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UVA is the major contributor to the photodegradation of tretinoin and isotretinoin: Implications for development of improved pharmaceutical formulations

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Abstract

The chemical stability of tretinoin (RA) and isotretinoin (13RA) in ethanol and dermatological cream preparations exposed to solar simulated light (SSL), UVA, and visible light has been studied. Photostability was monitored by an HPLC method that allowed simultaneous analysis of RA and 13RA, thus allowing photodegradation due to isomerization to other retinoids and photolysis to non-retinoid products to be monitored. Both retinoids undergo both isomerization and photolysis following SSL, UVA and visible light exposure but RA is more sensitive to photodegradation than 13RA. Degradation of both retinoids by photolysis is considerably greater in cream formulations than in ethanol and the photodegradation follows second order kinetics. Rate constants and half-lives for degradation of RA and 13RA in ethanol solution and cream preparations subjected to different light sources are reported. The UVA component of SSL is the major contributor to photodegradation. Since UVA penetrates deeply into skin, our results suggest that photodegradation of RA may contribute to the photosensitivity associated with RA therapy. Our studies suggest that development of improved formulations and the use of effective UVA sunscreens may reduce the side effects of RA therapy.

Keywords: Retinoic acid; Photostability; UVA; Photoisomerization; Photolysis

1. Introduction

Retinoids are involved in several biological processes, particularly proliferation and differentiation (Fisher and Voorhees, 1996). Three major isomers shown to be biologically active in human tissues include all-trans retinoic acid or tretinoin (RA), 13-*cis* retinoic acid or isotretinoin (13RA) and 9-*cis* retinoic acid (9RA) or alitretinoin (Lovat et al., 1997). Both RA and 13 RA are widely used in treatment of various dermatological conditions that include acne vulgaris, psoriasis, photodamaged skin and other skin disorders (Layton and Cunliffe, 1992; Orfanos et al., 1997; Chivot, 2005; Kang et al., 2005). Retinoids have been shown to undergo degradation when exposed to light generating RA isomers of which 13RA and 9RA are the most prevalent (Curley and Fowble, 1988; Lucero et al., 1994; Brisaert et al., 1995; Cahnmann, 1995; Brisaert and Plaizier-Vercammen, 2000; Ioele et al., 2005). Light exposure also leads to degradation to non-retinoid products (Curley and Fowble, 1988). The degradation of RA and 13RA in both the solid state and solution has been studied previously (Tan et al., 1992, 1993). The chemical stability of tretinoin in dermatological preparations has been investigated by Brisaert and coworkers who have observed that RA photodegradation in lotions is rapid with the most harmful wavelength for degradation at 420 nm and not the wavelength of maximum absorption at 350 nm (Brisaert et al., 1995; Brisaert and Plaizier-Vercammen, 2000). For 13RA, hydoxypropyl-β-cyclodextrin delays photodegradation and minimizes isomerization (Yap et al., 2005) and inclusion of RA in liposomes has been reported to protect against photodegradation (Brisaert et al., 2001). The chemical stability of RA in ethanol and niosomes under UV and artificial light conditions has been studied and incorporation of RA in niosomes

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led to reduce the rates of the photodegradation (Manconi et al., 2003). The photodegradation process of RA and 13RA in ethanol and liposomes has been studied by UV spectrophotometry and RA was found to rapidly isomerize in ethanol to 13 RA and 9RA with first-order kinetics (Ioele et al., 2005). Other studies also have reported RA degradation to follow first-order kinetics (Brisaert and Plaizier-Vercammen, 2000; Ioele et al., 2005) while studies reporting degradation to follow zero-order kinetics have appeared (Manconi et al., 2003).

We report here studies of the photodegradation of RA and 13RA using a recently developed analytical method (Tashtoush et al., 2007) that allow the distinction between degradation occurring by isomerization to other RA isomers from degradation occurring by photolysis to non-retinoid products. Our results show that the rates of photolysis are greater in cream formulations than in ethanol solution, that photodegradation of RA in ethanol solution and cream formulations follows second-order kinetics, and that the UVA portion of solar light accounts for a major portion of RA and 13RA photodegradation.

