# DOUBLESPHERE-RETINOL

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## ABSTRACT

The raw materials requiring new technology have been introduced to the market as highly functional cosmetics have been popularized recently. The doublesphere which is the new capsulation is thus born. The doublesphere is double-layered capsulation of which the primary film is the nanocapsulation of liposome and the secondary film is the visible capsulation. The main component of the primary film is lecithin and that of the secondary film is algin/carrageenans/agar/cellulose. The doublesphere is characterized by that it is possible to keep active beautifying components stable and to improve the moisturizing effect, penetration power into the skin, roughness of the skin, etc. in view of its efficacy and effects since it is double-layered capsulation. The doublesphere-retinol is made in order to measure the stability, efficacy, and effects in the present study.

**KEY WORDS: liposome, microencapsulation, Doublesphere-Retinol**

## 1. Introduction

The recent trend in the development of cosmetics has been directed to the cosmedical technology for

which high functionality is sought. This has led to the production of raw materials of which components are very sensitive to the light, heat, and oxygen. It is therefore required to have the technology of effectively prescribing active ingredients. The doublesphere is a double-layered capsulation, of which the primary film is the nanocapsulation of liposome and the secondary film is the visible capsulation. The Liposome of the primary film is characterized by that both hydrophilic and lipophilic substances may be contained in the closed packet of bi-layer and its size and shape may be easily varied. It is also used for the drug delivery system because it is non-toxic *in vivo* since phospholipid is used, which has been studied actively. Two lipophilic groups are attached to the hydrophilic part such as phospholipid, and a bi-layer, not spherical micell, is formed if it is dispersed into the water. Such closed packet is called vesicle, and that which is composed of double phospholipid is defined as liposome. The types of liposome are classified into the multilamella liposome (multilamella vesicle, MLV) and unilamella liposome (unilamella vesicle, ULV) according to the size and shape of the vesicle. The unilamella liposome if further divided into LUV (large unilamella vesicle) and SUV (small unilamella vesicle). The size of MLV is about 400-3,500nm, that if LUV is 200-1,000nm and that of SUV is 20-50nm.(Fig.1) The encapsulation efficiency in % of MLV is 5-15%, that of LUV is 35-65%, and that of SUV is 0.5-1.0%. The method of preparation for liposome includes the mechanical method, detergent removal method, solvent projection method, reverse phase evaporation method, etc. In more details, the method for the manufacture of MLV includes the vortexing method, hydration method, bangham method, and lipidfilm method; that of SUV includes the sonication method, and surfactant removal method (cholic acid removal method); that of LUV includes the reverse phase evaporation method, ethanol injection method, and ether infusion method; and there are other methods of preparation such as the French press method, freeze-Thaw method, ca Induced fusion method, etc.

In the cosmetics area, liposome is applied to smoother metabolism of substances and increased

absorption of beautifying components into the skin by increasing the fluidity of biological films by using phospholipid. There is also niosome which is similar to liposome. Niosome forms the closed packet by grafting the phosphate derivative to the non-ionic surfactant (polyoxyethylene alkylether, polyoxyethylene alkylester, siaccharose diester) and the glycerine frame to have a amphophilic property. The size of such niosome is 10-5,000nm, and it is possible to contain many active ingredients.

The primary film of the doublesphere is composed of liposome or niosome, and the secondary film is composed of visible capsulation. The composition of the capsulation is various including algin, carrageenans, gelatin, PVA, cellulose, chitosan etc., and there are very many ways of making the visible capsulation.(Fig.2) The size of capsulation is around 5-2,000μ, and the capsule has elastic, rigid, fragile, and strong properties.

The doublesphere is made by converting vitamins, whitenings, flavors, etc. which are active ingredients, into the liposome of the primary film and forming algin/ carrageenans/ agar/ cellulose of the secondary film with those converted to liposome. The appearance of doublesphere is shown in the figure.3. Based on this, the stability of each 1) general cream (O/W) containing 1% of retinol, 2) liposome of the primary film, 3) visible capsulation in which the primary capsulation of retinol is not performed, and 4) doublesphere-retinol is measured at 25℃ and 45℃, the change in the content of retinol in each formulation is analyzed quantitatively by means of HPLC, and the efficacy and effects of doublesphere are measured in the present study.

## 2. Experiment

## 2.1 Experimental materials

The 1,450,000IU/gr of retinol 50C of BASF is used in the present experiment. Lipoids S100-3 of Lipoid Company is used for lecithin to make liposome. Algin which is used for visible capsulation of the secondary film is keltone from the Kelco Company. Chondrus crispus of Carrageenan Company of the U.S.A. is used for carrageenan, and junsei chm is used for agar. The purified water which has passed through anion and cation exchange resin columns is used for water, and all materials used in the present experiment conform to the standards for cosmetics.

## 2.2 Experimental instruments

The particle size of liposome is measured to study the distribution of particles by using the laser light scattering system (Malvern, PCS4700, UK) which is a particle distribution-measuring instrument. The phenomena of liposome is measured with SEM, and the change of colors at 25℃ and 45℃ is measured by using the chroma meters (Minolta, CM-100R, Japan). HPLC (waters) is used for the quantitative analysis of retinol, and Microfluidizer (Microfluidics Crop, F-120, USA) is used for making vesicles of liposome.

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

## 2.3 Experimental method

### 2.3.1 Manufacture of various types of capsules

Formula 1) General cream (O/W)

| | |
|---|---|
| A) Carbomer | 0.40% |
| EDTA-2Na | 0.01% |
| Glycerine | 6.00% |
| DEA-cetyl phosphate | 0.70% |

| | | | |
|---|---|---|---|
| Methylparaben | | | 0.20% |
| Water | make | to | 100 |

| | |
|---|---|
| B) Stearic acid | 1.20% |
| Cetyl alcohol | 1.00% |
| Sorbitan stearate | 0.50% |
| Glyceryl stearate | 2.00% |
| Macadamia nut oil | 15.00% |
| C) Triethanolamine | 0.40% |
| D) Retinol | 1.00% |

Procedure: Heat A until all ingredients dissolve. Add B to A and emulsify. Add C to AB and neutralize. Allow ABC to cool to room temperature. Add D to ABC.

Formula 2) Liposome of the primary film

| | | |
|---|---|---|
| A)Lipoid S 100-3 | | 1.00% |
| Propylene glycol | | 5.00% |
| MCT | | 5.00% |
| B)Water | make to | 100 |
| C)Ethanol | | 10.00% |
| Retinol | | 1.00% |

Procedure: Heat A until all ingredients dissolve. Add B to A and emulsify. Allow AB to cool to room temperature. Add C to AB. Pass microfluidizer.

