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Stability of all-*trans*-retinol in cream

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Synopsis

Among the retinoids, we chose retinol (vitamin A) and studied the stability behavior of all-*trans*-retinol in creams. We observed thermal isomerization from all-*trans*-retinol to 13-*cis*-retinol, and this isomerization was a function of increasing temperature and oil content in creams. In addition, when the possibility of contact between retinol and water in creams was enhanced, retinol could be easily dehydrated to anhydrovitamin A. Therefore, a balance of oils and water is considered to be important for the stabilizing of retinol in cream. We found that the reduction of all-*trans*-retinol in cream was caused by compound factors: thermal isomerization by temperature, dehydration by water, decomposition by oxygen, and peroxide in the surfactants or oils used in creams.

INTRODUCTION

Among the retinoids, which are supposed to induce thickening of the epidermis and thin the stratum corneum and be effective for the treatment of skin diseases (1), retinol is regarded as desirable because of its lower stimulus compared to retinoic acid. However, retinol is said to be unstable in light and oxygen. Some studies on the stability of oil-soluble retinoids in oil are reported (2–4), but in these studies the phenomenon of decrease of retinoids was observed under mixed conditions affected by light, oxygen, lipid peroxide, temperature, and water. Therefore, in order to clarify the influence of each condition and the stabilization countermeasures for application in skin-care cream, we studied the stability behavior of all-*trans*-retinol, which has a higher physiological activity than other isomers, by means of reverse-phase high-performance liquid chromatography (HPLC). The possibilities of ultraviolet effects that can cause photoisomerization (5) of retinol and oxygen effects that can cause oxidation and decomposition of retinol are negated by using pure yellow-colored fluorescent light, brown-colored bottles, and a glove box and argon gas blanket for preparing the samples, preservation, and measurement.

MATERIALS AND METHODS

STORAGE TEST

Ten milliliters of 500 ppm retinol (3100 units/mg; FLUKA) in ethanolic solution or squalane (Nikko Chemicals) in a 20-ml brown-colored screw bottle sealed with a cap and 400 ppm of retinol in creams with 500 ppm butylated hydroxy toluene (BHT) (Wako Jyunyaku Kogyo Co.) in an aluminum tube sealed with a cap (about 15 g) were preserved under various temperature conditions. Table I shows the formula of sample water-in-oil creams. In order to investigate the influence of water and oil in cream on all-*trans*-retinol, we prepared water-oil creams, varying the relative quantities of the oil and water phases. Prepared retinol in ethanolic solution, and squalane and sample creams, were prepared in the absence of ultraviolet lights by using pure yellow-colored fluorescent light (National Co.) and in the absence of oxygen, using an argon gas blanket, blowing and bubbling with argon gas.

CONTACT OF RETINOL WITH WATER

Five hundred parts per million of retinol in 100%, 75%, and 50% ethanolic solutions were prepared and stored at 50°C for periods of ten days. In addition, 500 ppm of retinol in 50% ethanolic solution with 2% polyoxyethylene glycerol monoisostearate (60 mol) (Nihon Emulsion Co.), a surface-active agent, were prepared and compared with 500 ppm of retinol in 50% ethanolic solution without surface-active agent. This surface-active agent had to be fresh, and the sample for the storage test had to be prepared using blowing and bubbling argon. If not, the influence of peroxide in the surface-active agent (4) and that of oxygen could not be neglected.

EFFECT OF ANTIOXIDANTS

Butylated hydroxy toluene (BHT) (Wako Jyunyaku Kogyo Co.), butylated hydroxy anisole (BHA) (Wako Jyunyaku Kogyo Co.), vitamin E (Tokyo Kasei Kogyo Co.), and vitamin C (Wako Jyunyaku Kogyo Co.) were selected as antioxidants. Five hundred parts per million of retinol in ethanolic solution including 500 ppm of each of these antioxidants were stored at 50°C for periods of five days without using an argon gas blanket. When vitamin C was added to the retinol-ethanolic solution, the pH was adjusted at pH 7.0 with sodium hydroxide, since retinol is very unstable at low pH.

