STUDY ON STABILITY, EFFICACY, AND EFFECT OF A CREAM CONTAINING 5% OF RETINYL PALMITATE

Hong-Keun Ji, Young-Hwan Jeon
Han-Kook Cosmetics Co., LTD., R&D Center
36-1, SAMJEONG-DONG OJONG-GU,
BUCHUN, KYUNGGI-DO, KOREA

SUMMARY

Retinyl Palmitate, the skin normalizer, is useful to promote greater skin elasticity, to diminish lipid peroxidation and skin roughness following UV exposure, and promote a youthful general skin appearance. In manufacturing creams, Retinyl Palmitate(RP), which is a derivative of retinol, is used since retinol is easily oxidized by heat and light. However, only a small mount of retinyl palmitate is used since using a large amount of it may be harmful to its stability. In this study, thermal stability and UV stability of W/O-, W/S-, O/W-, and MLV-type creams containing 5% of retinyl palmitate and 10% of tocopheryl acetate(TA) are measured by Chroma Meters, and the content of RP is quantitatively analyzed by HPLC at 25°C and 45°C. Also, how RP has been changed by heat, light, etc. is measured by HPLC , and toxicity of the changed substance is studied. Particle size of each type of the cream is measured, cellular renewal is measured by using DHA(dihydroxyacetone) and Chroma Meters in order to study their efficacy and effect, moisture content is measured by using Corneometer and Tewameter, and how much wrinkles are improved is studied by using Image Analyzer. Development of MLV-type cream containing 5% of RP and 10% of TA, and satisfying conditions for better creams has been successful.

Key words: Retinyl Palmitate(RP), Multilamellar Vesicles(MLV)

1. INTRODUCTION

The development of cosmetics has been recently cosmeceutical pursuing high functionality. Among them, vitamin cosmetics which stand out from the points of view of environmental contamination, pollution, etc. have been reviewed in this paper.

Vitamin A is called axerophtol . Vitamin A present in the animal system are A_1 and A_2, and that present in the plant system is carotinoid. Vitamin A_2 (3-dehydro-retinol) has about 40% of the effect of A_1 (retinol), and both A_1 and A_2 exist in the form of an ester of fatty acid in nature. In the three-dimensional structure of Vitamin A_1, isoprene chains in Vitamin A1 are mostly trans-type, however, some Vitamin A_1's are cis-type. Vitamin A_1's in all trans-type have the strongest activity, and those in cis-type have 75% of the activity of those in all trans-types. Vitamin A is easily oxidized and loses its effect by oxygen in air, light, and heat (Fig.1).

RP is a skin normalizer, which increases elasticity of skin, reduces lipid peroxide, and prevents roughness of skin due to exposure of the skin to UV.

The metabolism of Vitamin A and its derivatives represent Fig.2. and Vitamin A transport and Vitamin A binding proteins symbolized Fig.3.

In the formula of a retinol cream, if oil phase is increased, thermal isomerization takes place, whereas, if water phase is increased, decomposition takes place. Therefore, for a retinol cream, the ratio of water phase and oil phase is very important. The reason for the reduction is reported to be due to thermal isomerization by temperature, dehydration by water, decomposition by oxygen, and surfactant used in water and oil phases (Fig.4).

The stability of Retinyl Palmitate in standard solution was compared with the behavior of other retinoid's solution, stored at the some experimental conditions. The obtained results were reported in Fig.5.

In the formula of RP cream, RP is changed to yellow by light and heat. Such change in color may be reduced by BHA and BHT, but is not always successful. In RP cream, activities of RP may be minimized and the oil phase may be stabilized by using antioxidants. At this time, dl-α-tocopherol and ascorbyl palmitate are used as anti-oxidants, however, if RP cream is used along with dl-α-tocopherol, change in color may be prevented under the conditions of 45°C, 25°C, and light. When using water in water phase, trace metals have to be removed by using distilled water or deionized water. A chelating agent is used in removing trace metals. The composition is stable at pH 5-6, and RP should be added at 35-40°C since it is sensitive to heat. However, this is also not always successful.

Based on the above, for W/O-, W/S-, O/W-, and MLV-type creams containing 5% of RP, stability against change in color at 45°C and 25°C is measured, change in the content of RP for each cream is quantitatively analyzed by HPLC, and cellular renewal is measured by using DHA.

2. EXPERIMENT

2.1 Materials for Experiment

Retinyl palmitate used in this experiment is BASF 1.0 million I.U./g; tocopheryl acetate is made by BASF; cholesterol, cholesteryl ester, lecithin, etc. are used as raw materials for lipid base; and PEG-5-soyasterol, (POE)n-cetylether, sorbitan stearate, glyceryl stearate, cetyl dimethicone copolyol, cetyl phosphate are used as surfactants. Purified water which has passed through anion-cation exchange resin column is used. The materials used in this experiment are used for cosmetic grades.

2.2 Instruments for Experiment

T.K. Auto Homo Mixer made by Tokushu Kika Kogyo in Japan is used for manufacturing microemulsion, and in the evaluation of emulsion stability, change in particle distribution is measured by using Laser Light Scattering System (Malvern, Model PCS 4700.,UK) which is an instrument for measuring particle distribution.

MLV phenomenon is reviewed by photographing Freeze-Fracture Scanning Electron Microscopy,

Electron Spin Resonance(ESR), and cellular renewal state and changes in color under 25°C, 45°C, and UV are measured by using Chroma Meters (Minolta, CM-1000R., Japan). Quantitative analysis of RP and confirmation of denatured RP are performed by using HPLC (Waters, Model 510) under the following conditions:

- Detection: Ultraviolet Spectrophotometer (280nm) (Waters, M441, U.S.A.)
- Column: μ-Bondapak C_{18} (3.9×300mm) (Waters, U.S.A.)
- Flow Rate: 1.0ml/min
- Mobile Phase: Methanol

Microfluidizer (Microfluidics Corp., U.S.A.) is used in order to make globule of MLV.

The measurement of effectiveness is used by CORNEOMETER CM 820 (Courage and Khazaka, Germany), TEWAMETER TM 210 (Courage and Khazaka, Germany), and IMAGE ANALYZER (NEXUS CO., Japan)

2.3 Method of Experiment

2.3.1 Manufacture of various types of cream (Table 1)

2.3.1.1 Method of Manufacture on the MLV liposomes formation(Fig.6.)

2.3.2 Measurement of change in color of W/O-, W/S-, O/W-, and MLV-type creams by heat and UV

Change in color of each cream under 25°C, 45°C, UV is measured by using Chroma meters after 1 month.

2.3.3 Measurement of change in content and conversion into another substance of W/O-, W/S, O/W-, and MLV-type creams

Quantitative analysis of each cream under 25°C and 45°C is performed by using HPLC to see how the content of 5% of RP has been changed, and whether RP is converted into another substance is analyzed by using HPLC.

2.3.4 Measurement of cellular renewal by using DHA

1) Apply each of 4 types of cream on a forearm for 15 days.

2) After 15 days, attach a 5×5cm² patch of 0.6ml of O/W-type cream containing 4% of DHA for 24 hours.

3) After 24 hours, detach the patch, and measure change in color by using Chroma Meters.

4) Check change in color every day for 20 days.

2.3.5 Measurement of formation of MLV liposome

In order to confirm possible formation of multi-lamellar vesicle when the vitamin made according to the manufacturing method of MLV liposome is put into vesicles, the samples are sent to Sero. Lab in France and measured by Freeze-Fracture Scanning Electron Microscopy.

2.3.6 Measurement of particle size

Four types of cream thus manufactured are kept in a 25°C thermostatic tub, and their particle sizes are measured. Laser Light Scattering System (PCS 4700 Malvern, UK) is used as a measuring instrument, and the measurement is performed at room temperature since an automatic temperature control device is attached to the instrument.

2.3.7. Measurement of wrinkle by using IMAGE ANALYZER

Analyzer it on the monitor by 3-Dimentional Skin System program and then, measure the number of wrinkle peak, the depth of the wrinkles.

2.3.8. Measurement of Electron Spin Resonance(ESR)

The objection of this evaluation was to verify the presence of liposome in two samples.
A stable spin label with along molecular axis was utilized to obtain electron spin resonance(ESR) spectra of two liposome formations. In aqueous dispersion with liposomes, the ESR spectrum will reflect the anisotropic motion of the label.

2.3.9. Measurement of Cell Toxicity .

Harvested cells by trypsinization were diluted by media contained 2% serum and 90ul of cell suspension(2,500~3,000cells/well)was Inoculated into each well of a 96 well tissue culture plate and cultured for 2days at 37°C, 5% CO^2 incubator. Then media was replaced with new one and cells were treated with 10ul of chemicals for 2 days. After chemical treatment 100ul of neutral red solution (50ug/ml)was add to each well and incubated 3hr.

