05% (w/v) tretinoin, 50 ml of propyleneglycol and 100 ml of ethanol. To protect this lotion from degradation, the following additives were added before the solutions were brought to 100 ml with ethanol: 10% (w/v) of each Brij®, 1.5% (w/v) β-cyclodextrin, 0.2% (w/v) Uvinul D 50, 0.1% and 1% (w/v) Parsol 1789, 1% (w/v) Parsol 1789 together with 5% (w/v) Acudyne™290 solenoids, 0.010–0.200% (w/v) chrysoin and 0.010–0.100% (w/v) fast yellow.

All these solutions were prepared with minor red light and stored in the dark to exclude isomerization before the irradiation with the xenon lamp was started.

2.3. Irradiation of the solutions

To irradiate the solutions, a XBO 450 W high pressure xenon lamp was used connected with a Siemens VX 501 r-5b apparatus to adjust the current at 14 A and the voltage at 22 V and a sample holder house with a quartz cuvet of 1 cm pathlength or one of 0.1 cm containing the solution, placed at a distance of 28 cm from the xenon lamp. The 0.1 cm cuvet was only used to irradiate the solution containing Acudyne™290 because of the milky character of this solution. The temperature in the cuvet during the irradiation never exceeded 36°C. At this temperature, thermal instability problems could not occur since a previous investigation (Brisaert et al., 1995) showed that the lotion, stored at 37°C, was stable for months.

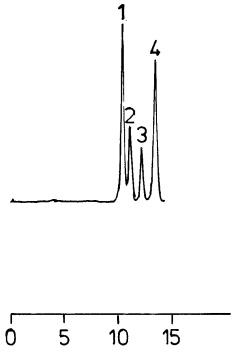

Fig. 1. Chromatogram of tretinoin and its isomers. Peak 1, isotretinoin; peak 2, isomer of tretinoin; peak 3, isomer of tretinoin; peak 4, tretinoin.

To define the wavelength of intrinsic instability, a Carl Zeiss M4 QIII monochromator was placed between the xenonlamp and the sample holder house using a slit width of 2 nm.

2.4. HPLC method

The HPLC method was based on a previous work (Mensink et al., 1987) and consisted 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 changed gradually from methanol–water–acetic acid (75–12.5–1 volumes) to acetonitrile–water–acetic acid (80–20–1 volumes) to separate tretinoin from its isomers. Subsequently, the flow was changed from 1.8 to 1 ml/min and the UV-detection was changed from 320 to 350 nm, the wavelength of maximum absorption of tretinoin.

With this HPLC system, four isomers of tretinoin could be separated as shown in Fig. 1, the first eluting being identified as isotretinoin (= 13-*cis*-retinoic acid) and the last eluting as tretinoin (= all-trans-retinoic acid), the two most important isomers. This separation of four isomers was as good as in a previous study (Elbaum, 1988) but other authors separated seven isomers (Mc Kenzie, 1978; Curley and Fowble, 1988). Nevertheless, in our study the quantitative determination of tretinoin itself was the most important and this isomer was well separated from the other ones.

Unfortunately, for the analysis of the tretinoin lotion containing the UV-A sunscreen, Parsol 1789 was eluting immediately before tretinoin, when using this HPLC system, in a very big peak that partly covered the peak of tretinoin, as shown in Fig. 2. Small changes in mobile phase composition, did not resolve this problem maintaining the separation of the isomers of tretinoin.

When we take a look at the absorption spectra of tretinoin and the sunscreens plotted in Fig. 3, we see that the big peak of Parsol 1789, used in a