2. Experimental methods

2.1. Chemicals and reagents

Tretinoin, isotretinoin and trifluoroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile and ethanol (HPLC grade) were purchased from EMD Chemicals Inc. (Gibbstown, NJ, USA). Brij-58, glyceryl monostearate, cetostearyl alcohol, white petrolatum, sorbic acid, butylated hydroxytoluene, simethicone, sorbitol 70% solution, propylene glycol and polyethylene glycol were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Double distilled de-ionized water was used.

2.2. HPLC instrumentation and conditions

An HPLC system consisting of Varian Pro-star solvent delivery system model 230 (Varian Chromatography systems, CA, USA), connected to a UV/Visible Spectroflow 757 absorbance detector (ABI, NJ, USA) and HP 3395 integrator (Hewlett-Packard, USA) was used for all experiments. The injector was fitted with a loop of 50 μl. Reversed-phase chromatographic separation was performed using a Nucleosil 5μ C18 100A, 250 mm × 4.6 mm column (Phenomenex, CA, USA). The detection wavelength was set to 342 nm. The mobile phase used was composed of 0.01% trifluoroacetic acid (TFA) and acetonitrile (15:85, v/v%) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45μ membrane filter (Advantec MFS Inc., CA, USA).

2.3. Cream preparations

Tretinoin (0.025%) and isotretinoin (0.025%) creams contained the following components: water, propylene glycol, sorbitol, sorbic acid, butylated hydroxytoluene, simethicone, white petrolatum, cetostearyl alcohol, Brij-58, glyceryl monostearate, polyethylene glycol and tretinoin or isotretinoin. The ratio of oil phase to aqueous phase was (27.5:72.5%, w/w). The water phase was mixed and dissolved in one container at 65–75 °C and the oil phase was melted and mixed in another container at 65–75 °C using a water bath. The oil phase was then added to the water phase and mixed until a cream was formed using an IKA mixer model RW 20DZM (IKA-works Inc. NC, USA). The cream was cooled to room temperature while stirring.

2.4. Standard stock solutions

Stock solutions containing 1 mM of tretinoin or isotretinoin were prepared in ethanol. To compare with cream preparations, solutions containing 0.025% tretinoin and 0.025% isotretinoin were prepared in ethanol for photostability studies.

2.5. Irradiation conditions

A kilowatt (kW) large area light source solar simulator, model 91293, from Oriel Corporation (Stratford, Connecticut) was used, equipped with 1000 W Xenon lamp power supply, model 68920, and a VIS-IR band pass blocking filter plus either an atmospheric attenuation filter (output 290–400 nm plus residual 650–800 nm, for SSL) or UVB and UVC blocking filter (output 320–400 nm plus residual 650–800 nm, for UVA), respectively. The output was quantified using a dosimeter from international light Inc. (Newburyport, MA), model IL 1700, with an SED240 detector for UVB (range 265–310 nm, peak 285 nm), or a SED033 detector for UVA (range 315–390 nm, peak 365 nm) at a distance of 36.5 cm from the source, which was used for all experiments. At 365 nm from the source, the SSL dose was 7.63 mJ/cm^2/s UVA and 0.40 mJ/cm^2/s UVB radiation. Using a UVB/UVC blocking filter, the dose at 365 nm from the source was 5.39 mJ/cm^2/s UVA radiation with residual UVB dose of 3.16 μJ/cm^2/s.

For irradiation with visible light, Luzchem expo Panels composed of 5 Sylvania 8-W cool white light tubes was used to deliver visible light at an irradiance of 2.34 mJ/cm^2/s. The irradiance in the visible region (400–700 nm) was determined using a digital light meter, Model SLM-110 from (A.W. Sperry Instruments Inc., NY, USA), at 20 cm from the light source. For photostability studies in ethanol, a 1.0 ml solution containing 0.025% tretinoin or isotretinoin in ethanol was placed into 1.5 ml Eppendorf centrifuge tubes. At the appropriate times of irradiation, a tube was placed into an amber container covered with aluminum foil until the time of analysis. For photostability studies in cream formulations, a 1 g sample of tretinoin or isotretinoin was spread uniformly over the cover of a 35 mm tissue culture dish. At the appropriate times of irradiation, the sample covered with aluminum foil until time of analysis.