Neutral red passes through the intact plasma membrane and concentrated in lysosome of living or undamaged cell. After that we replaced neutral red solution with 100ml of 1% formaline-1% $CaCl_2$ solution. Intracellular neutral red was extracted using 1% acetic acid-50% ethanol solution. We measured the Result of MLV Cream contained 5% RP and at 25°C, 45°C sample using microplate reader at 540nm.

2.3.10. Measurement of Moisturizing effect by using CORNEOMETER, TEWAMETER

5 female volunteers at the age of 22-43 years with healthy skin were included in the test. The participants were briefed on the study procedures and each gave written informed consent. Measurements were carried out at 22±1°C and a relative humidity of 60±10 %.Subjects were accustomed to ambient conditions for 20 min prior to any measurement. The test was carried out on the volar forearms. The skin of the forearms was treated with a 5% aqueous solution of sodium lauryl sulphate(SLS) and an occlusive dressing applied. The dressing was removed 2h later, and the regions gently washed with water and air-dried. After 30 min the measurements were done. Then the five test products were applied, one area remained untreated. The dose of

application was about 2 mg/cm². In the following 14 days a home application in the morning and evening took place. Measurements were evaluated during the treatment period on day 3, 7 and 14 one hour after the last daily application. Use of other cosmetic products was restricted on the test areas throughout the whole study.

3. RESULTS AND DISCUSSION

3.1 Stability in Color of W/O-, W/S-, O/W-, and MLV-type creams against 45°C and UV

Stability in color for each cream under 45°C and UV is shown in Fig. 7. As shown in Fig.7, color of each cream is changed by 45°C and UV as time goes on due to affect of RP. MLV-type cream showed increased stability in color by 3-13 times under 45°C and by 2-6 times under UV compared to other types of cream. This is because MLV forms stable multi-lamellar structure and the MLV contained 5% RP is stable.

3.2 Measurement of Change in Content of RP, Change into Another Substance, and Toxicity for W/O-, W/S-, O/W-, and MLV-type Creams

W/O-, W/S, O/W- and MLV-type creams containing 5% of RP are placed under 25°C and 45°C. Change in the content of RP is measured after 1 month and 1 year, and the results are shown in Fig.8. As shown in the Fig.8, the content of RP was changed greatly in the order of MLV- < W/S- < O/W- < W/O-type of cream.

Comparison of the HPLC peak of RP between the original sample and the denatured RP kept at 45°C for 1 year showed that RP has been changed into a new substance (Fig.9). This change of RP into another substance seems to affect toxicity of the cream. Change in toxicity for a cream kept at 25°C and 45°C for 1 year is shown in Fig.10. It is shown that 25°C cream has lower toxicity than 45°C cream. Therefore, in order to use RP effectively for a long time, using MLV-type cream, which maintains the content of RP of 5%, seems to be most effective for the skin. Reduction in the content of RP is suspected to be because of toxicity of the substance changed from RP according to HPLC analysis.

3.3 Measurement of Cellular Renewal

Measurement of cellular renewal for W/O-, W/S-, O/W-, and MLV-type creams indicates that MLV liposome is composed of more than 2 multi-layered membranes with the vesicle as one layer. Facial components are mixed into a solution, put into the hydrophilic or lipophilic portions, and form vesicles. MLV-type cream can maintain its effect for a long time, and increase penetration of moisturizer. MLV-type cream showed faster cellular renewal action by 1-3 days compared to other types of creams (W/O-, O/W-, and W/S-type creams)(Fig.11).

3.4 Measurement of Particle Size and MLV Liposome

In order to verify whether MLV-type cream forms liposome, it is photographed by Freeze-Fracture Scanning Electron Microscopy as shown in Fig.12. It was shown that the cream has a multi-layered lamellar structure. The interval of repetition of lamella is primarily due to contact with water. The particle size is 0.50-3.90μm at this time.

The particle sizes of W/O-, W/S-, O/W-, and MLV-type creams are shown in Fig.13. The particle size distribution of MLV vesicles is 0.08-0.90μm, and an average particle size is 0.20μm. It is seen that active ingredients are capsulated in the multi-layered hydrophilic portion of liposome vesicles. It is also seen that the active ingredients thus capsulated are very stable against change in color.

3.4.1. Measure of ESR

ESR spectra maybe obtained from paramagnetic molecules with an unpaired electron. In aqueous dispersions, this probe partitions preferentially into hydrophobic domains(kp>> 1 0 E 6). The long molecular axis of the probe is known to spontaneously align with ordered lipids. If the lipid phase is disordered, as in the cage of large mixed-detergent micelles and lipid droplets, then the probe will be free to tumble equally in all directions(isotropic motion). If the lipid phase is ordered, as in the case of liquid crystals, lipid bilayeres and very small micelles, then the motion of the probe will be disproportionately restricted in at least one axis(anisotropic motion). Evaluation of liposome formulations is showed Fig.14.

A) liposomes prepared from soy lecithin at a concentration of 20mg/ml
~ This spectrum A is provided as a reference for bona fide liposomes.
Note the two outer splittings have become broadened due to slow anisotropic motion narrow mark the position of the hyperfine extreme characteristic of liposomes.

B) MLV
This spectrum reveals antiotropic motion.
This is evident (arrow)as a shoulder on the low field (left)peak of the spectrum

3.5. Measurement of moisture effect

Measurement of moisture holding volume MLV, W/O, W/S, O/W cream, it is obvious that the emulsion type (ie MLV, W/O, O/W, W/S Type etc) may play a role. This is illustrated by Fig.15. The result of the base MLV Type cause higher moisturization than other base(O/W, W/O). This is linked with the higher occlusive effect of MLV . When compared to O/W, W/O emulsions and also with a better prevention of TEWL. Each and every TEWL value of MLV, O/W, W/O is shown at Fig.16. Therefore, MLV Cream produced a reduction of TEWL, accompanied with an increase of skin humidity.

3.6. Measurement of wrinkle-number and wrinkle-depth

The relations between number and depth of skin-wrinkle peak are shown at Fig.17.
As shown at fig.17., considering the number of wrinkle-peak is decreasing and the depth of wrinkle is decreasing in terms of measurement Value of MLV.

4. CONCLUSIONS

In this study, W/O-, W/S-, O/W-, and MLV-type creams containing 5% of RP are manufactured, and experiments to obtain the most stable type of base as well as the most efficacious and effective type of base are performed. The results of this study are shown below:

(1) Among W/O-, W/S-, O/W- and MLV-type creams, MLV-type cream formed liposome by using lipid base and microfluidizer showed superior stability against change in color due to formation of vesicles.

(2) Among W/O-, W/S, O/W, and MLV-type creams containing 5% of RP, MLV-type cream maintained stability without having the content of RP changed even after 1 year due to capsulation of RP, while W/O-, W/S-, O/W-type creams had rapidly reduced content of RP.

(3) According to HPLC measurement, the sample kept at 45°C for 1 year had RP converted into another substance as time goes on. The result of toxicity test showed that this another substance had higher toxicity compared to the sample kept at 25°C.

(4) In cellular renewal experiment, MLV-type cream showed the best effectiveness. It was proved that MLV-type cream, which has active ingredients capsulated, had extended effect and penetrated into the skin the most.

(5) MLV creams produced a decreasing of number peak and depth of skin-wrinkle and reduction of TEWL, accompanied with an increase of skin humidity.

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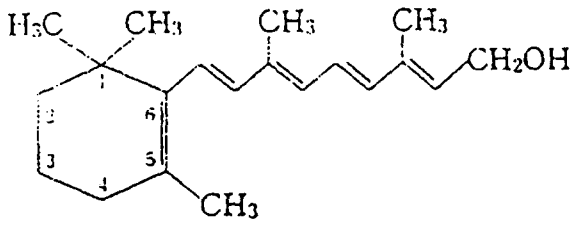
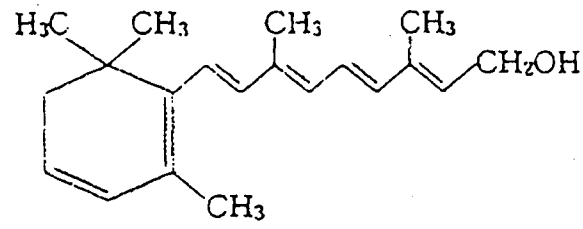
Table.1. Formulas of sample W/O, W/S, O/W, MLV creams.