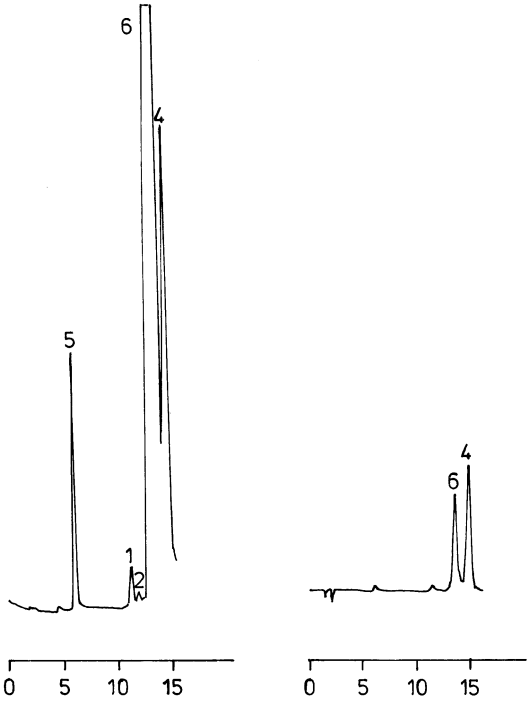

Fig. 2. Chromatograms of a tretinoin lotion containing Parsol 1789. Left side, detection wavelength is 350 nm; right side, detection wavelength is 403 nm. Peak1, isotretinoin; peak 2, isomer of tretinoin; peak 3, isomer of tretinoin (invisible); peak 4, tretinoin; peak 5, Parsol 1789 (first peak); peak 6: Parsol 1789 (second peak).

concentration of 1% (w/v), in the chromatogram is due to a very high absorption at 350 nm. From the same figure, it is seen that the absorption of Parsol 1789 becomes lower than that of tretinoin at about 400 nm. So the detection wavelength of the HPLC system was shifted to 400 nm and more and after some trials, the best resolution between the peaks of Parsol 1789 and tretinoin was obtained at a wavelength of 403 nm. The analysis of the tretinoin lotions containing the UV-A filter was always performed with the chosen HPLC system at a detection wavelength of 403 nm, chromatogram shown in Fig. 2. Because of the smaller peaks of both tretinoin and Parsol 1789 at this wavelength, the sensitivity should be increased for quantitative analysis.

3. Results and discussion

3.1. Degradation of tretinoin

The photodegradation of the tretinoin lotion is very fast under the used circumstances, as shown in Fig. 4. Photodegradation follows first order kinetics and reaches an equilibrium after 25–30 min of irradiation. At this photostationary state, only about 20% of the initial tretinoin concentration is left. Measurements after 1 and 1.5 h irradiation, revealed that the content of tretinoin was still about 20%.

The most important isomer is isotretinoin and since isotretinoin is also therapeutically active, the formation of this isomer as a function of time was followed too as shown in Fig. 4. It can be concluded from the figure that the formation of isotretinoin was not at the same extent as the

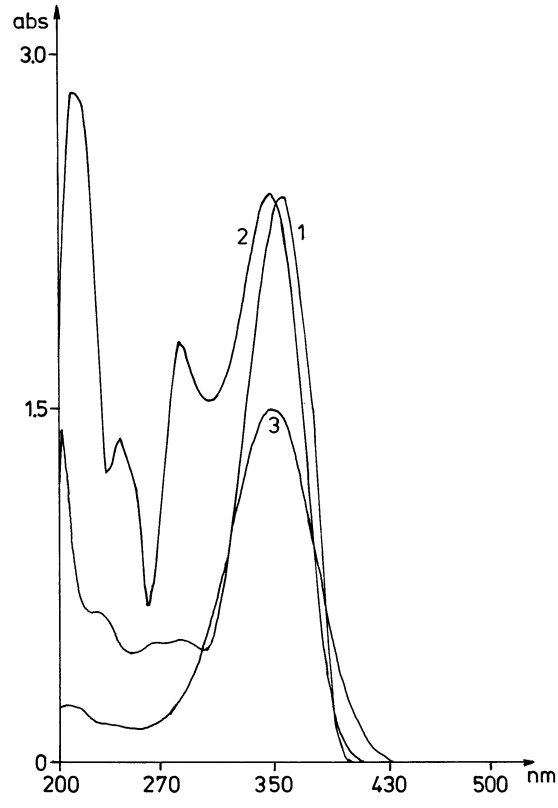

Fig. 3. Absorption spectra, (1) Parsol 1789 0.1% (w/v); (2) Uvinul D 50 0.2% (w/v); (3) Tretinoin 0.05% (w/v).