2.6. Sample preparation

A 0.5 gm sample of cream formulations was taken from the dish and weighed into a 50 ml conical centrifuge tube using a Mettler balance model PB-303S (Mettler-Toledo, Switzerland). The sample was dissolved into 30 ml acetonitrile by vortex mixing for 3 min then 1.5 ml was transferred to an Eppendorf

centrifuge tube and subjected to centrifugation at 10,000 rpm for 5 min using an Eppendorf microcentrifuge (Brinkmann Inst Inc., NY, USA). An aliquot of 50 μl of the supernatant was injected directly into the HPLC. For photostability studies in ethanol, 0.5 ml was withdrawn from the centrifuge tube and mixed with 30 ml ethanol, then injected directly into HPLC.

3. Results and discussion

3.1. Photostability of retinoic acids in ethanol solution

Studies of RA and 13RA in ethanol solution following exposure to SSL demonstrated that RA conversion to other products was very rapid as 80% of the original RA was gone by 2 min of exposure (Fig. 1A). A major product was 13RA as 40% of the RA originally present was rapidly converted to this product after which a slow loss of both RA and 13RA was observed. The chromatographic conditions used in this study were able to detect 9RA and other RA isomers and no significant amount of these RA isomers were detected. Compared to RA, the loss of 13RA was slower as 40% of 13RA still remained after 5 min and only 20% of the 13RA originally present was recovered as RA and a slow loss of RA was observed with increasing times of irradiation (Fig. 1B). These results demonstrate that two types of reactions were occurring for both RA and 13RA, photoisomerization to other RA isomers and photolysis to non-retinoid degradation products. Similar rates of photodegradation for RA and 13RA were observed when only the UVA portion of SSL was used as an irradiation source (Fig. 1C and D). Following exposure to visible light, rates of photodegradation of both RA and 13RA were slower than irradiation by SSL and UVA, but again the rate of RA loss was greater than that of 13RA (Fig. 1E and F). Our results show that in ethanol solution that RA is less photostable than 13RA, photoisomerization between RA and 13RA is a major but not exclusive component of degradation, and the UVA portion of the SSL accounts for the majority of the degradation caused by SSL.

3.2. Photostability of retinoic acids in cream preparations

The photodegradation of RA and 13RA cream preparations was also carried out under SSL, UVA and visible light irradiation. Irradiation of RA by SSL caused rapid isomerization to 13RA but 13RA also was degraded following its formation.