Table I
Formula of Sample Water-in-Oil Creams

Ingredient	Cream A	Cream B	Cream C (% by weight)
Water	65.03	54.03	42.03
Glycerin	15	15	15
Oils	15	26	38
Stearyl alcohol	(4)	(4)	(4)
Petrolatum	(4)	(8)	(12)
Liquid petrolatum	(7)	(14)	(22)
Polyoxyethylene glyceryl monostearate	4.6	4.6	4.6
Ethyl parahydroxy benzoate	0.2	0.2	0.2
retinol	0.04	0.04	0.04
Butylated hydroxy toluene	0.05	0.05	0.05
perfume	0.08	0.08	0.08

DETERMINATION OF ALL-*TRANS*-RETINOL AND 13-*CIS*-RETINOL

We weighed, to the nearest 0.1 mg, approximately 0.3 g of sample into a 25-ml volumetric flask, added methanol/ethyl acetate solvent (1:1) including 0.5% (w/v) BHT, and dissolved using ultrasonics. The sample solution was obtained by filtering through a 0.45-micron filter. The sample solution was analyzed by HPLC under the following conditions:

- Detection: Ultraviolet spectrophotometer (325 nm) (U-best 55, Japan Spectroscopic Co.)
- Column: Vydac 201 TP 104-C18 (25 × 0.46 cm) (Vydac Co., U.S.A.)
- Flow Rate: 1.0 ml/min
- Oven Temperature: 35°C
- Mobile phase: 0.136% (w/v) potassium phosphate monobasic and 0.034% (w/v) potassium phosphate dibasic solution/acetonitrile (3:7)

The ratio of the value (peak response of all-*trans*-retinol and 13-*cis*-retinol in the sample solution obtained from the chromatogram/sample weight in grams) after the storage test to that before the test is evaluated as % remaining. Standard 13-*cis*-retinol (Sigma Chemical Co, St. Louis, U.S.A.) was used for identification of 13-*cis*-retinol. Since retinol is light-sensitive to photoisomerization, sampling was done in a room having pure yellow-colored fluorescent light and using brown-colored volumetric flasks and autosampler.

RESULTS

INFLUENCE OF OILS IN CREAM ON RETINOL

Figure 1 shows an HPLC chromatogram (325 nm) of water-in-oil cream with 400 ppm of retinol after six months storage at 40°C. The cream storage test result shows decrease of all-*trans*-retinol and increase of 13-*cis*-retinol, a main isomer of all-*trans*-retinol. This conversion from all-*trans*-retinol to 13-*cis*-retinol is thermal isomerization (6), which can

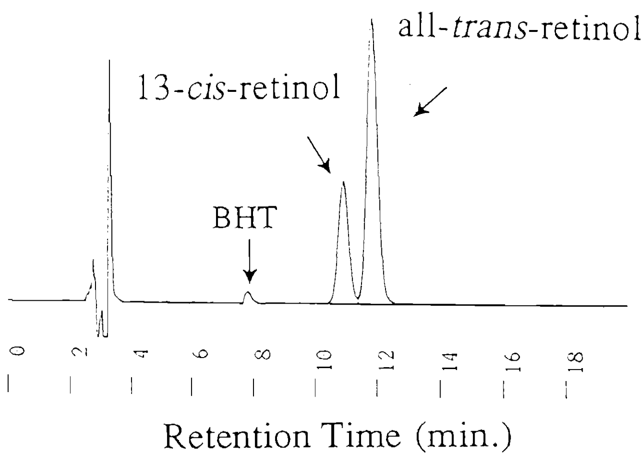


Figure 1. Chromatogram of HPLC (325 nm) of water-in-oil cream with 400 ppm of retinol after six months storage at 40°C.

be observed when all-*trans*-retinol in oil is preserved at higher storage temperatures. Figure 2 shows the stability of all-*trans*-retinol in squalane during ten days storage at 0°C, 25°C, 50°C, and 80°C. Thermal isomerization was accelerated due to increase of temperature. This isomerization was observed in other oils, including liquid petrolatum, jojoba oil, macadamia nut oil, and silicone oils. Figure 3 shows the relation between the weight percentage of total oils in cream and percent increase of the original amount of 13-*cis*-retinol after two months storage at 50°C. The larger the percent of oils in the cream formula became, from 15% to 38%, the more thermal isomerization from all-*trans*-retinol to 13-*cis*-retinol occurred proportionally. This result shows that the quantity of the oil phase had influence on the degree of thermal isomerization.

INFLUENCE OF WATER IN CREAM ON RETINOL

In order to investigate the influence of water in the cream on all-*trans*-retinol, the relation between the weight percentage of water in cream and the percent remaining of the original amount of all-*trans*-retinol after two months storage at 50°C was examined (Figure 4). As a result, it was clarified that the larger the percent of water in the cream formula became, from 42.03% to 65.03%, the lower was the percent remaining of all-*trans*-retinol. Apparently the result of thermal isomerization seems to contradict this result. But we can infer that the influence of water is stronger than that of thermal isomerization under this condition. Therefore, it is considered important for the stabilizing of all-*trans*-retinol in cream that the appropriate balance of oil and water be identified.