Formula 3) Visible capsulation in which the primary capsulation of retinol is not performed

| | | | |
|---|---|---|---|
| A)1.water | | | 29.50% |
| 2.Algin/ carrageenans/ agar/ cellulose | | | 0.50% |
| 3.Retinol | | | 1.00% |
| 4.Liquid paraffin | | | 20.00% |
| 5.CaCl2 | | | 0.20% |
| 6.Water | | make to | 400 |

Procedure: material 2 was added to 1. After dissolving, adding 3 mixture. Obtained mixture was added to 4 and mixed. This mixture was added to the Solution which were prepared by adding material 5 to 6, and stirred at about 150rpm for 20-30minutes.

Formula 4) Doublesphere-retinol

| | | | |
|---|---|---|---|
| A)Lipoid S 100-3 | | | 1.00% |
| Propylene glycol | | | 5.00% |
| MCT | | | 5.00% |
| B)Water | | make to | 100 |
| C)Ethanol | | | 10.00% |
| Retinol | | | 10.00% |
| D)1.water | | | 29.50% |
| 2.Algin/ carrageenans/ agar/ cellulose | | | 0.50% |
| 3.FORMUlA 2 | | | 10.00% |
| 4.Liquid paraffin | | | 20.00% |
| 5.CaCl2 | | | 0.20% |

6.Water make to 400

# 3. Review and results

## 3.1 Conformation of liposome

Liposome is measured with SEM to confirm its formation, which is shown in the figure.4. As shown in the figure.4, it is seen that the unisphere is formed in a nanoemulsion. The particle size distribution of liposome is also shown in the figure.5, where the distribution of particle sizes is 50-200nm.

## 3.2 Measurement of change in colors by heat of capsules in Formulas 1, 2, 3, and 4

The measurement of change in colors of each sample at 25℃ and 45℃ after one month with a chroma meter is shown in the figure.6. (COLOR INTENSITY=| at 45℃ color intensity – at 25℃ color intensity|). As shown in the figure.6, the color changes at 45℃ as the time passes by due to the affect of retinol, where the double sphere is 4-16 times more stable than other formulas. This is due to that the factor for stabilization of liposome in the primary film brings about the synergistic effect to the stability of the secondary film.

## 3.3 Quantitative analysis of retinol of the capsule in Formulas 1, 2, 3, and 4

The change in the content of the capsule having 1% retinol at 25℃ is analyzed quantitatively by means of HPLC.(Fig.7) The result of measurement of the content of retinol in the capsules in Formulas 1, 2, 3, and 4 containing 1% retinol stored at 25℃ for one month is shown in the figure.8. As shown in the figure.8, it is seen that the content of retinol is maintained in order of Formula 4 (doublesphere)>2>3>1. This result is the same as that of measurement with a chroma meter in 3.2.

3.4 Measurement of the efficacy and effects of doublesphere

3.4.1 Moisturizing effect

The figure.9 shows the result of measurement of the state of skin with respect to the moisturizing effect before and one week after using the doublesphere by using an image analyzer. It is seen that there is a large amount of keratin prior to the use of doublesphere, but it disappears one week after the use.

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/cm$^2$ . In the following 6 days a home application in the morning and evening took place. Measurements were evaluated during the treatment period on day 2, 4 and 6 one hour after the last daily application. Use of other cosmetic products was restricted on the test areas throughout the whole study.(Fig.10)

3.4.2 Wrinkle reduction effect

The results of measurement of the depth or wrinkles with an image analyzer prior to and one month after the use of doublesphere are shown in the figure.11. It is seen that the depth of wrinkles is significantly reduced one month after use compared to that prior to use.(Fig.12)

# 4. Conclusion

The following conclusion is drawn from the manufacture of the doublesphere and the measurement of its stability, efficacy, and effects in the present study:

1) The primary film of doublesphere is liposome of which main components are lecithin and active ingredients, while the secondary film is composed of algin/carrageenans/agar.
2) The doublesphere is very stable against heat. That is, neither the color of active ingredients is changed greatly although the time passes by, nor the content of active ingredients as the result of quantitative analysis by means of HPLC.
3) The doublesphere is superior in its moisturizing effect as well as wrinkle reduction effect.