TYPE	MATERIAL NAME		PRODUCTION METHOD
O/W	(A)CARBOMER #934	0.40	(A)→HEAT/DISSOLVE ↓ EMULSIFICATION←(B) ↓ NEUTRALIZATION←(C) ↓ COOLING ↓ O/W
	EDTA-2NA	0.10	
	GLYCERINE	6.00	
	DEA-CETYL PHOSPHATE	0.70	
	METHYL PARABEN	0.20	
	PURE WATER	q.s	
	(B)STEARIC ACID	1.20	
	CETYL ALCOHOL	1.00	
	SORBITAN STEARATE	0.50	
	GLYCERYL STEARATE	2.00	
	BHT	0.05	
	TOCOPHERYL ACETATE	10.00	
	RETINYL PALMITATE	5.00	
	MACADAMIA NUT OIL	12.00	
	JOJOBA OIL	3.00	
	(C)TRIETHANOLAMINE	0.40	
W/O	(A)CETYL DIMETHICONE COPOLYOL	3.00	(B)→HEAT/DISSOLVE ↓ EMULSIFICATION←(A) ↓ DISPER←(C) ↓ COOLING ↓ W/O
	MACADAMIA NUT OIL	12.00	
	JOJOBA OIL	3.00	
	TOCOPHERYL ACETATE	10.00	
	RETINYL PALMITATE	5.00	
	STEARIC ACID	1.20	
	CETYL ALCOHOL	1.00	
	BHT	0.05	
	(B)SODIUM CHLORIDE	0.50	
	METHYL PARABEN	0.20	
	PURE WATER	q.s	
	(C)POLYACRYAMIDE/ISOPARAFFIN/ LAURETH-7	3.00	

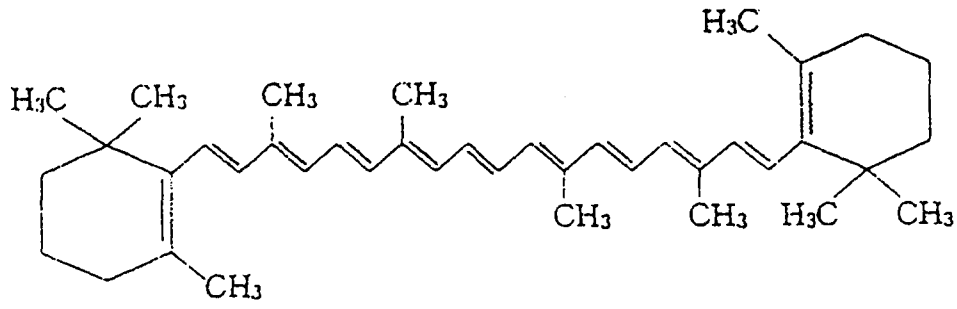
TYPE	MATERIAL NAME		PRODUCTION METHOD
W/S	(A)DECAMETHYL/ CYCLOPENTASILOXANE	6.00	(B)→HEAT/DISSOLVE ↓ EMULSIFICATION←(A) ↓ COOLING ↓ W/S
	METHYL POLYSILOXANE	5.00	
	POLYOXYETHYLENE/ METHYLPOLYSILOXANE	4.00	
	TOCOPHERYL ACETATE	10.00	
	RETINYL PALMITATE	5.00	
	BHT	0.05	
	(B)GLYCERINE	4.00	
	METHYL PARABEN	0.20	
	SODIUM PCA	2.00	
	PURE WATER	q.s	
MLV	(A)LIPID BASE	10.00	
	(B)PROPYLENE GLYCOL	5.00	
	CETYLPHOPHATE	0.50	
	PURE WATER	q.s	
	(C)**TOCOPHERYL ACETATE**	10.00	
	RETINYL PALMITATE	5.00	
	BHT	0.05	
	(D)POLYACRYLAMIDE/ISOPARAFFIN/ LAURETH-7	3.00	



Vitamin A_1



Vitamin A_2



β -carotene

Fig.1. The structure of Vitamin A.