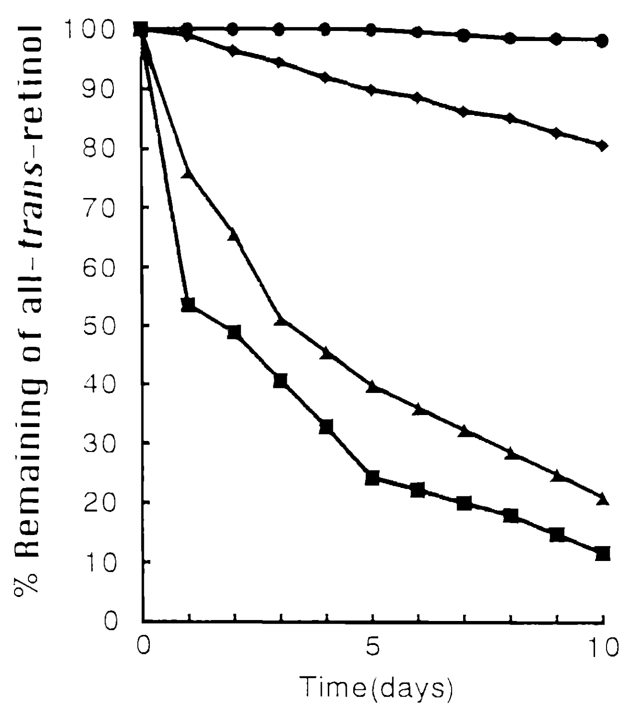


Figure 2. Percent remaining of all-*trans*-retinol in 500 ppm of retinol in squalane during ten days storage at 0°C ●, 25°C ♦, 50°C ▲, and 80°C ■ (with argon gas blanket).

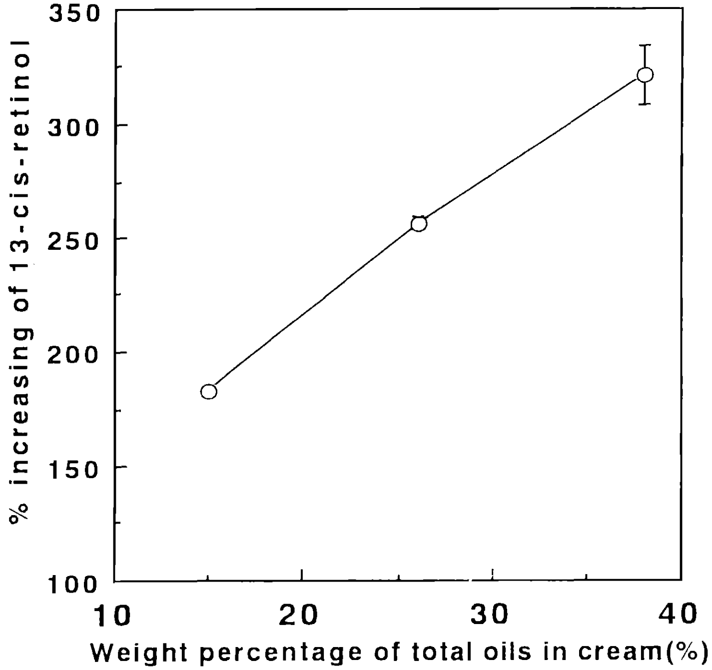


Figure 3. Weight percentage of total oils and percent increase of 13-*cis*-retinol in 400 ppm of retinol in oil-in-water cream after two months storage at 50°C.

Next, we checked the influence of the possibility of contact of water with all-*trans*-retinol by controlling the quantity of water or adding a surface-active agent in retinol-ethanolic solution. Figure 5 shows the relation between the percent of water in the retinol-ethanolic solutions, with or without a surface-active agent, and the percent remaining of all-*trans*-retinol in the solutions after ten days storage at 50°C. All-*trans*-retinol in ethanolic solutions decreased as the contact between all-*trans*-retinol and water was enhanced by an increase of water and surface-active agent. As all-*trans*-retinol was decreasing, one main product was found on the HPLC chromatogram. Figure 6 shows the UV spectra of this product in retinol ethanolic solution with a surface-active agent, after ten days storage at 50°C. This product was identified as anhydrovitamin A by using experimentally obtained standard anhydrovitamin A (7). This result corresponds with the findings of Anmo *et al.*, who reported that anhydrovitamin A was recognized as the product of purified vitamin A alcohol when the stability of vitamin A alcohol ethanolic solutions was investigated (8). The contact between all-*trans*-retinol and water accelerated the increase of dehydration and decreased all-*trans*-retinol without increasing 13-*cis*-retinol. We supposed the contact between 13-*cis*-retinol and water also promoted the decrease of 13-*cis*-retinol, and we could not observe an increase of 13-*cis*-retinol. The structural formulas of all-*trans*-retinol, 13-*cis*-retinol, and anhydrovitamin A are shown in Figure 7.