## Fig.1.The types of liposome

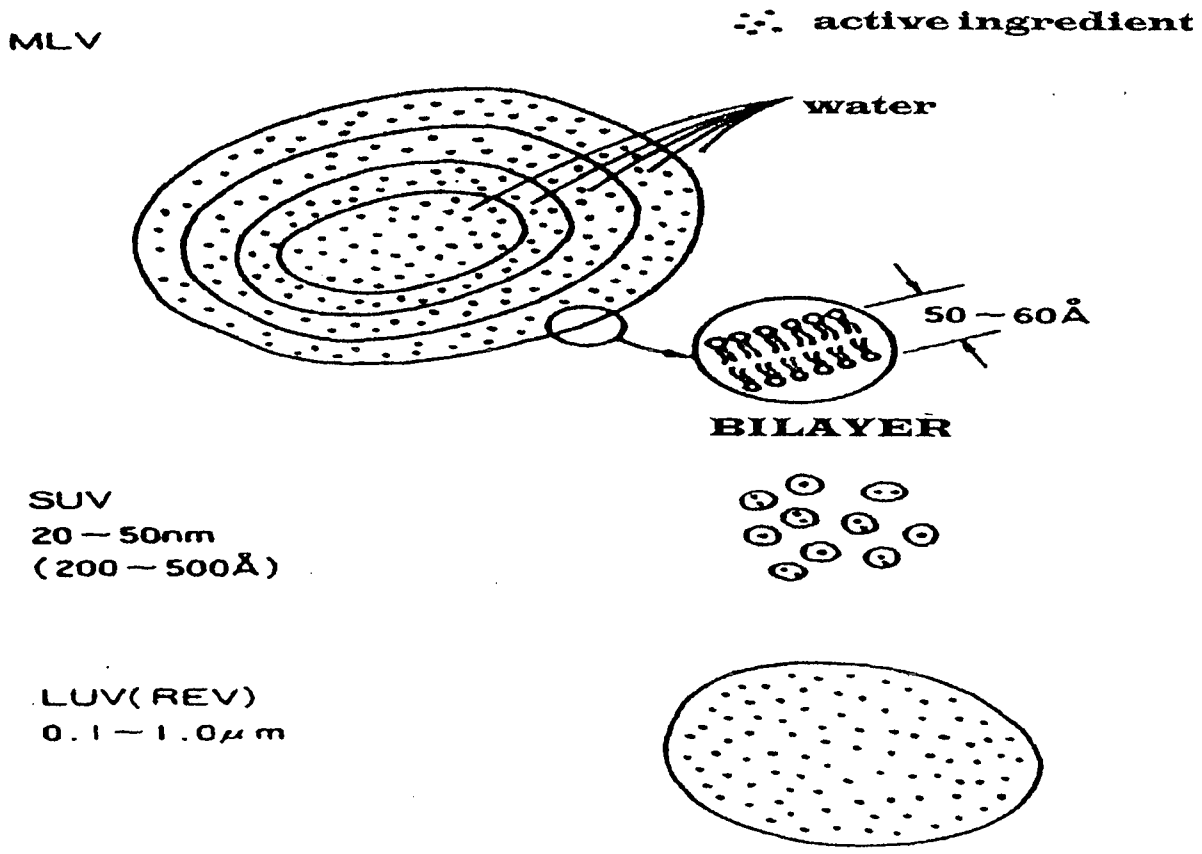
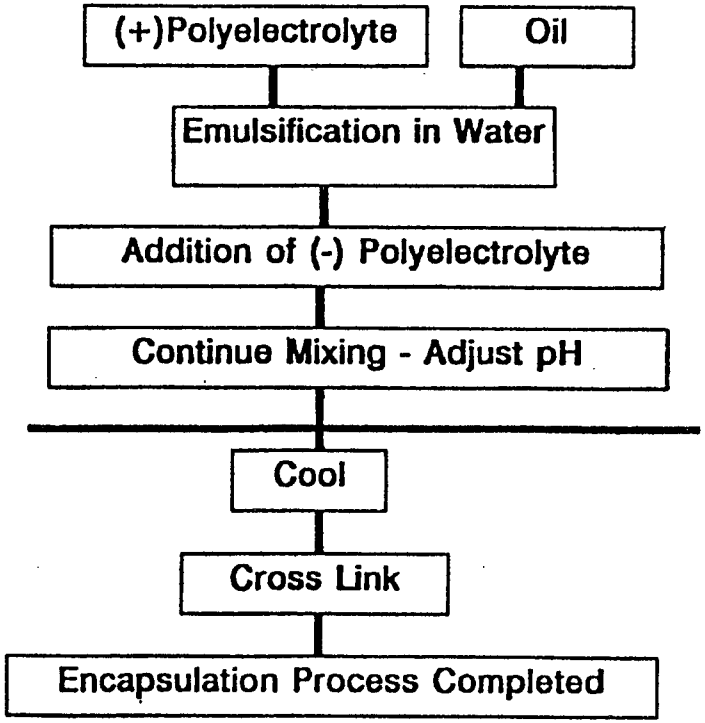
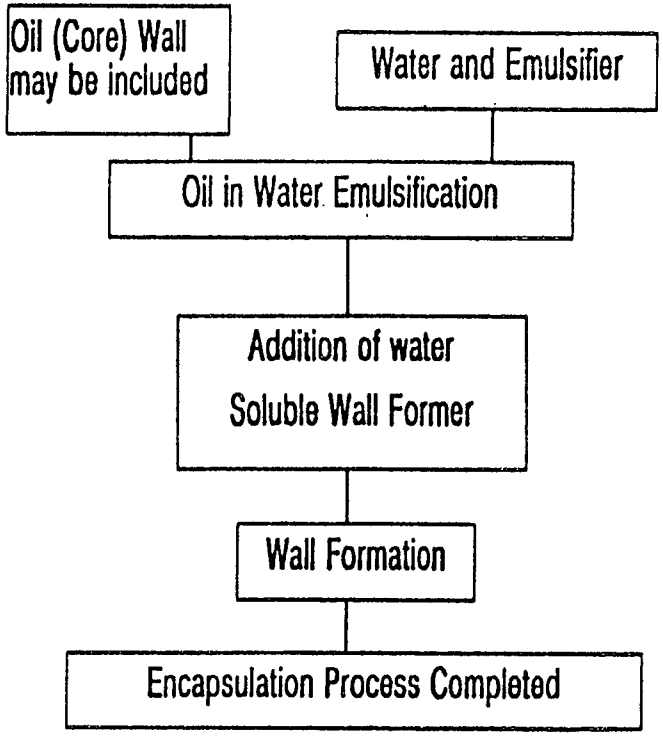


**Fig.2. Microencapsulation process**
**a)Coacervation , b)interfacial polymerization**




a)




b)