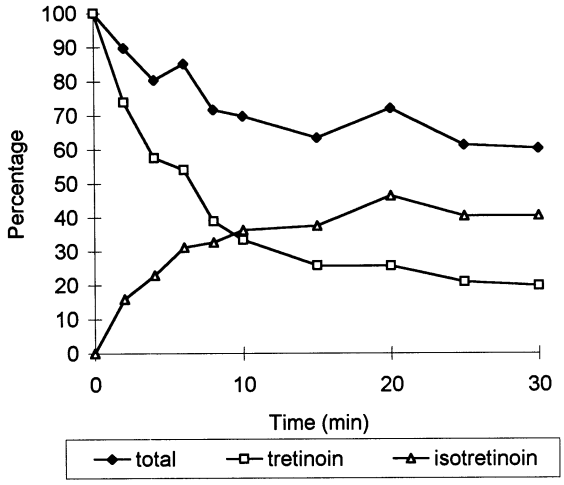

Fig. 4. Concentration of tretinoin and isotretinoin in an irradiated tretinoin lotion as a function of irradiation time.

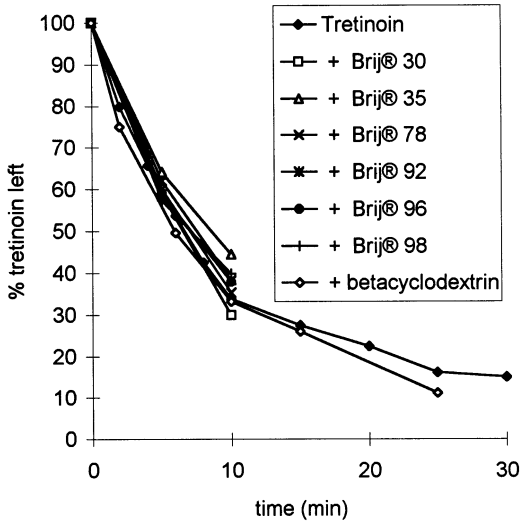

Fig. 5. Degradation curves of tretinoin in addition of surfactants and cyclodextrin.

degradation of tretinoin. At the photostationary state, about 40% of tretinoin is isomerized to isotretinoin. Plotting the sum of the content of tretinoin and isotretinoin as a function of time reveals that there is a loss in active compound anywhere and at the photostationary state, about 40% of the initial tretinoin concentration is isomerized to inactive isomers. This loss in active compound makes it useful however to protect the lotion against photodegradation.

3.2. Photoprotection by physical processes

Some surfactants (Brij®s) were added to the lotion because they can form micelles in which molecules can be incorporated and we assumed that the incorporated tretinoin could be protected from irradiation by that way. For the same reason also β-cyclodextrin was used as it can be seen as a cylinder in which inside a molecule can be entrapped. Unfortunately, neither of these additives has a real effect on the photodegradation of tretinoin, as shown in Fig. 5. However, the curve of Brij® 35 seemed to be statistical different from the tretinoin curve as tested with a Student's *t*-test.

The fact that tretinoin is too well soluble in the ethanol-propyleneglycol mixture of the lotion so that it is not placed in the inside of the surfactant micelles or the cyclodextrin, can be an explanation of the failure of photoprotection by these additives since the lotions were prepared as such by mixing all the ingredients together.

The addition of bovine serum albumin, described to inhibit the photodegradation of tretinoin completely even after 3 h of irradiation (Curley and Fowble, 1988), was not tried because we did not work in physiologic like solvents and the concentration of tretinoin used in our experiments was ca. 2500 times higher than in the article so that we suspected this to cause problems.

Since the additives tried to protect tretinoin against photodegradation in a physical way did not satisfy, chemical substances were investigated in a second step, more specifically UV-filters.

