Stability and Formation Mechanism for MLV liposomes with Phospholipid Film by Use of the Microfluidizer

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ABSTRACTS

The MLV liposomes have been developed in many drugs and cosmetics fields. The phospholipid base is made from ceramides, cholesterol, cholesteryl ester, lecithin, lanolin ester, and β-sitosterol, and surfactants are made by using (PEG)n-sitosterol(n=5) and K-cetyl phosphate. We made vesicles stable by passing samples through Microfludizer and created multilamellar vesicles to make MLV liposomes similar to the structure of men's skin. In order to make MLV liposomes, we created lipid membrane films which a mixture of phospholipid base and polyol group was reacted above Tc(95°C) by gelation for 3 hours. As the optimum conditions of Microfluidizer, we figured out 700 bar for the passing pressure of samples, 40°C for its temperature, and 3 times of frequency to pass through samples. Our MLV liposomes is stable on conditions of a low temperature(5°C) and a high temperature(45°C), which is not to be split in a large range. We produced our own moisturizing cream which has a good affinity to skin by means of this system.

1. Introduction

Since the discovery of Bangham's[1,2] which are composed of lipid bimolecular layers of surfactant phospholipid suspension using bimembrane. Such vesicles have been called as liposomes and succeeded in being vesiclated by means of lecithin's swelling reaction to phospholipid contained in water phase[3]. Two hydrophobic groups are, like phospholipid attached to hydrophilic group and shaped with bimolecular membrane and no prototypical micelle if diffused in water. Such phospholipid vesicles under this process are defined as liposomes. The skin's intercellular lipids are said to be 50% sphingolipids, 15% cholesterol, 10% cholesteryl ester, 20% free fatty acid and squalene, wax ester and triglycerides as a component of lipids of sebaceous glands. Sphingolipids are 95% of ceramides and 5% of glycolipids. Epidermis consist of several cell layers which are a primarily water and lipids(water insoluble materials). These lipids contain about 10% of stratum corneum and we have to notice how these lipids play important a role on the protection of the skin in cosmetic products[4].

1.1 Merits of MLV liposomes.

In several merits of MLV liposome system using phospholipid[5], first, it has a merit of capacity to contain water soluble as well as oil soluble materials in closed vesicles of bimolecular layers. Second, liposome in that system can be easily modified in its size and shape. Third, there's no toxicity within an organism by means of using phospholipid. Recently, there have been many researches on drug delivery system using their particular characteristics[6~10].

1.2 Elements and characteristics of MLV liposome.

Liposomes can be classified into multilamellar vesicles; MLV and unilamellar vesicles;ULV by its size and description. In particular, unilamellar vesicles are divided up by LUV(large unilamellar vesicles) and SUV(small unilamellar vesicles)(Fig.1). The size of liposomes in MLV is 200-3,500nm, in LUV about 100-1,000nm. And the range including active ingredients distributes 5 to 15% in MLV, 35 to 65% in LUV, and 0.5 to 1.0% in SUV. Skin horny layers, composed of lamellar structure, are deeply associated with barrier function of horny layers and skin moisturizing effect. The purpose of application of multilarmellar system using phospholipid in the field of cosmetic products is to increase its mobility of biomembrane for more active metabolism of matters. It is reported that longevity duration of cells has extended when conversing active ingredients in skin into liposomes through In-vivo and In-vitro test. As the results, the effect of liposome system is 5 times in epidermis and 3 times in dermis as high as the effect of general emulsification[11]. Though several microemulsion methods using phospholipid have been presented, to create lipid spheres, Guy Vanlerberghe and some colleagues succeeded in making microemulsion through Microfluidizer that is safety designed to disperse cholesterol and sodium dicetyl phosphate. And the method of making multilamellar vesicles using lecithin, cholesterol, and K-cetyl phosphate has lively been created by many researchers. On the basis of that process, this research is to use selectively most similar components to skin epidermis structure in forming the structural frame of MLV liposomes. This is to gain skin's good affinity and safety by creating stable vesicles. After forming lipid membrane film by means of gelation reaction of phospholipid, polyol group, and amphiphile surfactant above Tc, we made stable multilamellar vesicles using Microfluidizer. Also, we have experimented on each optimum condition with gelation temperature, smaples passing pressure, frequency of Microfluidizer passing through samples, and particle size and stability of vesicles. As application of cosmetic products, we compound oil phase and water phase of vesicles with each additive in order ot create moisturizing cream.