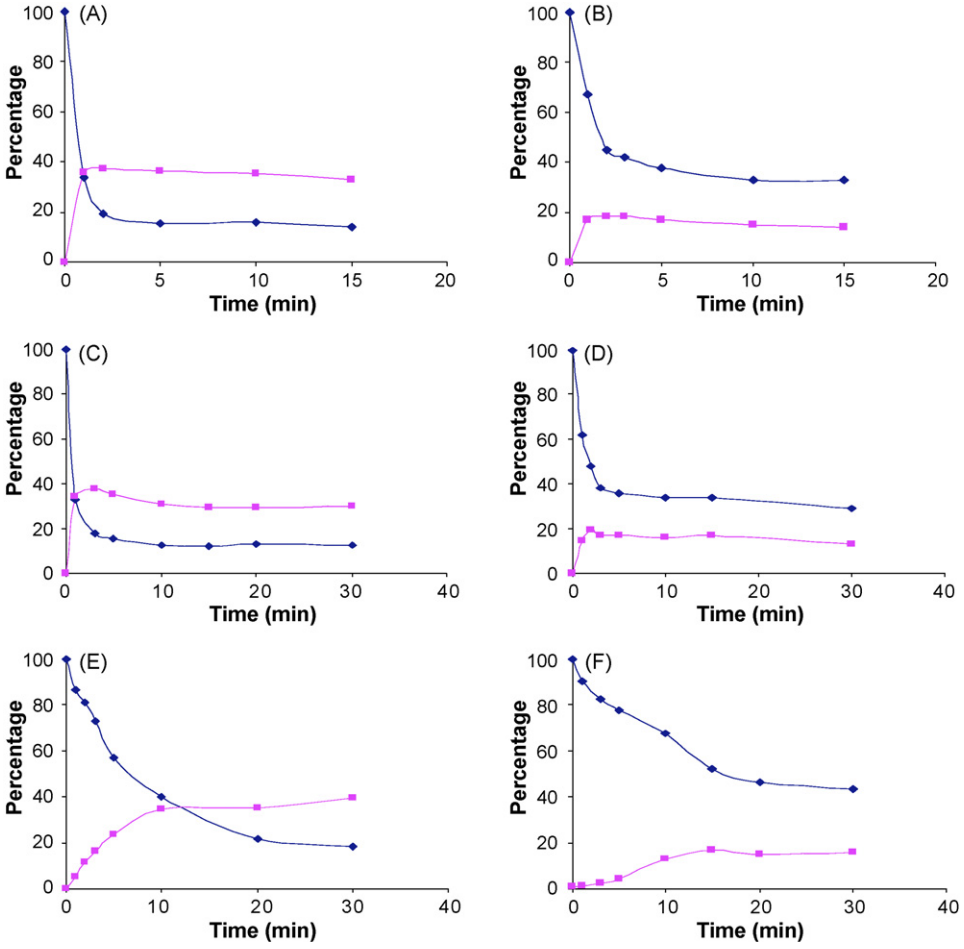

Fig. 1. Concentration of RA (diamonds) and 13RA (triangles) as a function of time in irradiated ethanol solutions. Degradation of RA formulations is shown in panels (A), (C), and (E) and degradation of 13RA formulations is shown in panels (B), (D), and (F). Panels (A) and (B) show SSL, panels (C) and (D) show UVA and panels (E) and (F) show visible light.

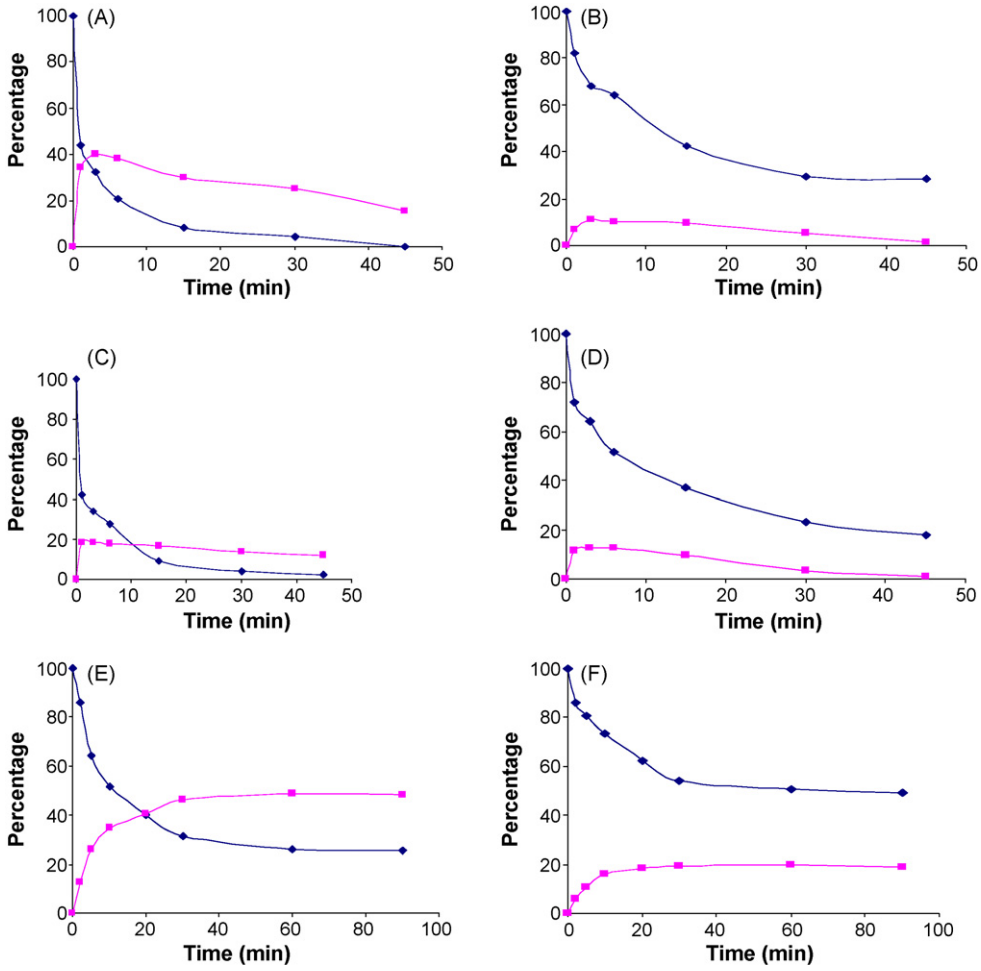

Fig. 2. Concentration of RA (diamonds) and 13RA (triangles) as a function of time in cream formulations. Degradation of RA formulation is shown in panels (A), (C), and (E) and degradation of 13RA formulations is shown in panels (B), (D), and (F). Panels (A) and (B) show SSL, panels (C) and (D) show UVA and panels (E) and (F) show visible light.