Fig.3. The appearance of Doublesphere

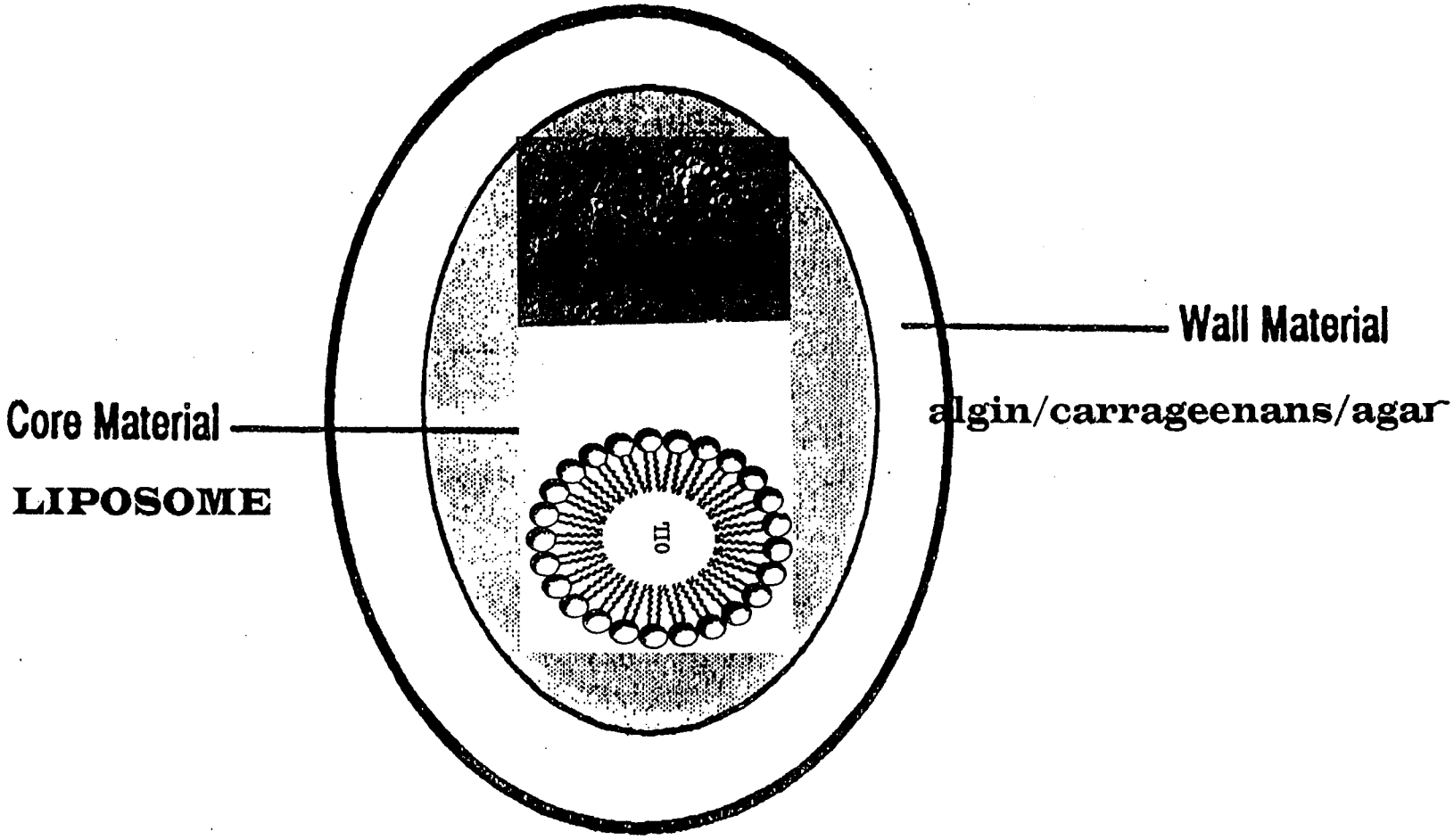


**Fig.4.Scanning electron microphotographs of liposome from formula 2, obtained after