3.3. Photoprotection by UV-filters

One of the most used chemical substances for photoprotection are the sunscreens or the UV-screens. Thoma and Klimek described the principle of photoprotection by spectral overlapping (Thoma and Klimek, 1991). This means that since tretinoin shows an absorption maximum around 350 nm, as shown in Fig. 3, a protective effect should be achievable by using substances that absorb in this region. We have chosen for one

UV-B filter (Uvinul D 50) and one UV-A filter (Parsol 1789), both having an absorption maximum around 350 nm and thus covering the spectrum of tretinoin pretty well, as you can see in Fig. 3.

Also the effect of a new polymer that will increase the Sun Protection Factor value of sunscreens, Acudyne™ 290, was investigated.

As Fig. 6 demonstrates, the UV-B filter Uvinul D 50 has no visible protecting effect on the photodegradation of tretinoin and also the protecting effect of the UV-A filter, used in the same concentration (0.1% w/v) as in Fig. 3 (degradation curve not shown) and even in a ten times higher concentration (1% w/v), is very small. Moreover, we can not say that the combination of Parsol 1789 and Acudyne™290 is really better than the sunscreen alone. This can be explained by the fact that the polymer is actually a bead with a core, that in the emulsion form is filled with water. Upon application of the sunscreen product containing the polymer on the skin and with drying of the normal sunscreen film, the water irreversibly migrates from the center, leaving an air-filled center. This entrapped air acts as an efficient scattering center to increase the pathlength of the radiation through the sunscreen formulation. In our experiment, on contrary, the solution is not applied on the skin and for that matter, the inside of the polymer is still filled with water, so that it can not scatter the radiation.

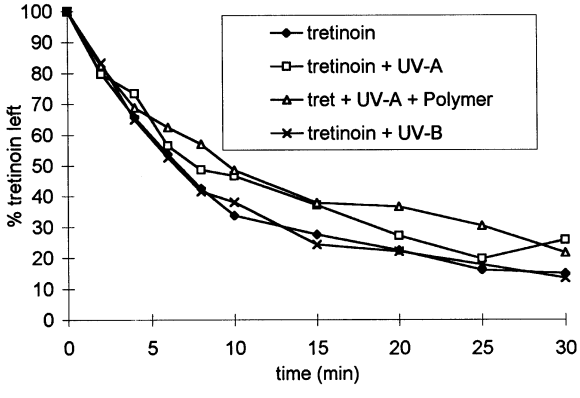

Fig. 6. Degradation curves of tretinoin in addition of UV-filters.

An explanation of the failure of the photoprotecting effect of the UV filters, despite the fact that the spectrum of tretinoin is reasonably well covered, is not found yet.

In a next experiment, we will investigate if the wavelength region of maximal absorbance corresponds with the region of intrinsic instability of the product as it was assumed earlier.

3.4. Determination of the wavelength region causing photodegradation

Since the results were not satisfactory until now, it seemed to be interesting to investigate which wavelength of the spectrum is responsible for the photodegradation of tretinoin. In a first experiment, the tretinoin lotion was irradiated for 2 h with monochromatic light of, respectively 350, 400, 450, 500, 550 and 600 nm. We irradiated now for a longer time as we did with the whole spectrum because the intensity and the energy of the radiation at a specific wavelength is lower. After 2 h of irradiation, there was not enough isomerization to determine quantitatively but we compared the chromatograms obtained. The isomers were best distinguishable at the chromatogram when the lotion was irradiated at 400 and 450 nm. At the irradiation wavelengths of 350 and 500 nm the isomers were less clear at the chromatogram while no isomers at all were distinguishable when irradiated at 550 and 600 nm although the intensity of the xenonlamp is higher in this higher wavelength regions than at lower regions. This reveals already that the wavelength of instability is situated around 400–450 nm and not at the wavelength of maximum absorption (350 nm) as we assumed. Secondly, the lotion was irradiated during 3 h at wavelengths between 380 and 460 nm, and the percentage of tretinoin was determined quantitatively and plotted as a function of irradiating wavelength, as shown in Fig. 7. From this figure, it can be deduced that the wavelength of maximal degradation is situated more specifically at 420 nm. This can be the reason why the UV-sunscreens do not have any effect on the photodegradation, because they do not absorb at that wavelength.