The degradation of RA when irradiated by SSL showed that no detectable RA material remained after 45 min (Fig. 2A). Again, 13RA was degraded at a slower rate than RA as approximately 40% of the original material remained after a 45 min exposure (Fig. 2B). Qualitatively similar results were observed for UVA (Fig. 2C and D) and for visible light (Fig. 2E and F) although the greater degree of photodegradation observed in the cream formulation for SSL and UVA was not observed for visible light.

3.3. Determination of kinetic parameters of photodegradation

The photochemical reaction in ethanol solution demonstrated that RA isomerizes to 13RA and vice versa, with both reactions reaching equilibrium by 15 min. Therefore the rate of photodegradation was calculated from the initial rate by measuring the concentration remaining of either RA or 13RA. As can be seen in Table 1, the rate constants were obtained from a slope of a curve fit of a plot of 1/concentration remaining versus time. The calculated half-life values were calculated according to the equation describing second-order kinetics and were found to be 7.21, 0.93, and 0.65 min when RA was irradiated by visible light, UVA, and SSL, respectively. The corresponding half-life values for 13RA were 19.5, 2.40, and 2.26 min, demonstrating that degradation of RA is higher than 13RA under all conditions of irradiation. The rate constants and half-lives of RA and 13RA in cream formulations irradiated with different light sources (Table 2) show that, while the rates of degradation are more rapid in the cream formulations relative to ethanol, the same relative pattern of degradation for RA and 13RA was observed. The photodegradation of RA in ethanol and cream formulations was found to follow second-order kinetics as a plot

Table 1
Rate constants of degradation of for RA and 13RA in ethanol solution[a]

Retinoic acid	Source	k (l/mmol min)	$t_{0.5}$ (min)	r^2
RA	SSL	1.82 ± 0.17	0.65 ± 0.08	0.998
	UVA	1.28 ± 0.13	0.93 ± 0.10	0.982
	Visible	0.16 ± 0.02	7.22 ± 0.19	0.986
13RA	SSL	0.53 ± 0.12	2.26 ± 0.12	0.985
	UVA	0.49 ± 0.18	2.40 ± 0.27	0.997
	Visible	0.06 ± 0.01	19.5 ± 0.93	0.985

[a] $n = 3$.

Table 2
Rate constants of degradation of for RA and 13RA in cream preparations[a]

Retinoic acid	Source	k (l/mmol min)	$t_{0.5}$ (min)	r^2
RA	SSL	1.11 ± 0.16	1.07 ± 0.20	0.997
	UVA	0.92 ± 0.13	1.30 ± 0.15	0.986
	Visible	0.09 ± 0.01	13.1 ± 0.32	0.988
13RA	SSL	0.08 ± 0.01	14.0 ± 1.31	0.992
	UVA	0.11 ± 0.01	11.3 ± 0.94	0.994
	Visible	0.03 ± 0.005	41.0 ± 2.13	0.981

[a] $n = 3$.

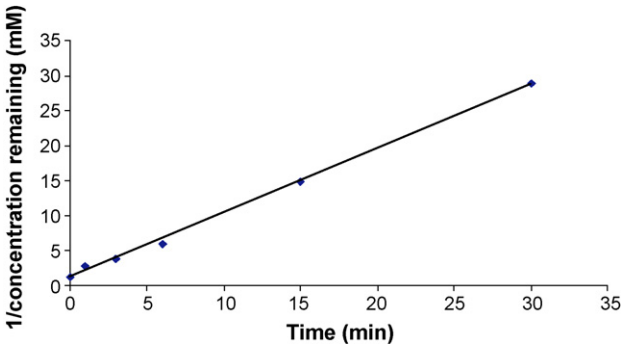

Fig. 3. Kinetic analysis of photodegradation of RA in a cream formulation irradiated with SSL.

of 1/concentration remaining versus time gave a straight line with $R^2 = 0.998$ (Fig. 3). This observation is in contrast to earlier studies (Brisaert and Plaizier-Vercammen, 2000; Manconi et al., 2003; Ioele et al., 2005), most likely due to the different conditions present in relatively dermatological preparations.