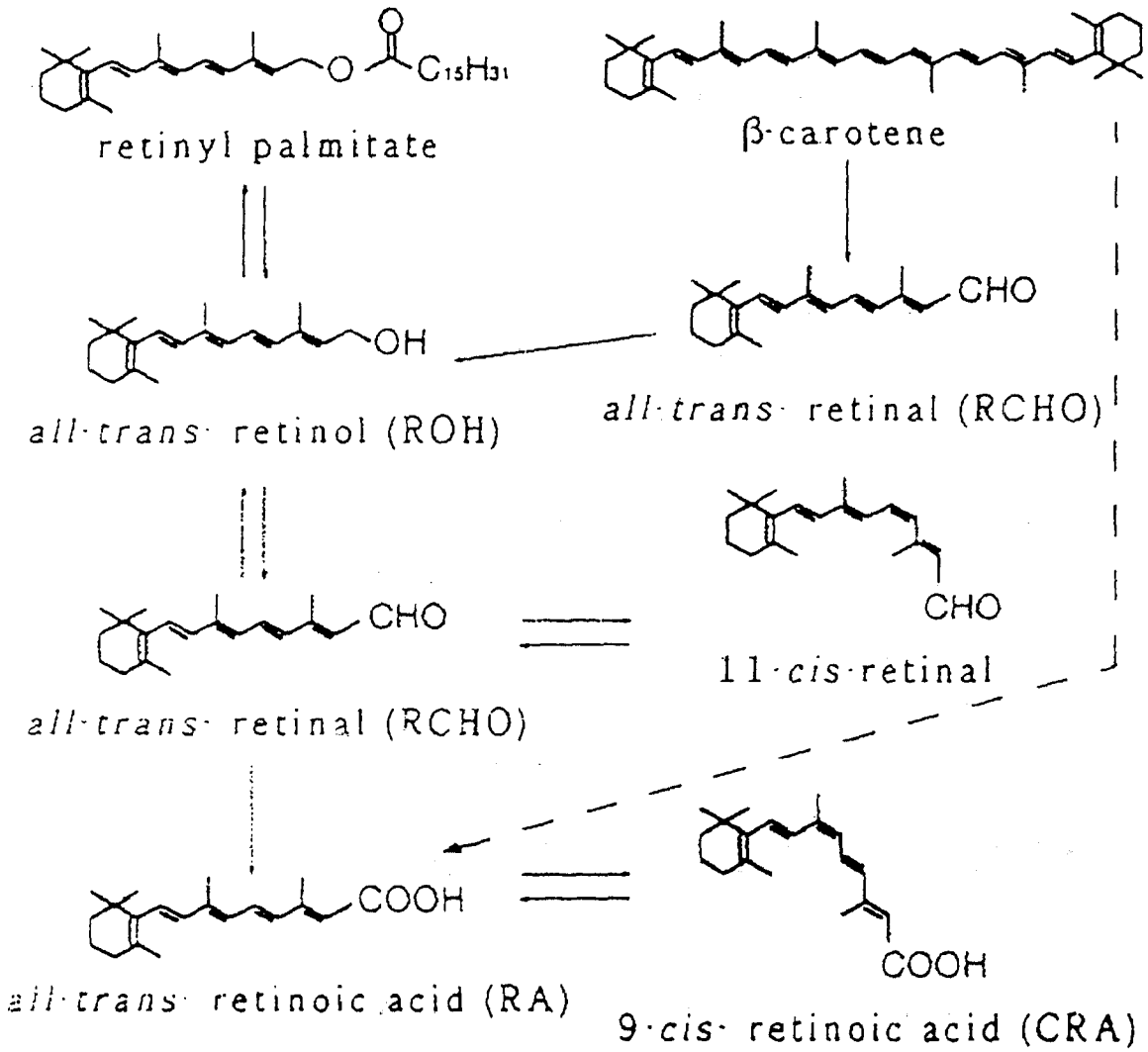


Fig.2. Metabolism of Vitamin A and Its Derivatives

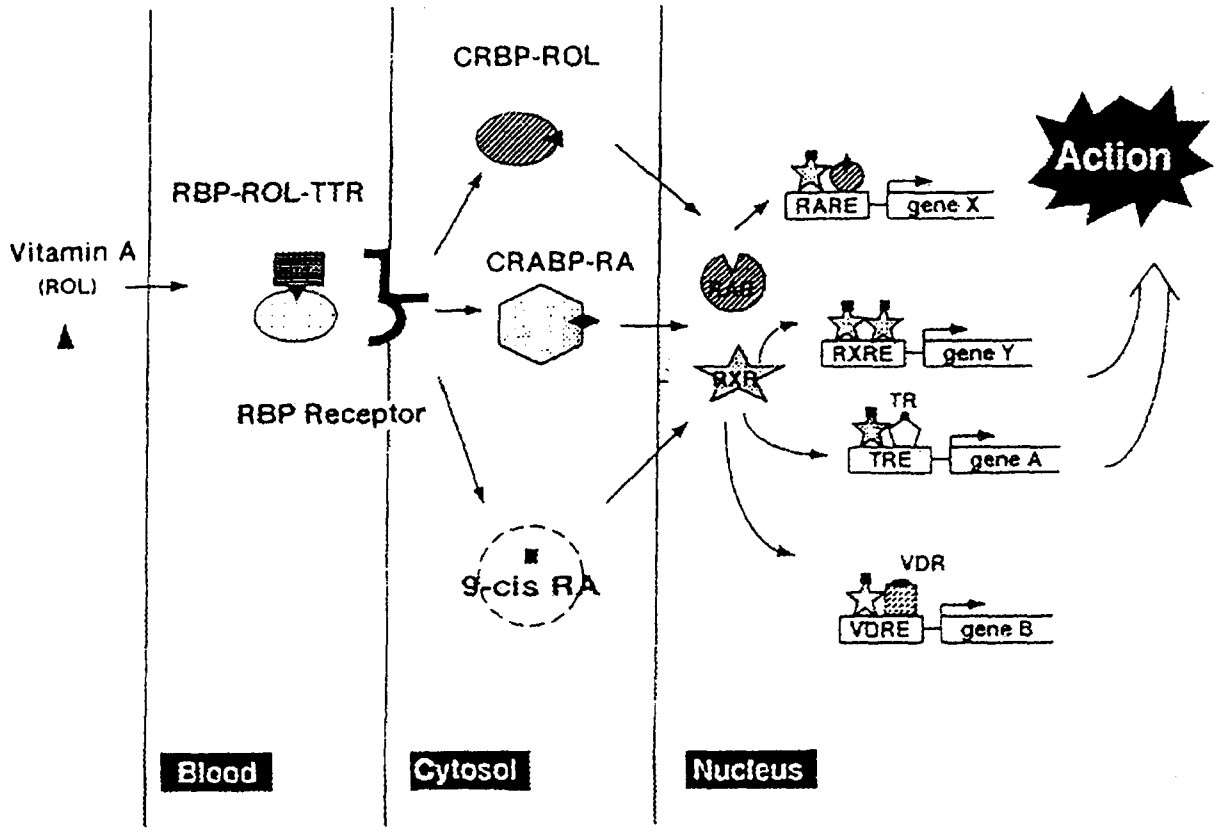
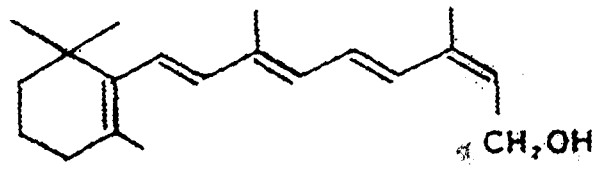
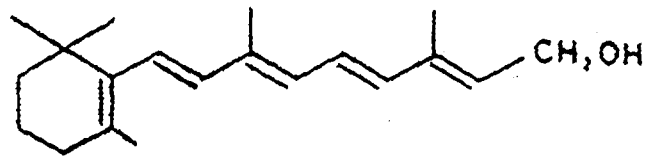


Fig.3. Vitamin A Transport and Vitamin A Binding Proteins



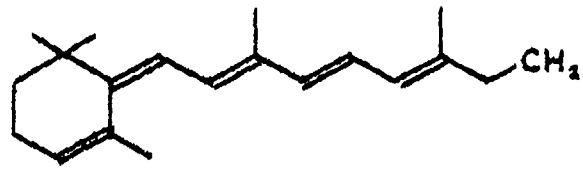
13-*cis*-retinol

THERMAL ISOMERIZATION



all-*trans*-retinol

DEHYDRATION



anhydro-vitamin A.

Fig.4. The structure formulas of all-trans-retinol, 13-cis-retinol, and anhydrovitamin A.

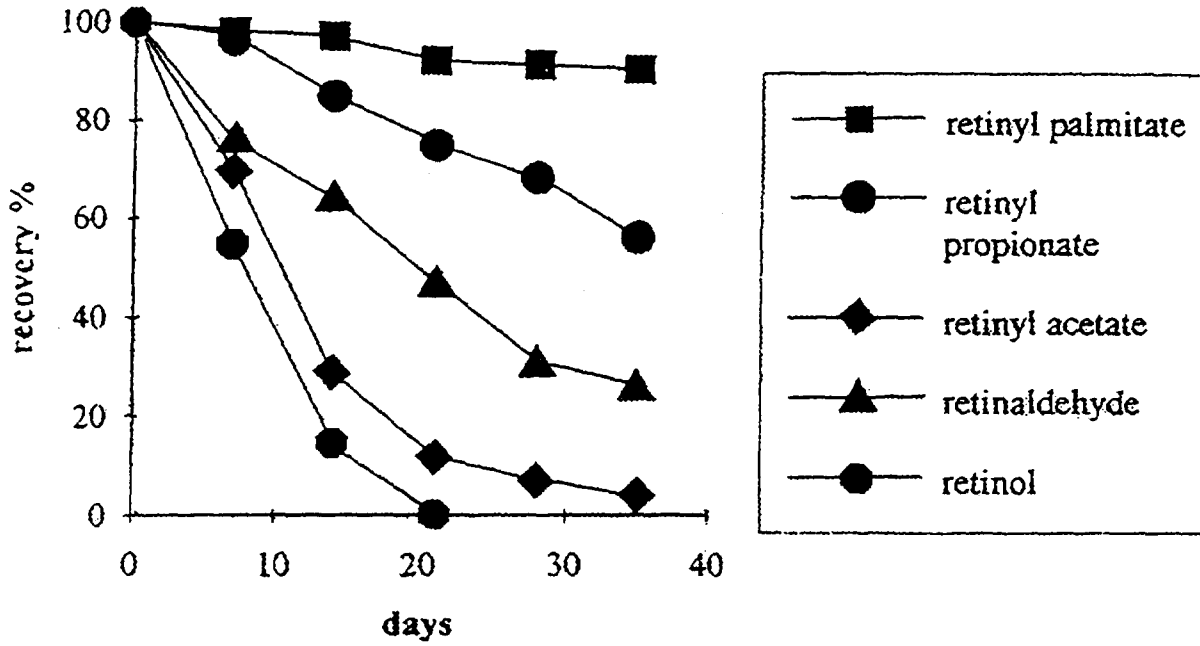


Fig.5. Stability trends vs time of different retinoids standard solutions stored room temperature

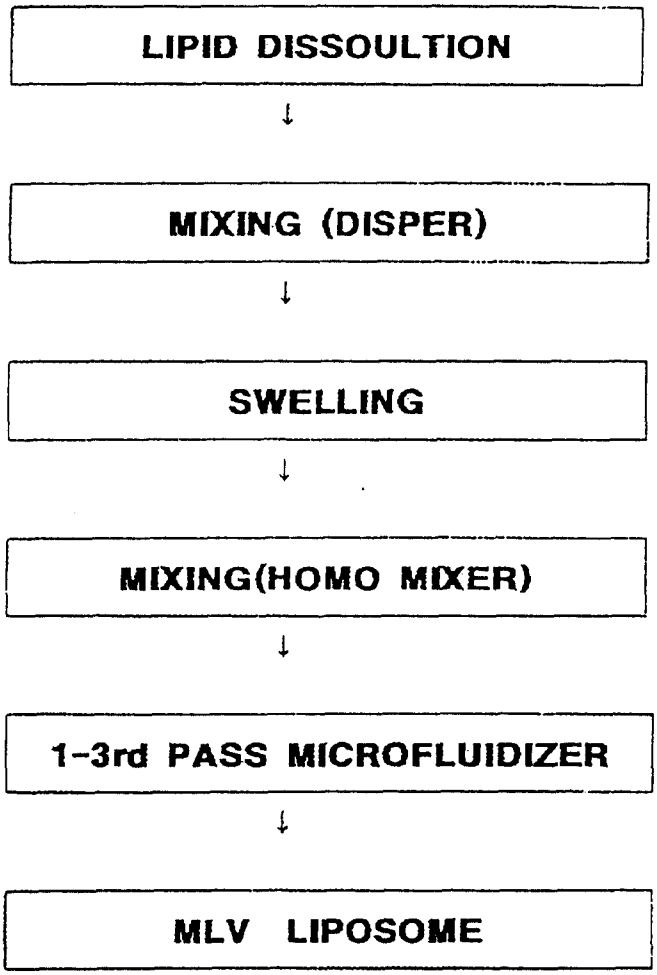
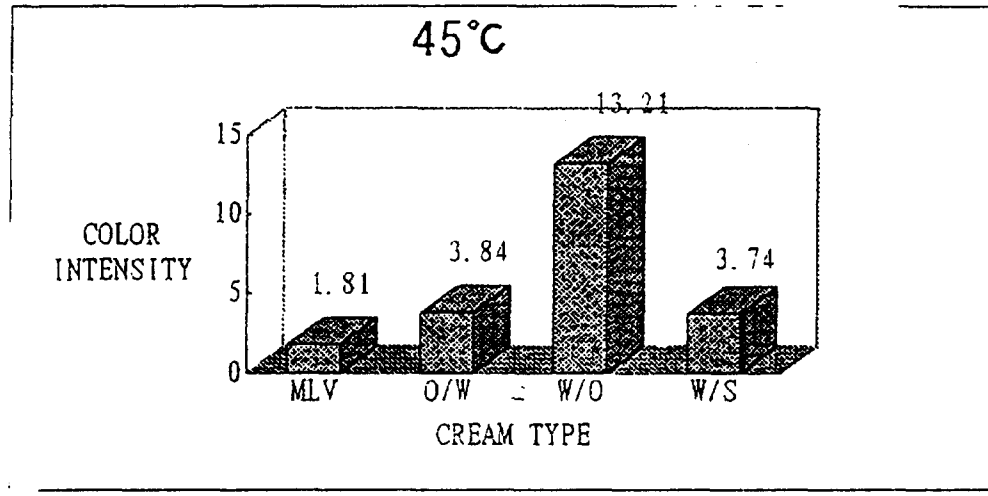
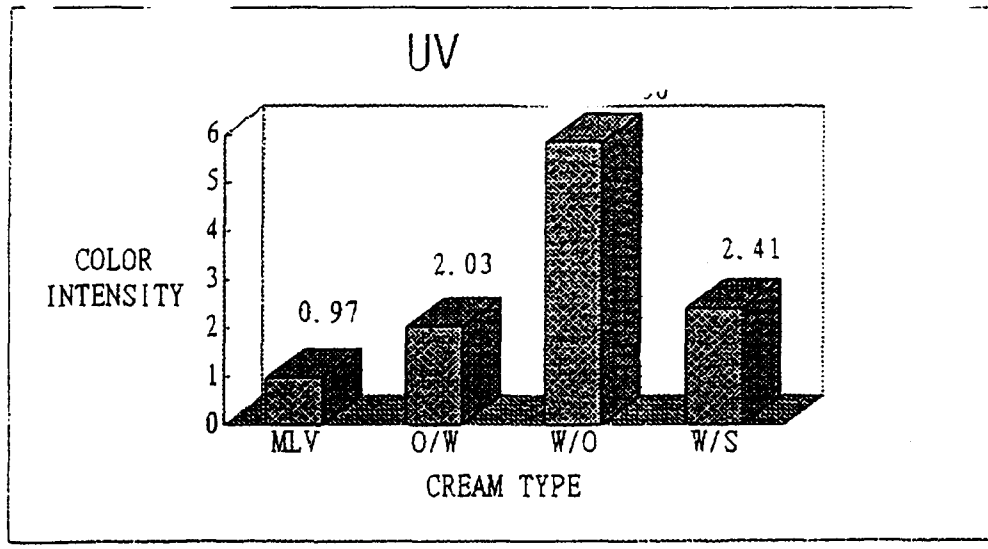