freeze-fracture(X58,000)**

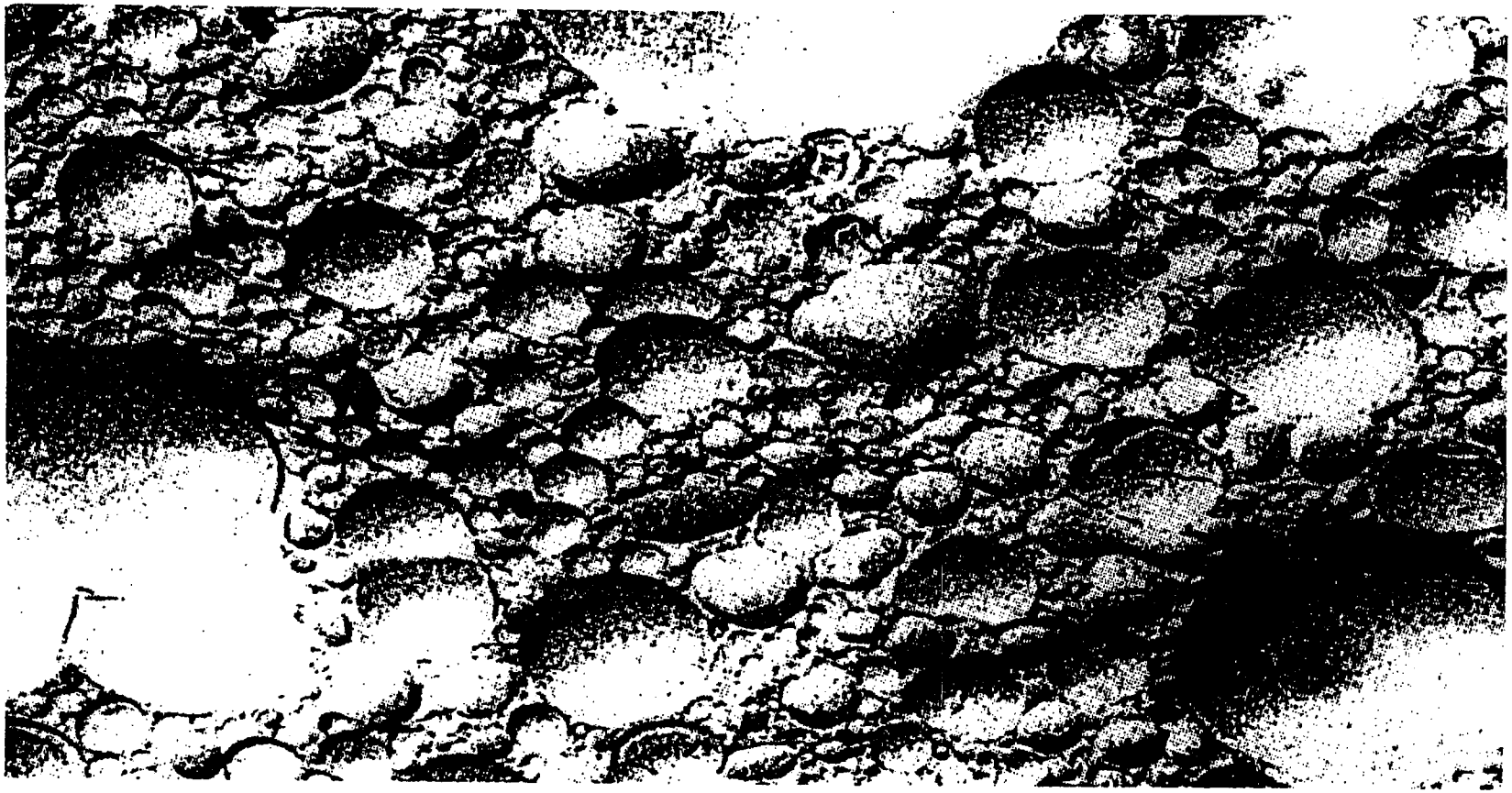


**Fig.5. Particle size distribution of liposome**

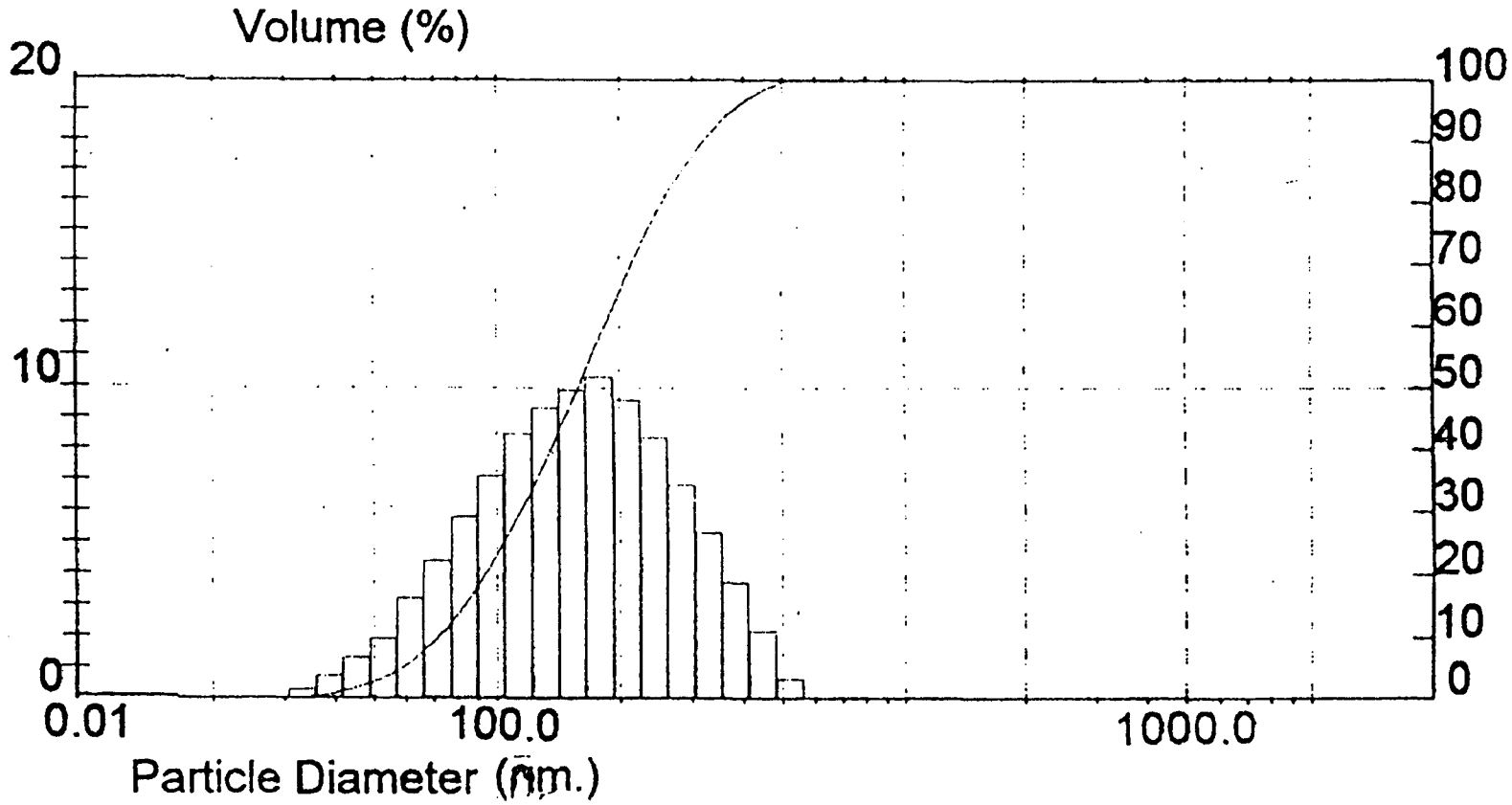


**Fig. 6. Color stability