2. Experimental

2.1 Materials

Phospholipid base conducted on this experiment is the following: ceramides(sero lab.), cholesterol(Henkel), lanolin ester(Nippon fine chem.co.,LTD.), β-sitosterol(kaukas), cholesteryl ester(Henkel), lecithin (UPI). We used (PEG)n-sitosterol(n=2-12), and K-cetyl phosphate for surfactant. All the materials we used in this experiment meets standard requirement of cosmetic products.

2.2 Equipment's

We took a photograph of the process of forming MLV liposomes with Freeze-Fracture Electron Microscopy and used Laser Light Scattering System (Malvern UK, DCS4,700, USA) in distribution of vesicles and also used Centrifugal in measuring the stability of particle size.

2.3 Experimental methods

Making methods of liposomes have been studied by many researchers up to now, such as MLV using Voltexing method[12], SUV using Pre-vesicle method[13], Sonication method[14], Ethanol-injection method[15], French press extrusion method[16], W/O/W-emulsion method[17], and Reverse-phase evaporation method[18]. In this research, we created a highly purified base through a process of completely dissolving phospholipid base in chloroform, followed by evaporating solvent in it. We could get some amount of gel of lipid after swelling reaction above Tc with polyol by passing the sample through Microfluidizer. Stable MLV liposomes are made by putting active additives such as AHAs, vitamin-A palmitate, and vitamin-E acetate in there.

Table 1. Composition of phospholipid base.

Order	Ingredients	Contents(wt,%)
1	Ceramides	10.00
2	Lecithin	20.00
3	Squalane	Q.S
4	Cholesterol	10.00
5	Cholesteryl ester	5.00
6	Lanolin ester	5.00
7	b-sitosterol	5.00
8	(PEG)n-sitosterol(n=5)	20.00
9	K-cetyl phosphate	5.00
	T O T A L	100.00

This is expected to bring an excellent action and great effect toward skin. Descriptions of MLV liposomes and Microfluidizer's schematic diagram are presented in Fig.2 and Fig.3. And compositions of phospholipid appears on Table 1.

2.4 Size and stability of particles.

We have for 6 months observed a sample under the condition of a low temperature (5°C) and high temperature (45°C) as well, and also observed the distribution of particle size with Laser Light Scattering System. We never used any samples that ever used and figured out the measuring frequency as the mean value of 10 time's measurement. For observing stability of MLV liposomes, stability of vesicles was tested with centrifugal and Image Analyzer.

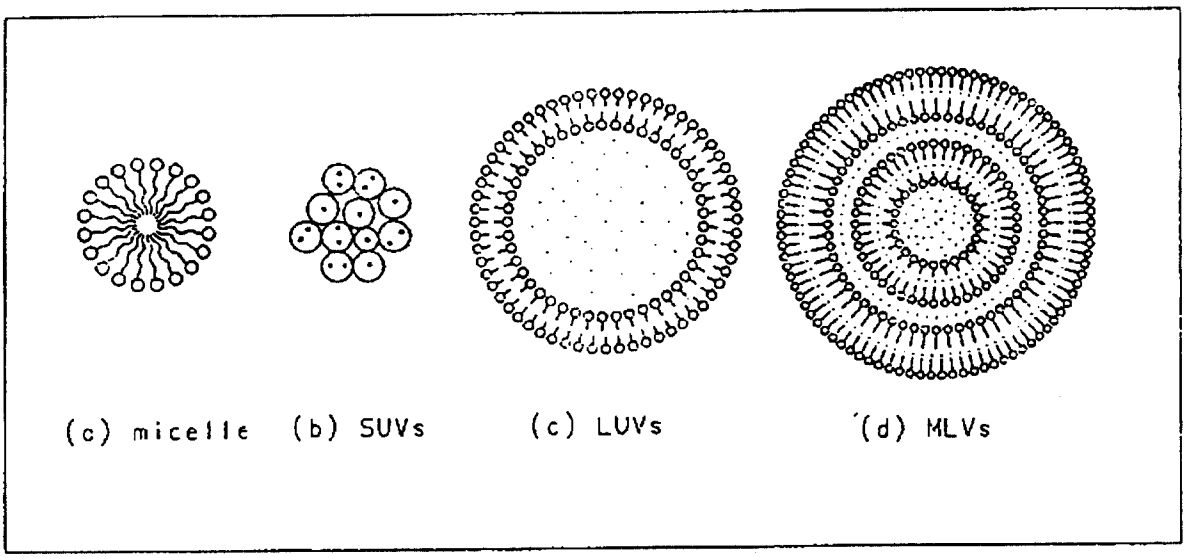

FIGURE 1. SCHEMATIC DIAGRAM OF MANIFOLD VESICLES.