Fig.6. Method of manufacture on the MLV liposomes formation



(a)



(b)

Fig.7. The color stability of W/O, W/S, O/W, MLV creams after 1 month storage at (a)45℃,(b)UV.

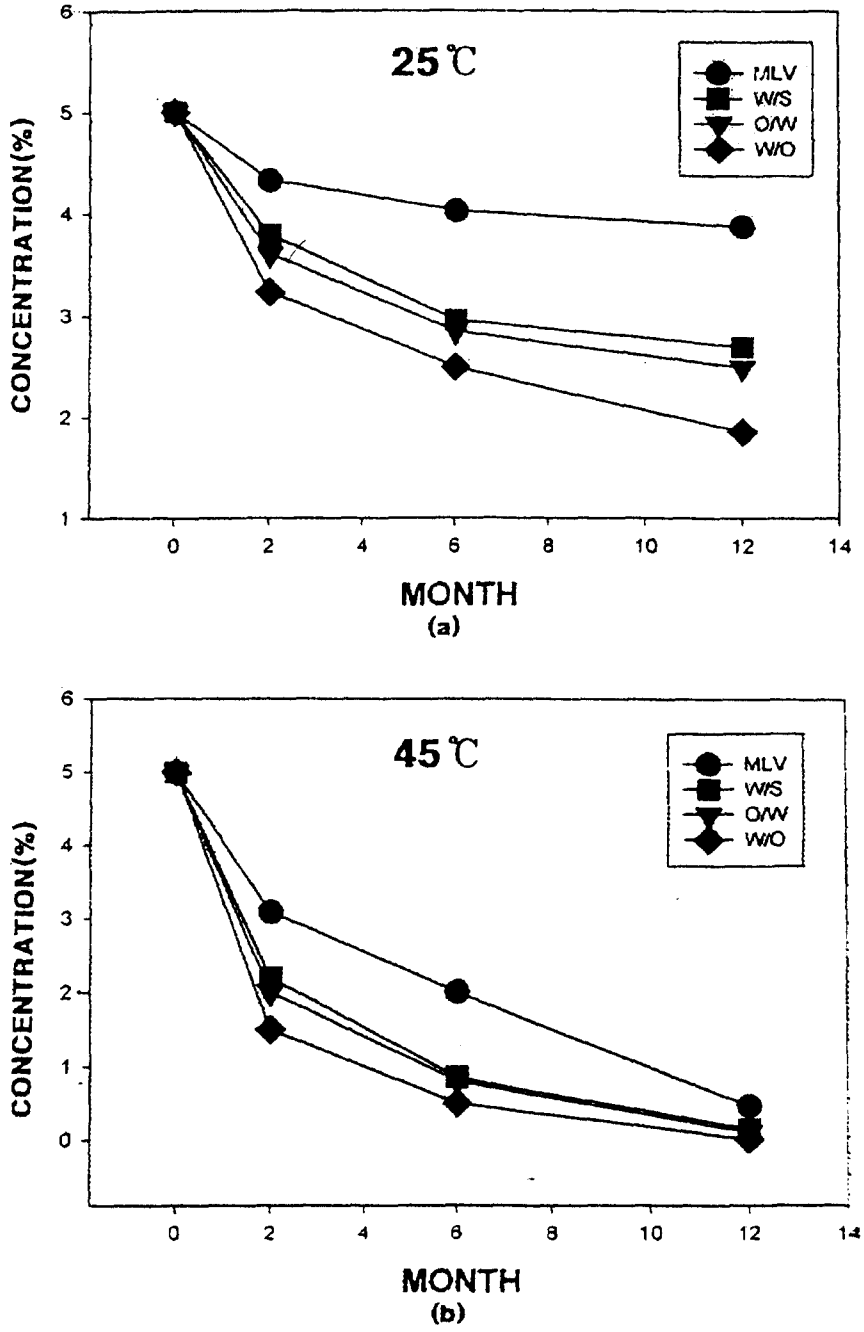
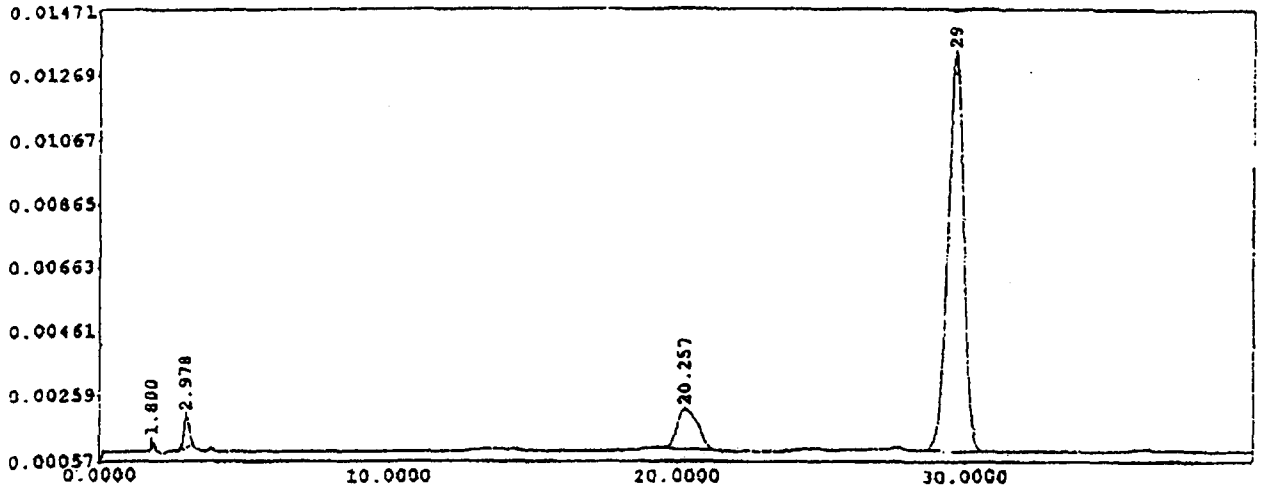
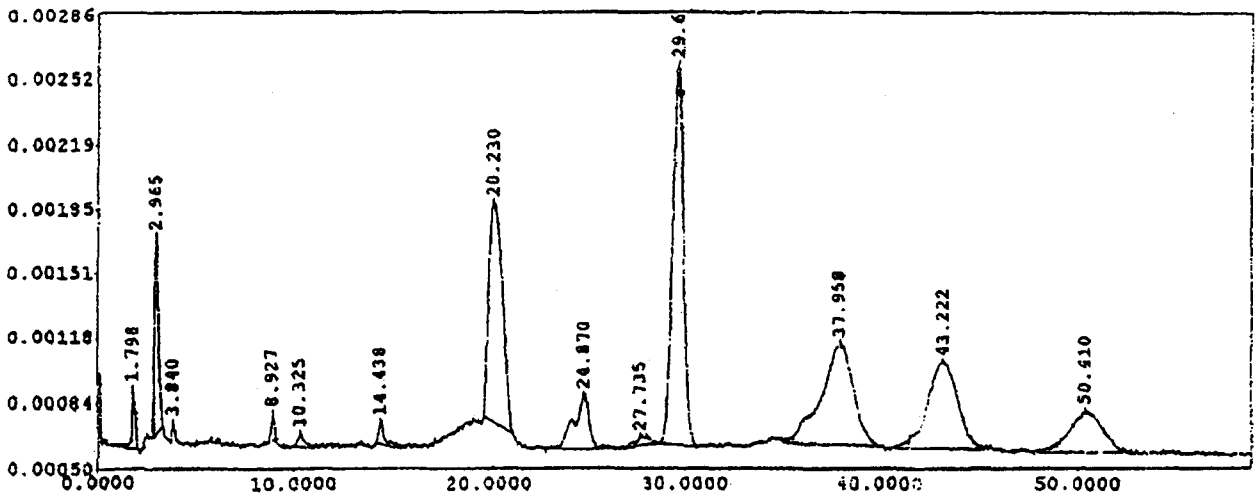


Fig.8. Percent remaining of retinyl palmitate in W/O, W/S, O/W, MLV creams during 12 months storage at (a)25℃,(b)45℃.