of tested formula after one month storage at 45C**

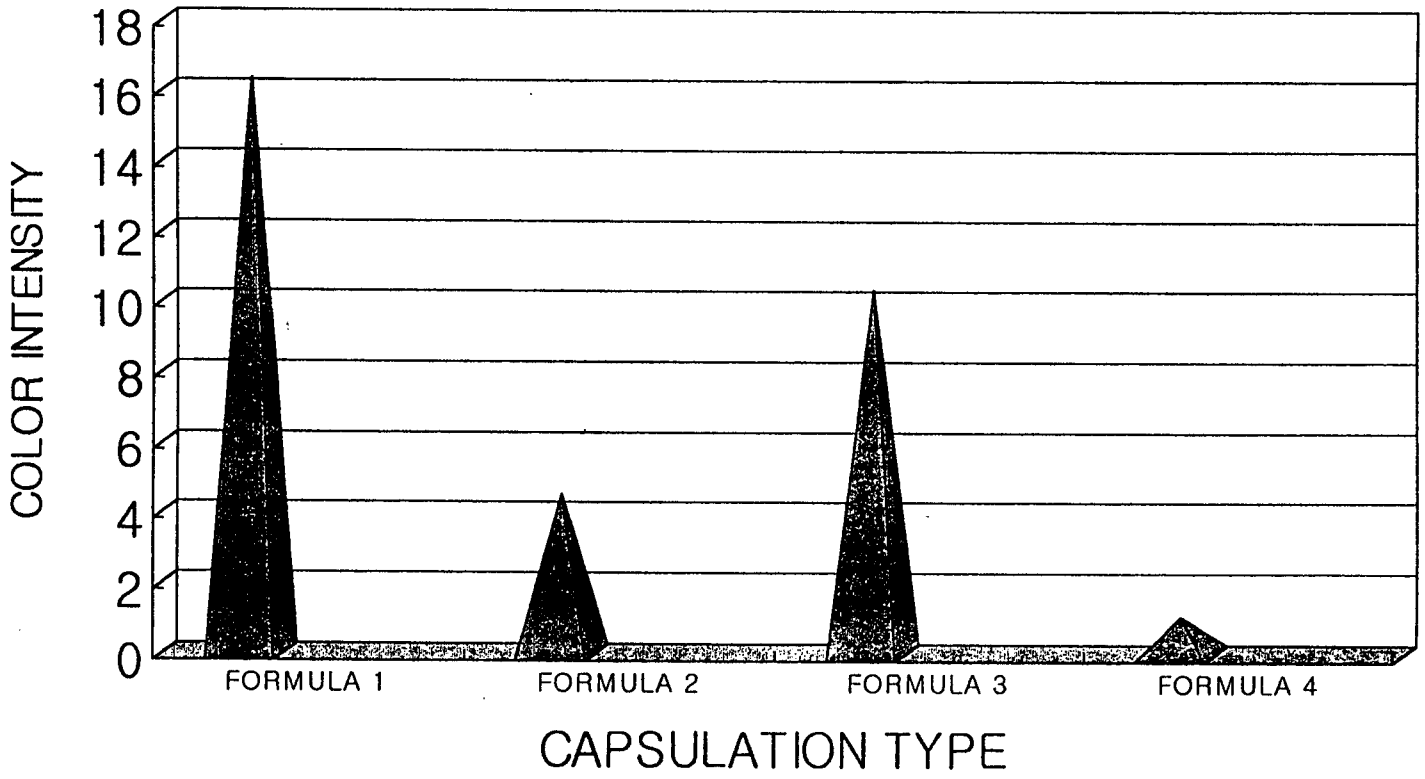


**Fig.7. Chromatogram of HPLC of Retinol after one month**

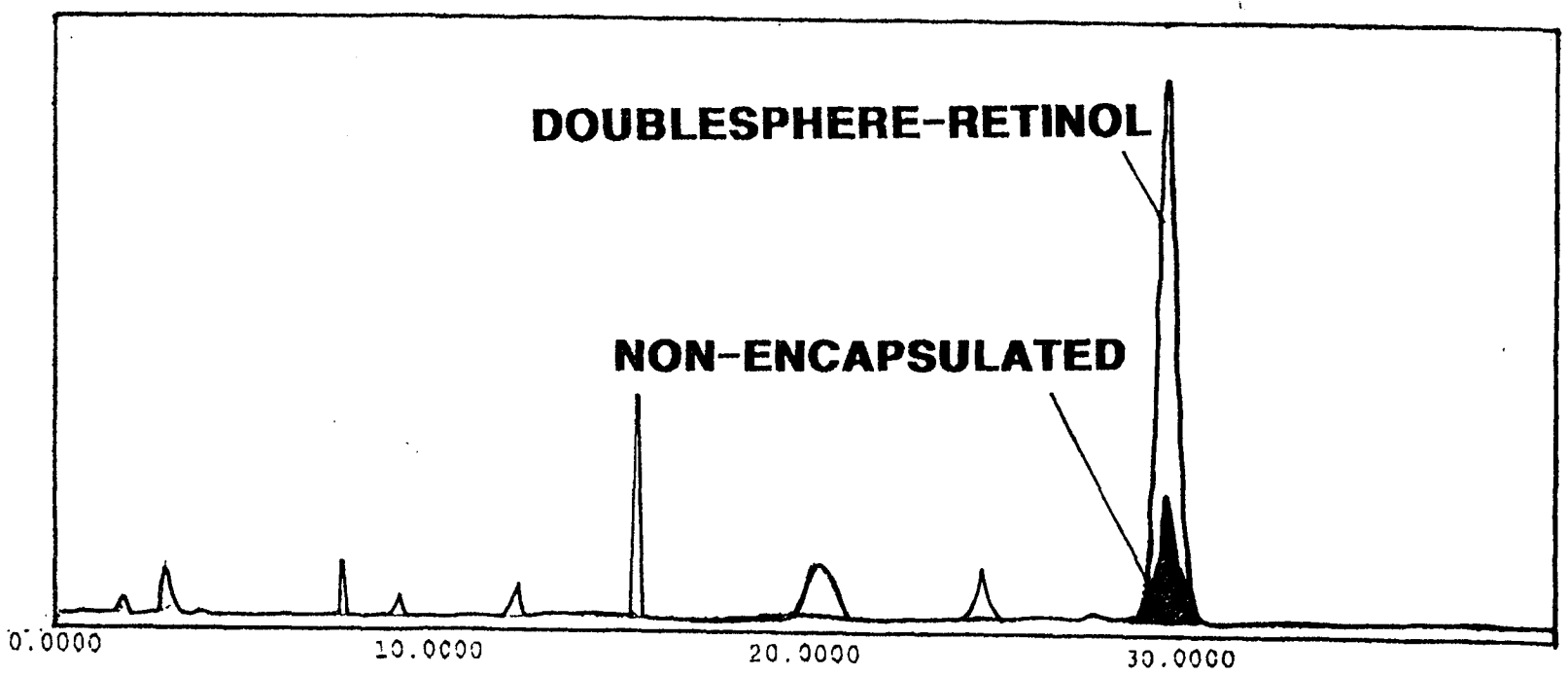