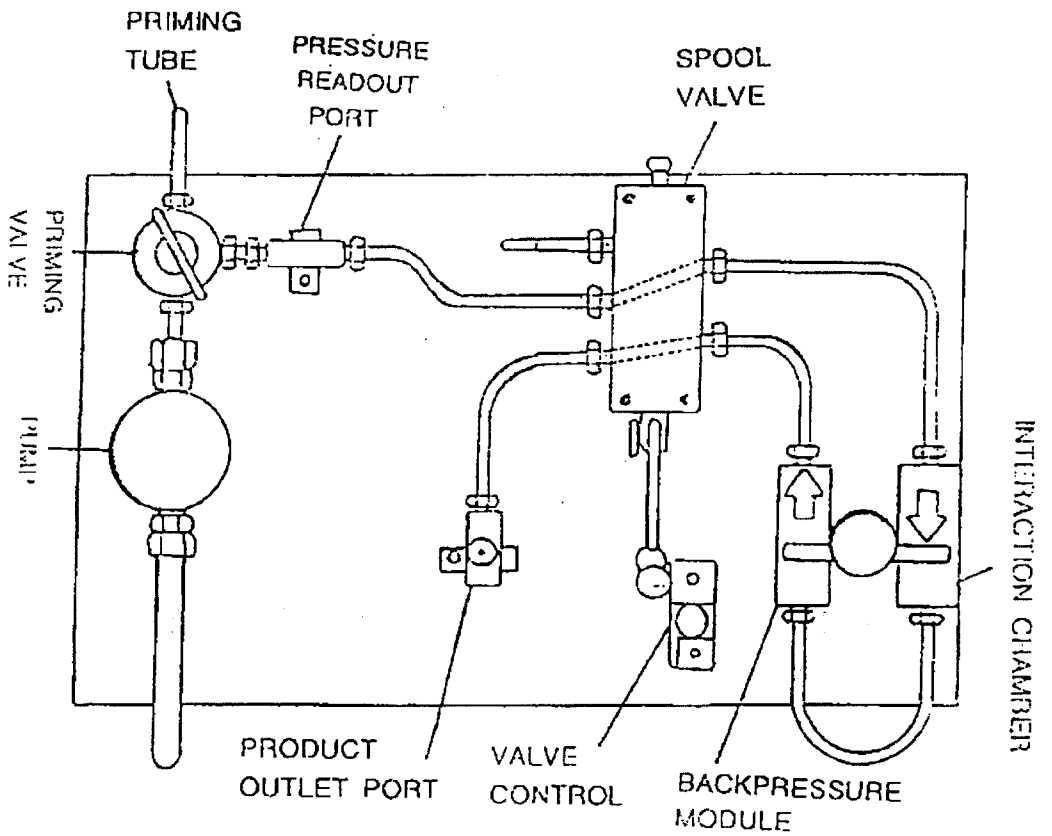

FIGURE 2. SCHEMATIC DIAGRAM OF THE MICROFLUIDIZER.