(a)



(b)

Fig.9. Chromatogram of HPLC(325nm) of retinyl palmitate after 12 months storage at (a)25℃,(b)45℃.

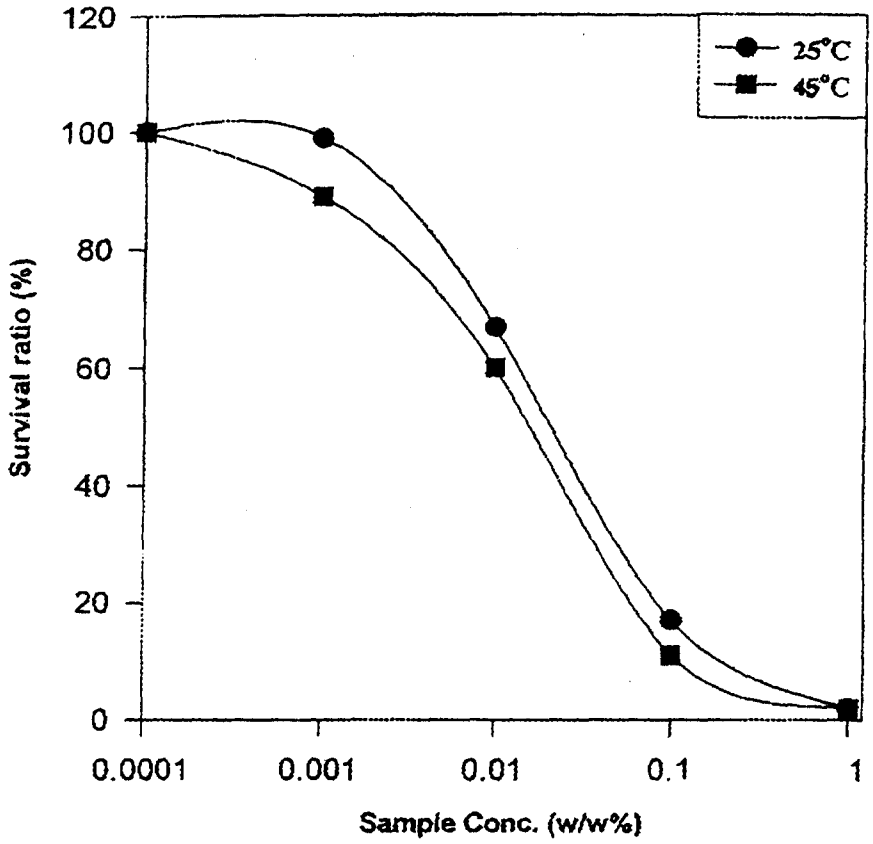


Fig10. Cytotoxicity measurement of retinyl palmitate in cream during 12months storage at (a)25℃,(b)45℃.

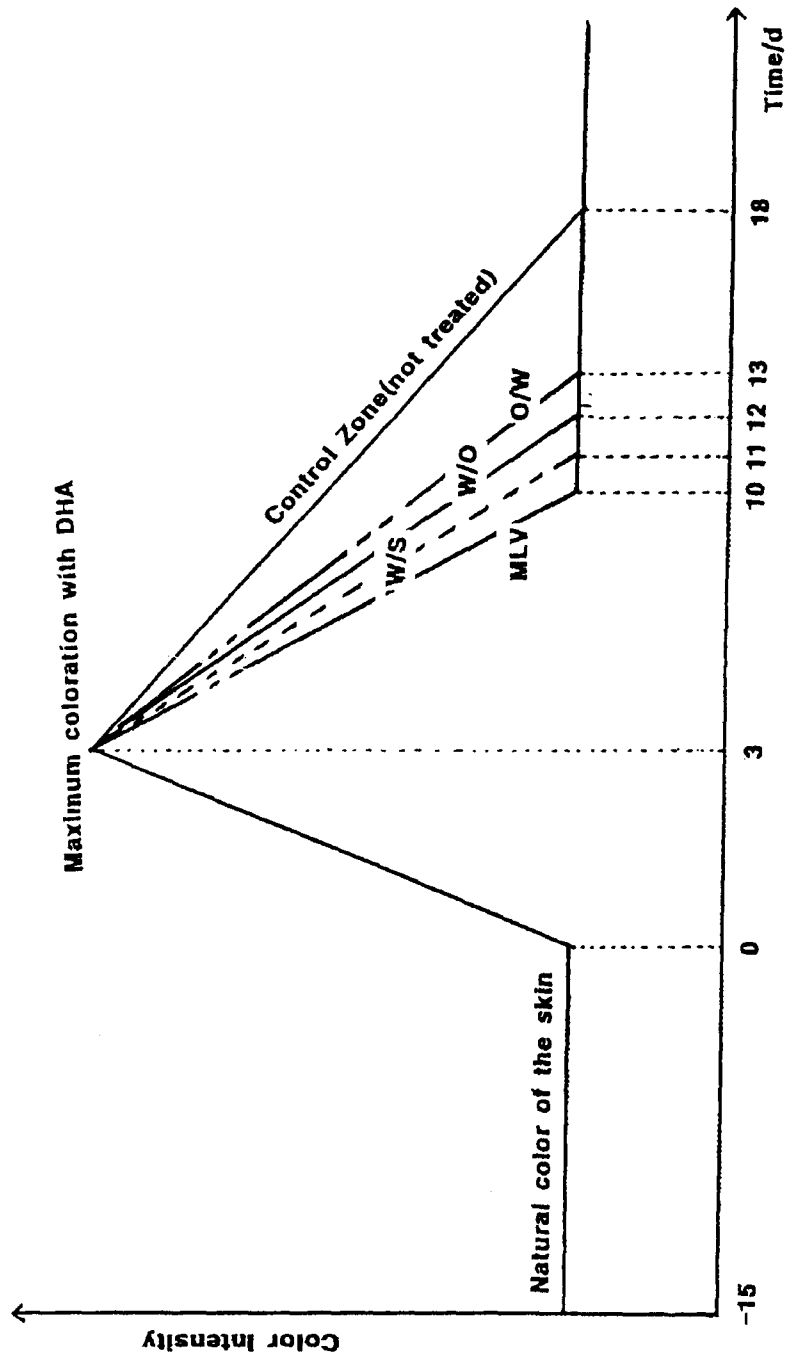
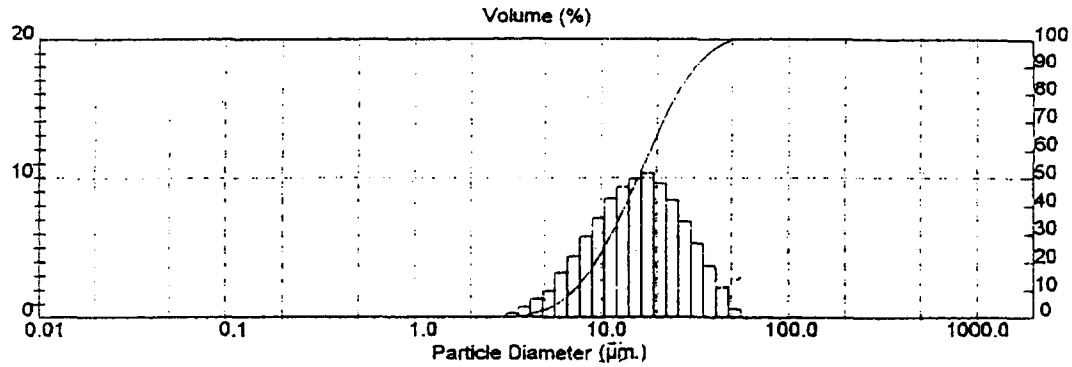
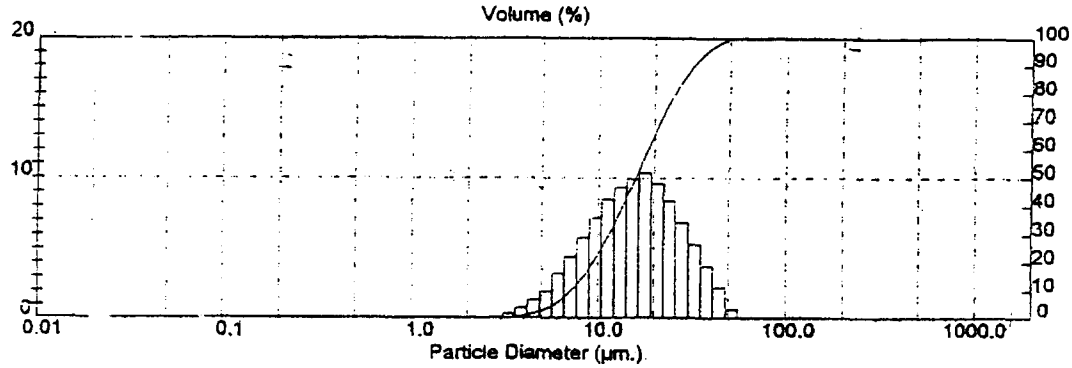


Fig.11.The disapperance of DHA treated skin coloration should be hasten by the use of W/O, W/S, O/W, MLV creams.