**Fig.8. Percentage of Retinol remaining in tested formulas during one month**

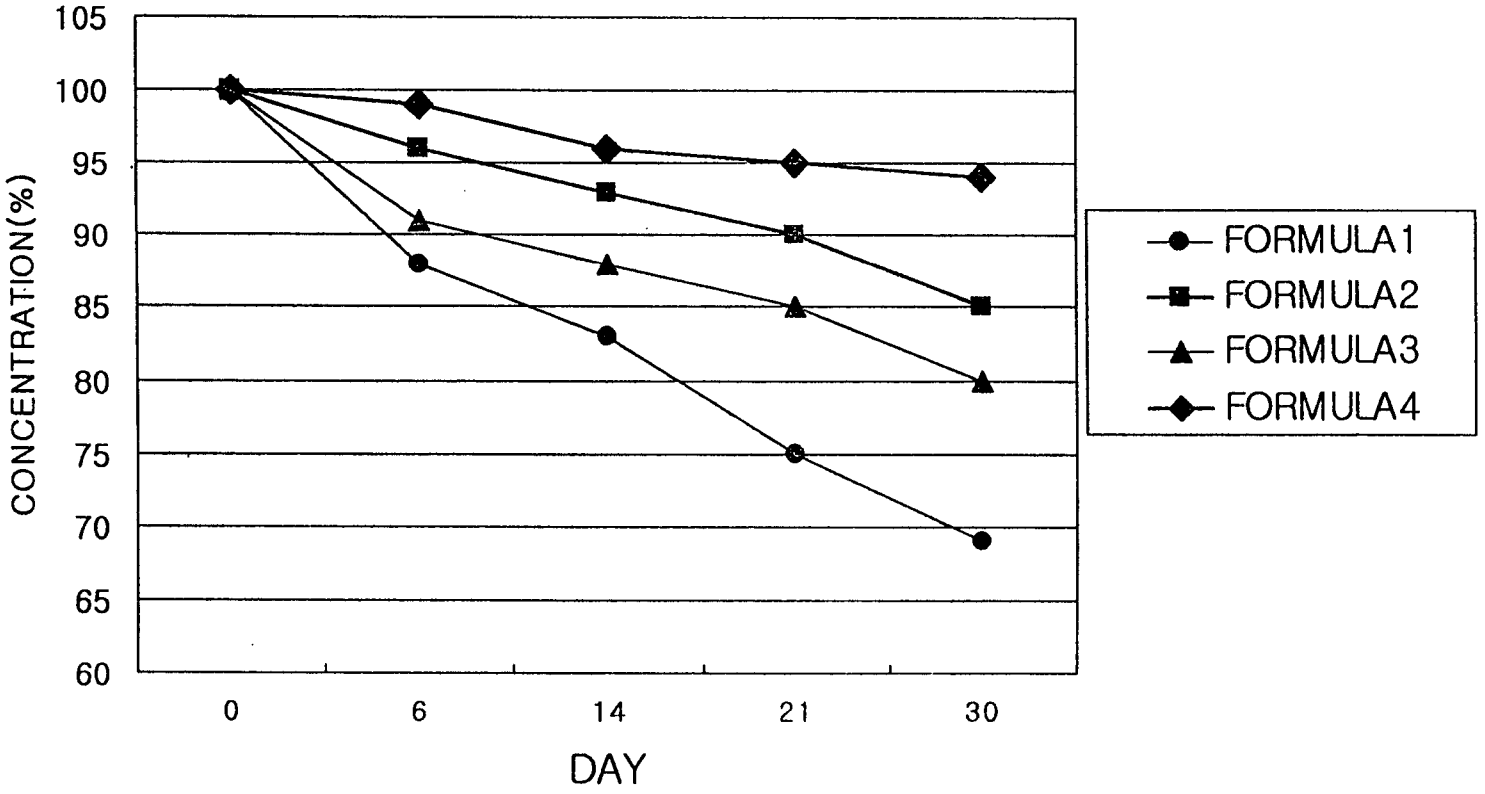
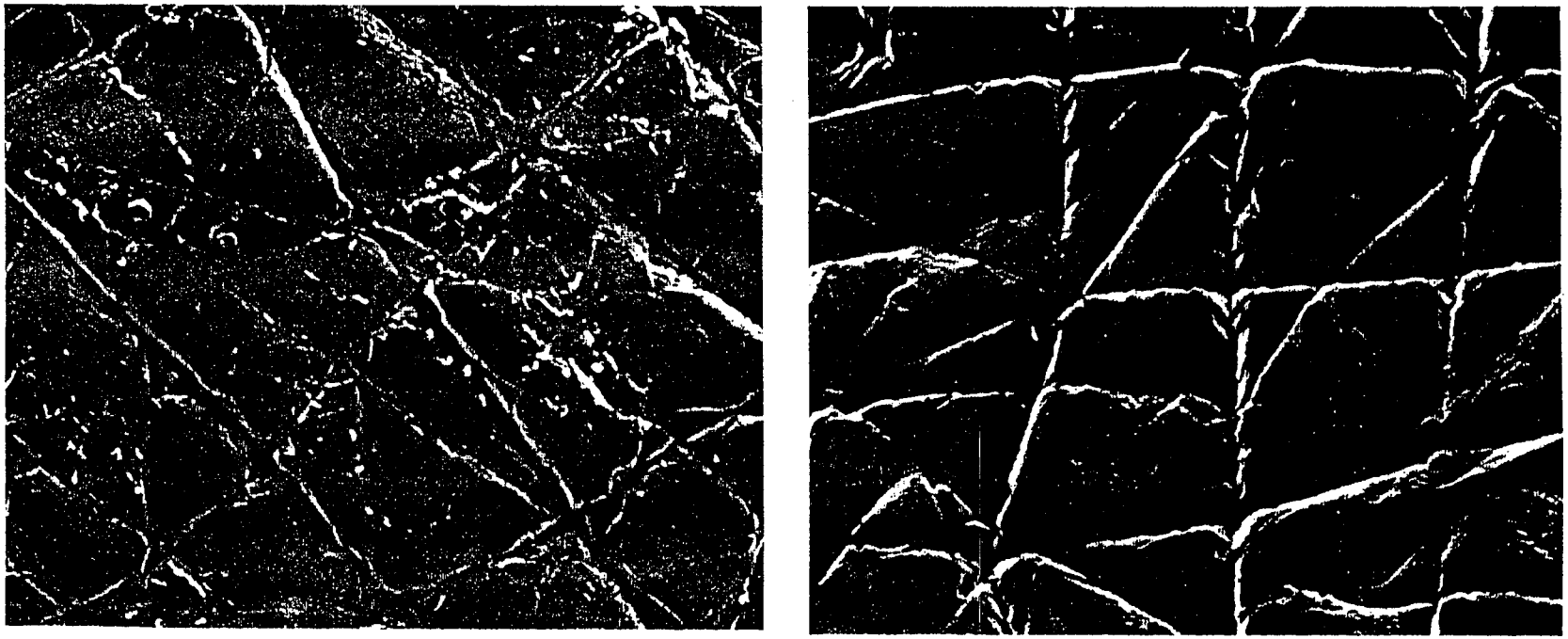