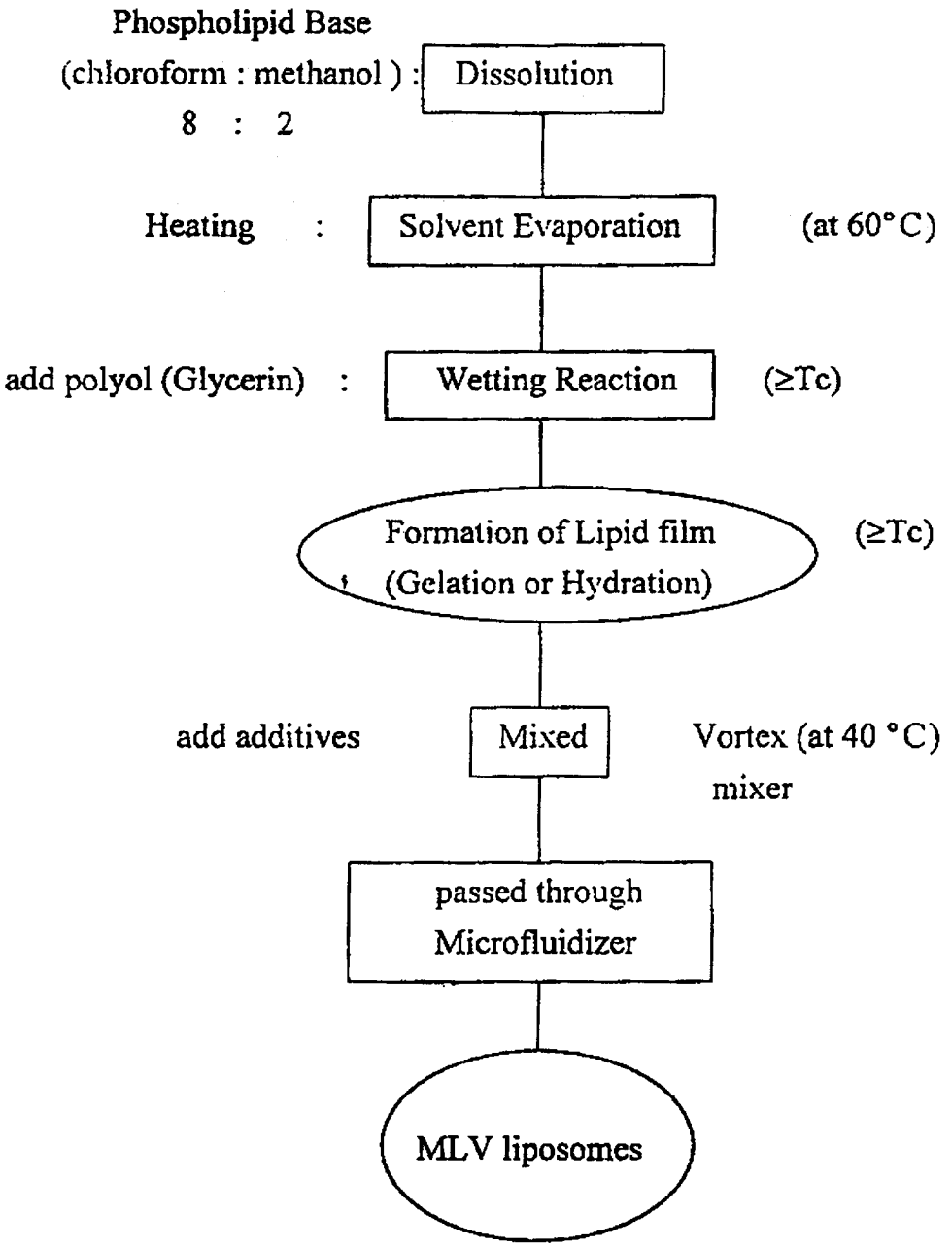

FIGURE 3. FLOW SHEET OF PREPARATION METHOD FOR MLV LIPOSOMES.