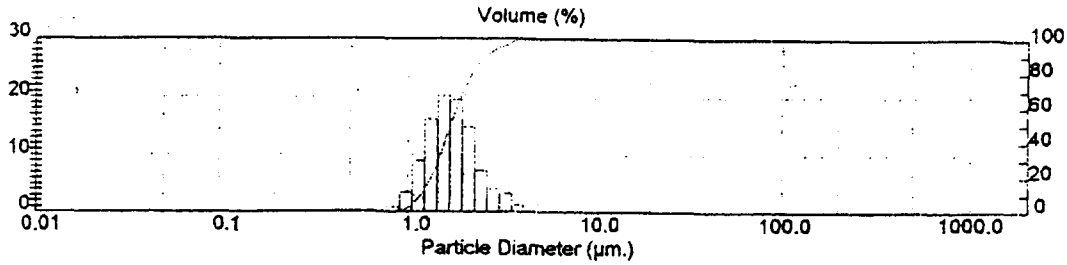
Fig. 12. Scanning electronic microphotograph obtained after freeze-fracture of the MLV(X83,000)



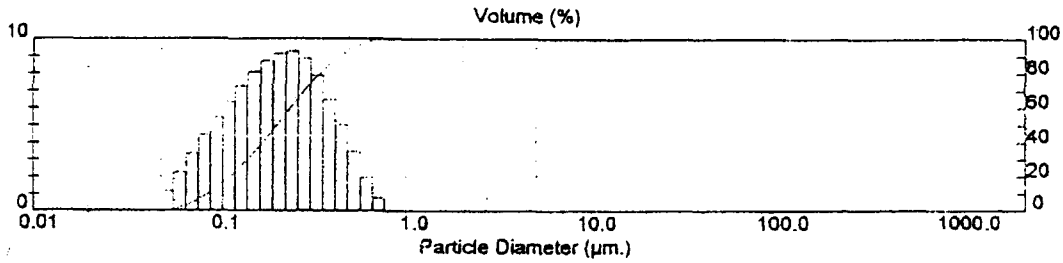
(a)



(b)

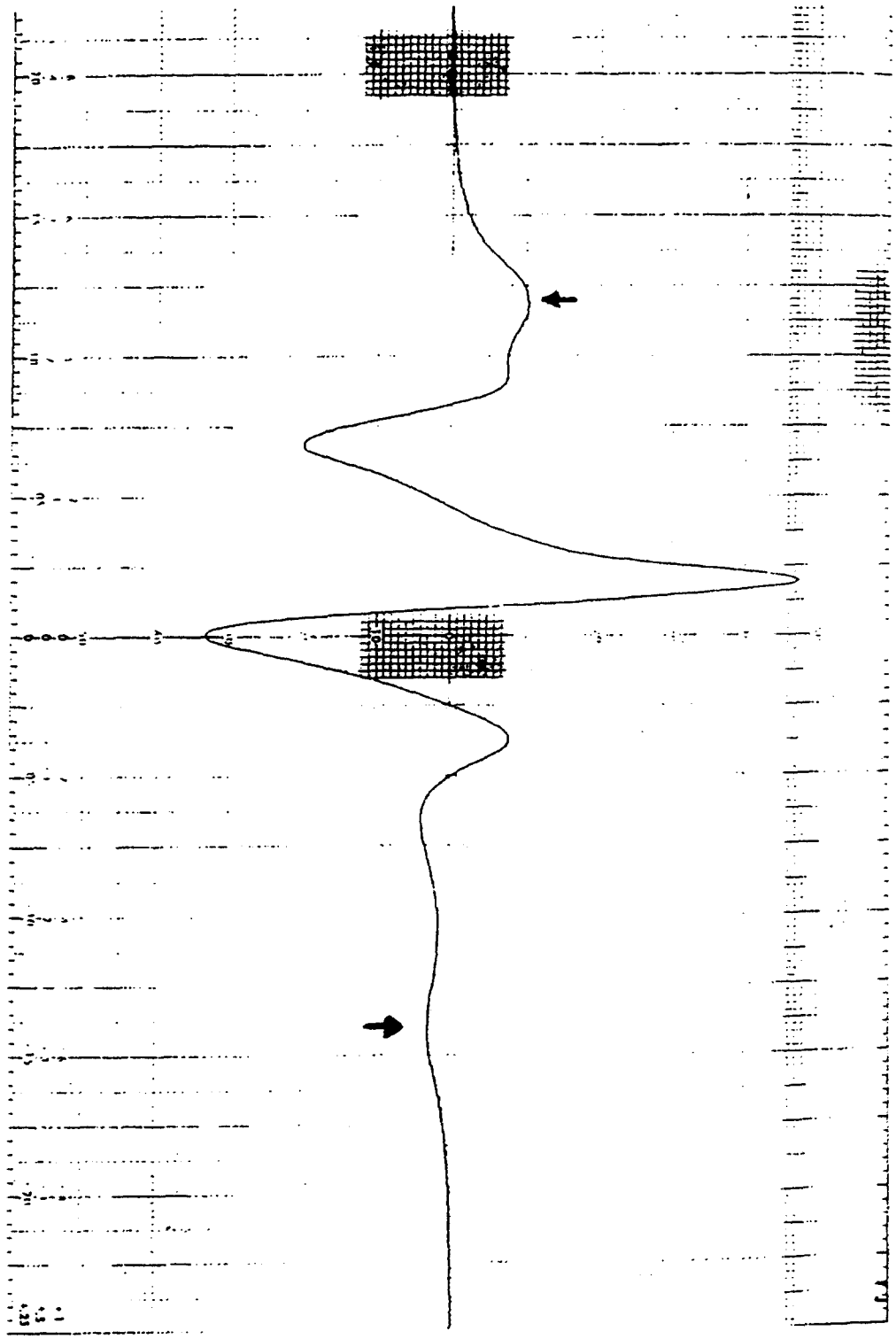


(c)