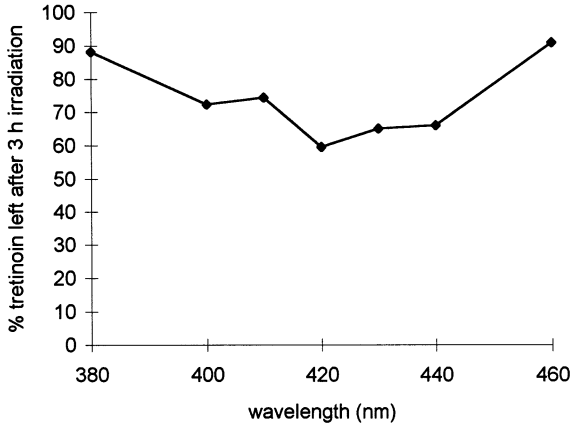

Fig. 7. Determination of the most harmful wavelength for photodegradation.

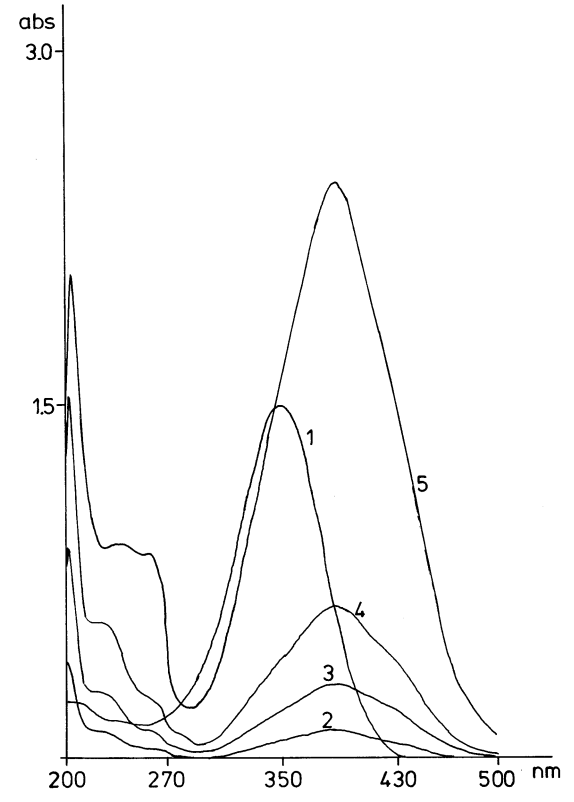

Fig. 8. Absorption spectra, (1) tretinoin 0.050% (w/v); (2) chrysoin 0.010% (w/v); (3) chrysoin 0.025% (w/v); (4) chrysoin 0.050% (w/v); (5) chrysoin 0.200% (w/v).

Now that we know this new element, we have to look for protecting substances that absorb specifically around 420 nm. Thoma and Klimek demonstrated in their work (Thoma and Klimek, 1991) that the yellow colourants mainly absorb in the region of 400–450 nm, and therefore the effect of some yellow colourants is investigated in a next part of the study.

3.5. Photoprotection by yellow colourants

A first yellow colourant used, was chrysoin. The absorption spectrum of different chrysoin concentrations (0.010, 0.025, 0.050, 0.200% (w/v)) dissolved in the lotion and 50 times diluted with ethanol, is given in Fig. 8, together with the spectrum of a tretinoin 0.05% (w/v) lotion identically diluted. The figure reveals that only the 0.0200% (w/v) concentration covers the spectrum of tretinoin almost completely, while the other concentrations only cover the relevant long wavelength region of tretinoin starting from about 400 nm. The reason why we tested different concentrations is because the yellow colourants colour the skin when the lotion is applied, so that we want to use an as low concentration colourant as possible but that still has photoprotecting properties.

The degradation curves of the tretinoin lotion with different concentrations of chrysoin are given in Fig. 9 and the degradation constants are given in Table 1. From the figure and the table, we can deduce that all the concentrations of chrysoin do protect tretinoin against photodegradation. The concentration of 0.200% (w/v) protects the most, the degradation constant ($k = 0.006$ min^{-1}) is eight times lower than when no colourant is added ($k = 0.048$ min^{-1}). The lotions with chrysoin concentrations of 0.050, 0.025 and 0.010% (w/v) are, respectively about five times ($k = 0.009$ min^{-1}), 3.5 times ($k = 0.013$ min^{-1}) and 2.5 times ($k = 0.018$ min^{-1}) more stable than the native lotion.