An advantage of the analytical method used in this study was that the relative rates of photoisomerization and photolysis could be determined. Since the degradation rate was much slower under visible light exposure, the rates of photolysis and photoisomerization were determined for this condition (Table 3). As shown in Table 3, k_{total} was calculated from the slope of a curve fit of a plot of 1/concentration remaining of either RA of 13RA versus time and $k_{photolysis}$ was calculated from a slope of a curve fit of a plot of 1/concentration remaining of either (RA-13RA) in case of RA degradation or (13RA-RA) in case of 13RA degradation versus time. Finally $k_{photoisomerization}$ was determined from the difference of ($k_{total} - k_{photolysis}$). These results show that the rates of photoisomerization are higher than the rates of photolysis in ethanol solution but that the rates of photolysis are higher than the rates of isomerization in cream formulations.

Table 3
Rate constants of total degradation, photolysis, and photoisomerization of RA and 13RA cream preparations irradiated by visible light[a]

Retinoic acid	Preparation	k_{total} (l/mmol min)	$k_{photolysis}$ (l/mmol min)	$k_{photoismerization}$ (l/mmol min)
RA	Ethanol	0.165 ± 0.021	0.056 ± 0.011	0.109 ± 0.020
	Cream	0.091 ± 0.011	0.068 ± 0.006	0.023 ± 0.002
13RA	Ethanol	0.061 ± 0.012	0.007 ± 0.001	0.054 ± 0.003
	Cream	0.029 ± 0.005	0.021 ± 0.007	0.008 ± 0.001

[a] $n = 3$.

Table 4
Rate constants of degradation of retinoid from ethanol solution[a]

Retinoic acid	Source	k (l/mmol min)	$t_{0.5}$ (min)	r^2
RA	SSL	0.330 ± 0.021	3.60 ± 0.29	0.994
	UVA	0.214 ± 0.017	5.57 ± 0.31	0.958
	Visible	0.038 ± 0.009	31.7 ± 1.39	0.986
13RA	SSL	0.247 ± 0.011	4.81 ± 0.73	0.958
	UVA	0.246 ± 0.023	4.85 ± 0.36	0.987
	Visible	0.030 ± 0.007	39.8 ± 1.16	0.976

[a] $n = 3$.

Table 5
Rate constants of degradation of retinoid from cream preparation[a]

Retinoic acid	Source	k (l/mmol min)	$t_{0.5}$ (min)	r^2
RA	SSL	0.116 ± 0.010	10.3 ± 0.76	0.996
	UVA	0.156 ± 0.021	7.63 ± 0.89	0.978
	Visible	0.012 ± 0.004	97.6 ± 1.32	0.980
13RA	SSL	0.068 ± 0.007	17.6 ± 1.12	0.996
	UVA	0.100 ± 0.023	11.9 ± 0.92	0.995
	Visible	0.012 ± 0.002	96.0 ± 2.18	0.965

[a] $n = 3$.

Since Both RA and 13RA are biologically active, the rates of disappearance of total retinoid from ethanol solution and cream preparations were calculated as shown in Tables 4 and 5, respectively. These results demonstrate that photodegradation occurs in RA and 13RA preparations upon exposure to light that can lead to loss of active material in such preparations.

4. Conclusions

Both RA and 13RA undergo both photoisomerization and photolysis following SSL, UVA and visible light exposure but RA is more sensitive to degradation than 13RA. Degradation of both retinoids by photolysis is considerably greater in cream formulations than in ethanol and the photodegradation follows second order kinetics. While studies in ethanol solution are valuable for determination of mechanisms of photodegradation of retinoids, the greater instability in cream formulations, where multiple components are present that can react with the retinoid, is of considerable practical significance as the identification and replacement of components of the formulations that accelerate RA photodegradation may allow the development of improved formulations. This possibility is supported by a recent study of Brisaert and Plaizier-Vercammen (2007). The UVA component of SSL is the major contributor to photodegradation. Since UVA penetrates deeply into skin, our results suggest that photodegradation of RA may contribute to the photosensitivity associated with RA therapy. Our studies suggest that the use of effective UVA sunscreens also may reduce the side effects of RA therapy.

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