EFFECT OF ANTIOXIDANTS

The effect of BHT, BHA, vitamin E, and vitamin C as antioxidants against oxidation

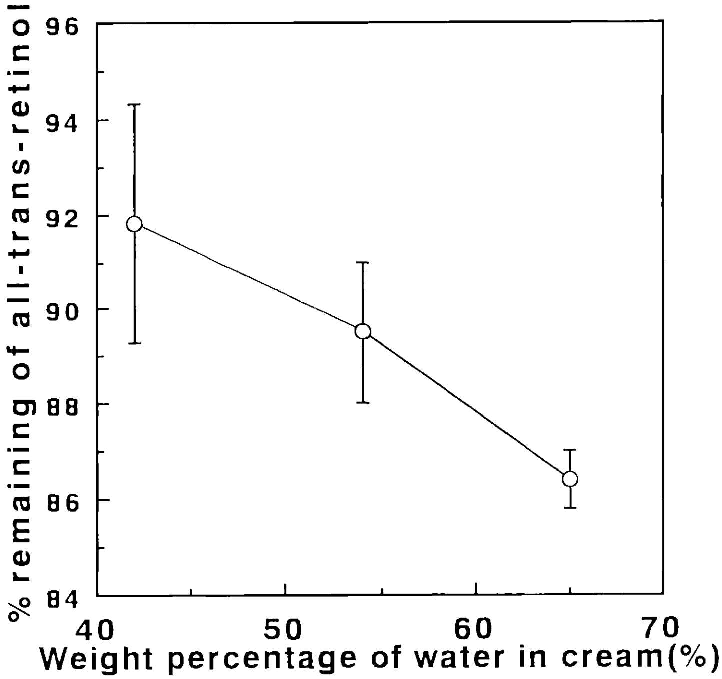


Figure 4. Weight percentage of water and percent remaining of all-*trans*-retinol in 400 ppm of retinol in oil-in-water cream after two months storage at 50°C (with argon gas blanket).

was evaluated in retinol-ethanol solution (Table II). These antioxidants had a high effect against oxidation of all-*trans*-retinol. Since oil-soluble antioxidants also have an effect against thermal isomerization and decomposition by lipid peroxide (9), use of antioxidants in cream seems to be invaluable.

DISCUSSION

Besides thermal isomerization, decomposition by contact with water, and oxidation, we have already known that peroxide in oil or surface-active agent aggravated the instability of all-*trans*-retinol (4,7). From the above results, we could construct a concept that if ultraviolet light is completely shielded, the decrease of all-*trans*-retinol in creams is more or less caused by these factors: thermal isomerization by temperature, dehydration by water, and decomposition by oxygen and lipid peroxide. And for stabilizing all-*trans*-retinol in creams, it is important 1) for prevention of thermal isomerization, to pay attention to the total amount of oil phase in cream and to avoid high temperatures during the manufacturing process of creams and not to preserve creams at high temperature; 2) for prevention of decomposition by water, to lower the possibility of contact between retinol and water by controlling the concentration of water and surface-active agents in creams; 3) for prevention of oxidation, to blow and bubble an inert gas through the ingredients of creams; 4) for prevention of decomposition of lipid peroxide, not to use oxidized or easily oxidizable oils and surface-active agents in creams; and 5) not only for prevention of oxidation but also for prevention of thermal isomerization and decomposition by lipid peroxide, to use oil-soluble antioxidants like BHT and BHA.