These results match with Thoma's proposal of spectral overlapping but it is remarkable that the photoprotecting capacity of a 0.010% (v/v) chrysoin solution is much lower than that of a 0.025% (v/v) solution as shown in Fig. 9. This can be explained by the absorption spectra showed in

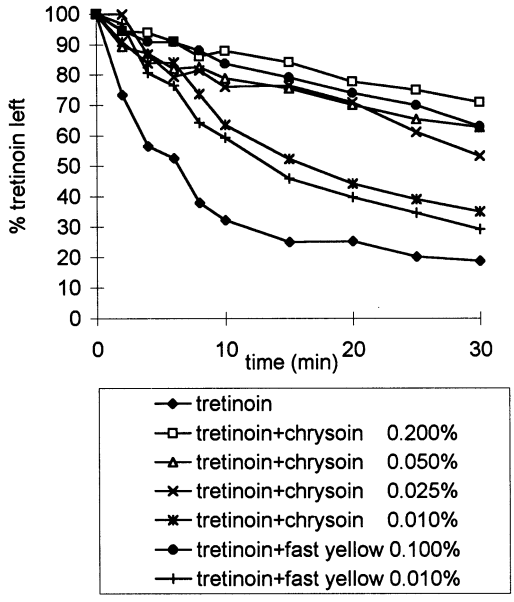

Fig. 9. Degradation curves of tretinoin in addition of yellow colourants.

Fig. 8: at 420 nm, the wavelength of intrinsic instability as stated previously, the absorption of the 0.010% (v/v) chrysoin solution is roughly identical to the absorption of the tretinoin solution, while the other chrysoin concentrations have a far higher absorption than tretinoin at this specific wavelength.

Application of the different concentrations on the skin, showed a yellow-orange colouring of the skin for the concentrations 0.200 and 0.050% (w/v) but not for the 0.025% and the 0.010% (w/v) solution. So the concentration of 0.025% (w/v) seemed the most suitable concentration of chrysoin to be used since it did not colour the skin and it did protect tretinoin to a greater extent than the 0.010% (w/v) concentration.

Also another colourant, fast yellow, was added to the tretinoin lotion and this substance also protected tretinoin against photodegradation as shown in Fig. 9 and Table 1, although less than chrysoin. A 0.100% (w/v) concentration of fast yellow retarded the photodegradation of tretinoin about seven times ($k = 0.007$ min^{-1}) and the 0.010% concentration two times ($k = 0.024$ min^{-1}).

Table 1
Degradation constants of different tretinoin lotions containing colourants

Composition of the lotion	Degradation constant (min^{-1})
0.05% (w/v) tretinoin lotion	0.048
0.05% (w/v) tretinoin lotion + 0.200% (w/v) chrysoin	0.006
0.05% (w/v) tretinoin lotion + 0.050% (w/v) chrysoin	0.009
0.05% (w/v) tretinoin lotion + 0.025% (w/v) chrysoin	0.013
0.05% (w/v) tretinoin lotion + 0.010% (w/v) chrysoin	0.018
0.05% (w/v) tretinoin lotion + 0.100% (w/v) fast yellow	0.007
0.05% (w/v) tretinoin lotion + 0.010% (w/v) fast yellow	0.024

4. Conclusions

This study has shown that the photodegradation of a tretinoin lotion placed in front of a xenon lamp was very fast causing different isomers and resulting in a loss of active compound. Many additives like surfactants, cyclodextrins and UV-filters failed in the photoprotection of the drug. But since the wavelength that causes the degradation is situated at 420 nm, the only additives that can be used to retard this photodegradation are yellow colourants like chrysoin and fast yellow. These colourants, however, have to be used in as small concentrations as possible so that they do not colour the skin.

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