**Fig.9. a)the untreated skin area and b)the skin area shown in a) 5hour after application of the Doublesphere-Retinol**



a)

b)

**Fig.10. Effect of tested formulas on forearm skin hydration**

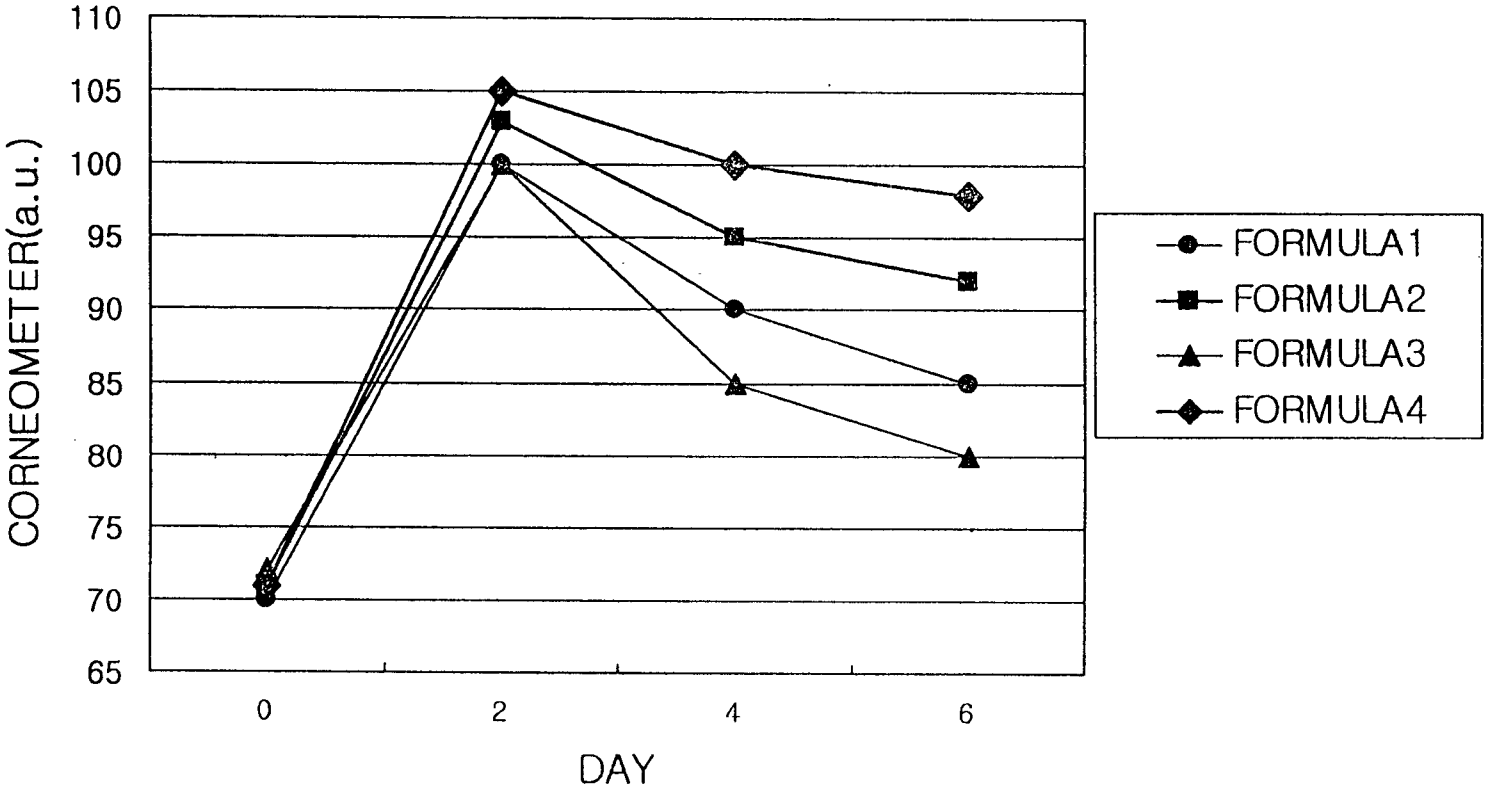


**Fig.11. Effect of tested formulas on depth of wrinkles**

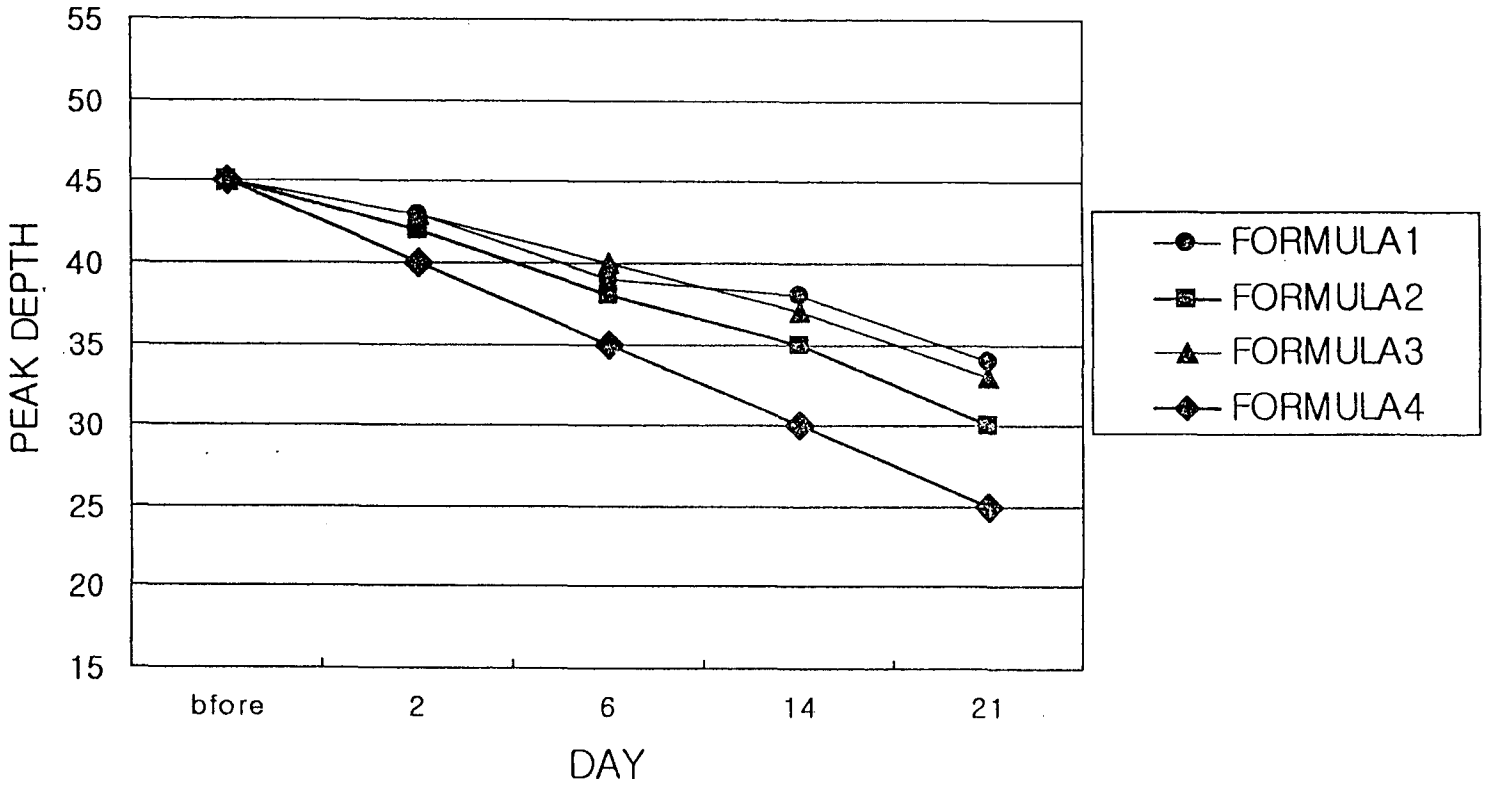
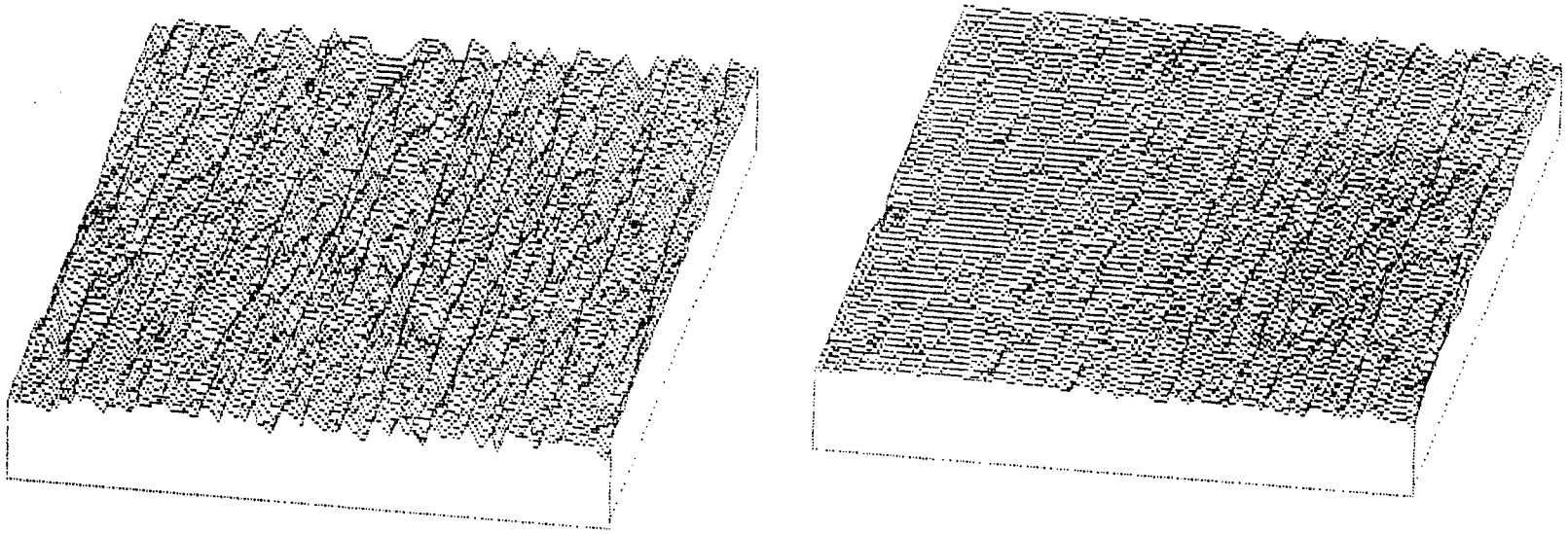


**Fig.12. Measurement of the wrinkles with an Image analyzer**



- before

after