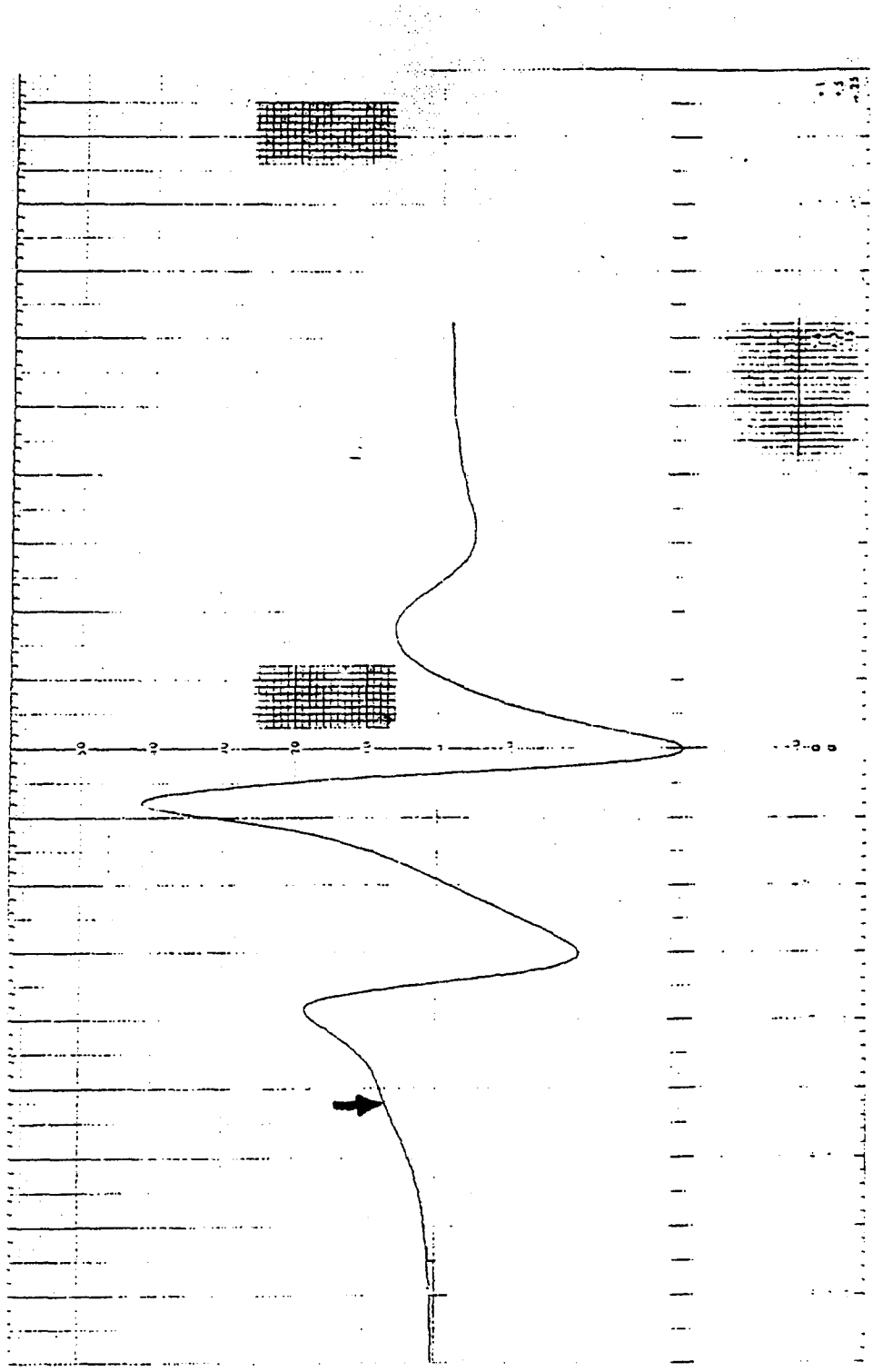


(d)

Fig.13-Particle size distribution of a(a)W/O, (b)W/S, (c)O/W, (d)MLV creams.



(A) SOY LECITHIN LIPOSOMES



(B) MLV

Fig.14. Evaluation of liposome formulations is showed (ESR study)

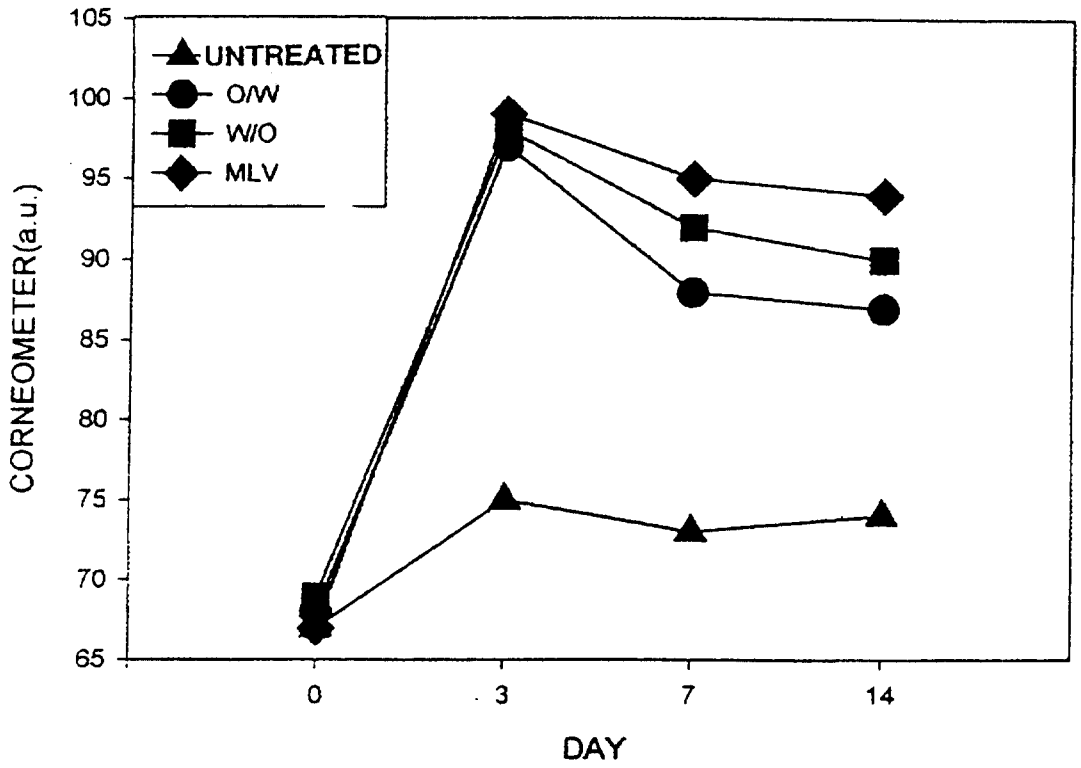


Fig.15. The effect of different creams on forearm skin hydration

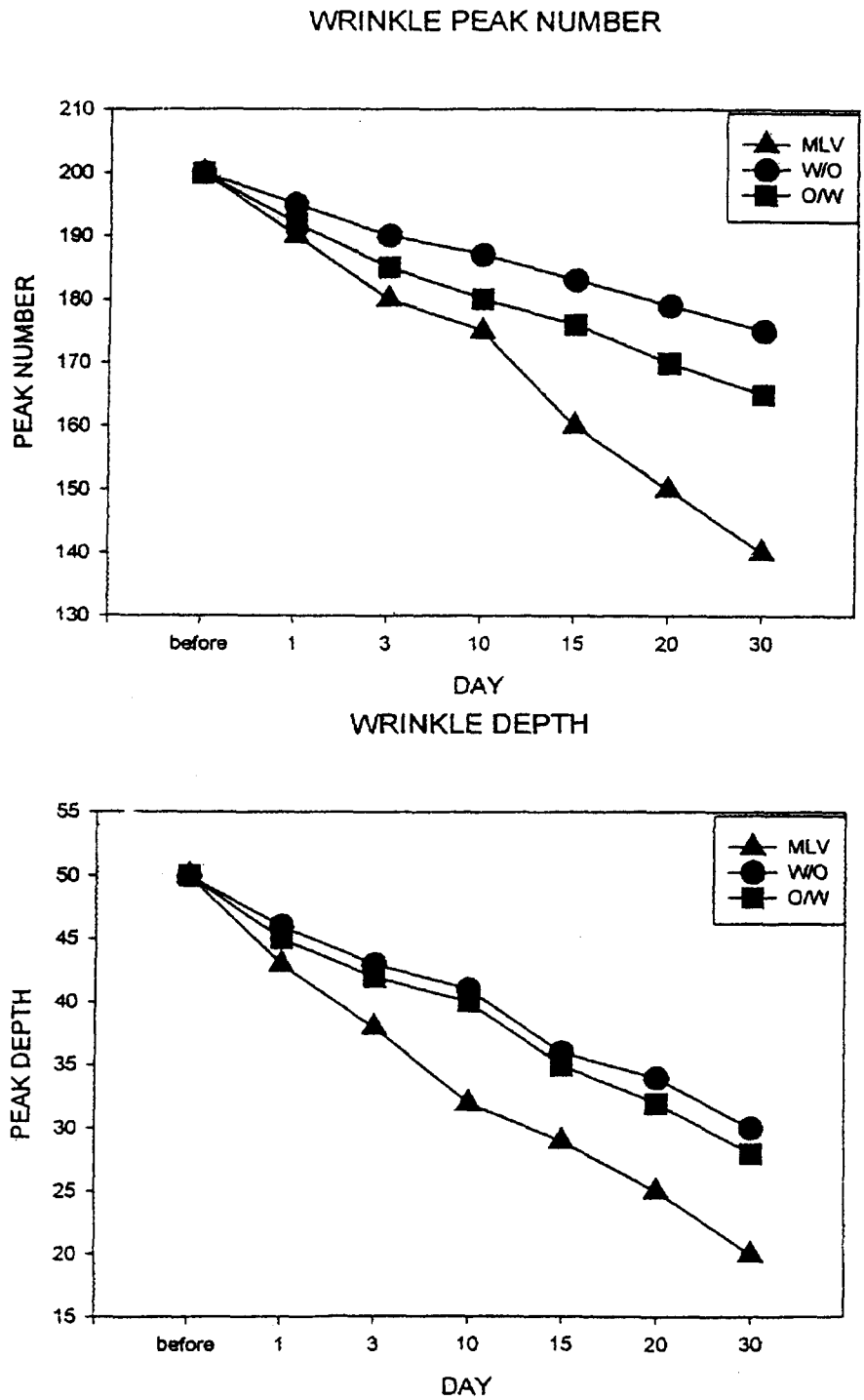


Fig.17. The measurement result of wrinkle efficacy