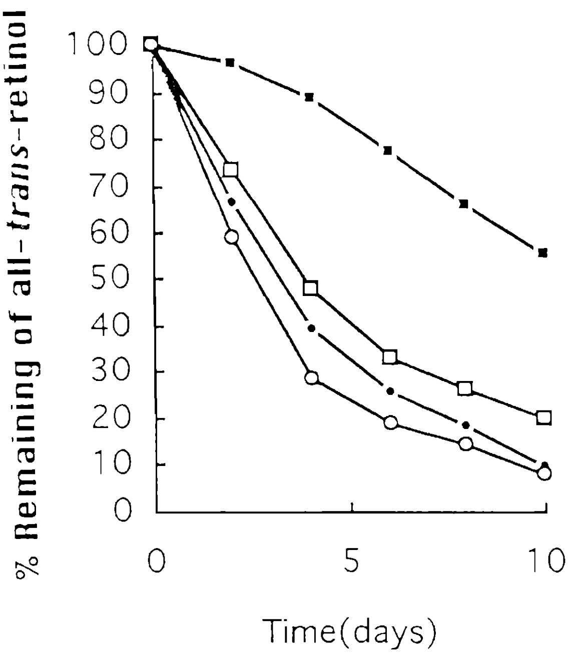


Figure 5. Percent remaining of all-*trans*-retinol in 500 ppm of retinol in 100% ethanolic solution. ■, 75% ethanolic solution; □, 50% ethanolic solution; ●, 50% ethanolic solution with 2% polyoxyethylene glycerol monoisostearate (60 mol); ○, after 10 days storage at 50°C (with argon gas blanket).

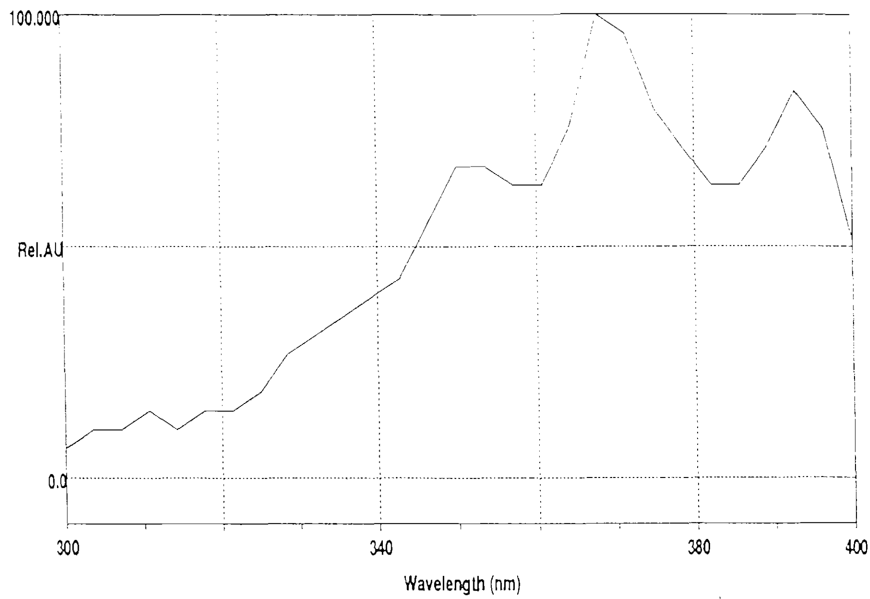
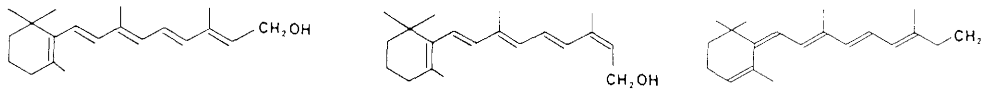


Figure 6. UV spectra of products in 500 ppm retinol 50%-ethanolic solution with 2% polyoxyethylene glycerol monoisostearate (60 mol) after ten days storage at 50°C (with argon gas blanket).



all-*trans*-retinol 13-*cis*-retinol anhydro-vitamin A.

Figure 7. The structural formulas of all-*trans*-retinol, 13-*cis*-retinol, and anhydrovitamin A.

Table II
Percent Remaining of All-*Trans*-Retinol in Retinol (500 ppm)-Ethanolic Solution With and Without Antioxidants During Five Days Storage at 50°C (without argon gas blanket)

Antioxidants	After 3 days storage	After 5 days storage
Without	1.6%	—
500 ppm BHT	94.4% (σ = 0.71)	92.0% (σ = 0.46)
500 ppm BHA	99.9% (σ = 1.32)	97.4% (σ = 1.73)
500 ppm Vitamin E	96.4% (σ = 2.67)	89.6% (σ = 0.30)
500 ppm Vitamin C (pH 7.0)	97.6% (σ = 0.02)	93.1% (σ = 0.24)

σ: Standard deviation.

In prescribing the formula of retinol-cream we have a dilemma because the increase of oil phase can cause thermal isomerization and the increase of water can cause decomposition. Therefore, a balance of oils and water in cream is considered to be very important.

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