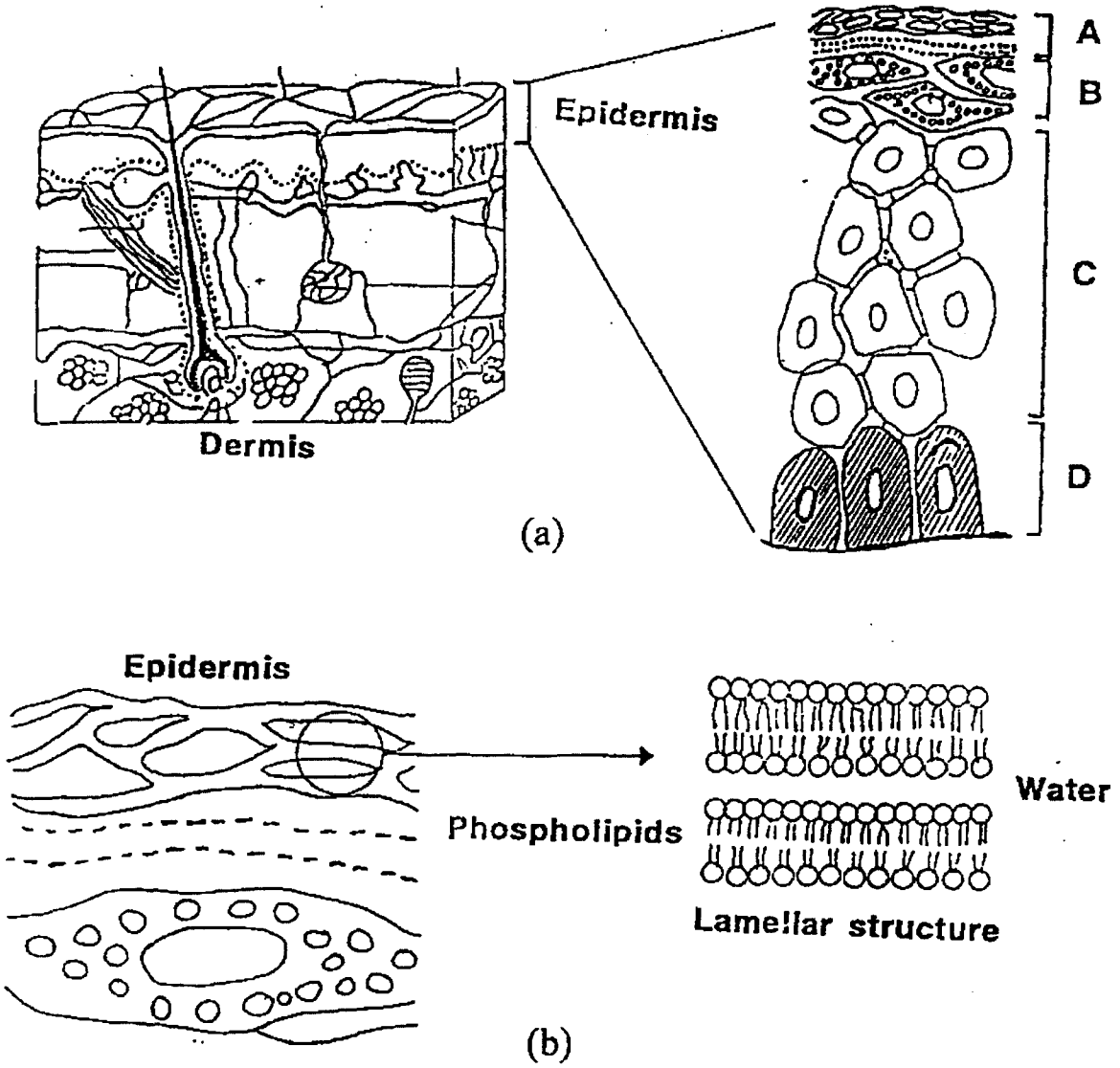

FIGURE 4. SCHEMATIC STRUCTURE OF MAN'S SKIN EPIDERMIS.
A;HORNY LAYER, B;GRANULAR LAYER,
C;PRICKLE CELL LAYER, D;BASAL LAYER.

3. Results and Discussion

3.1 A Mechanism composing MLV liposomes of phospholipid base.

As anyone get old, human epidermis also indicate its symptoms of senility. This pheonomenon is, more than any other things, needed to be prevented by taking nutritive chemical substances into skin Man's epidermis structure are as indicated in Fig. 4b, classified into epidermis and dermis[19]. Epidermis is again divided into 4 sectors of layer, which are Horny layer, Granular layer, Prickle cell layer, and Basal layer. epidermis(Fig.4b) is composed of linear lamella structures. Phospholipid base is indispensable in order to make those similar structures, and we should conduct swelling reaction above Tc for 3 hours after adding Polyol-group in there, in order to make very well below Tc, and it is most important that phospholipid base should be conversed into gel in this process. Only complete and regular components make stable lipid films. We added in below 40°C for the risk of decomposition and deterioration of active ingredients above Tc.

3.2 The pressure condition on samples passing through Microfluidizer.

One must pay a great attention to operating Microfluidizer. We measured if vesicles formed in the range of 100-1,000bar in order to find the pressure condition when ready-made phospholipid base passed through Microfluidizer. We found that the pressure in 700bar was most stable for vesicles(Fig.5). In the pressure as below as 500bar was irregular its distribution of vesicles, and we found that the liposome vesicles tend to be split in such a high pressure as above 800 bar.

3.3 The optimum conditions of temperature

We observed MLV liposomes whether they were properly formed in conditions of the pressure 700bar and the shifting temperature (10-100°C). As temperature goes up, particle size is somewhat getting smaller inversely, and we fixed the temperature passing through samples with 40°C. We considered of a disposition of dismantling additive ingredients and found no other problems with forming of vesicles.

3.4 The effect of MLV liposomes on increasing frequency passing samples through Microfluidizer

One must take a notice of the pressure, temperature, and frequency passing through Microfluidizer when forming MLV liposomes. We measured particle size distribution 1 to 10 times of sample frequency in order to make vesicles stable and regular. Fig.6 has shown that MLV liposomes would be most stable in the frequency of 3 times or more.

More than 4 times of frequency results in waste of fuel and in effeciency and less than 2 times of frequency brings about unstability of particle size distribution: 3 times of frequency is most proper.

3.5 Stability of MLV Liposomes

As stated above, we have been looking for the optimum conditions on several factors to make the most stable MLV liposomes. Fig. 7 indicates to prove whether this condition creates liposomes or not, using Freeze Fracture Electron Microscopy. The picture is magnified by 16,000 times, and we found multilamellar vesicles and unilamellar vesicles formed in it. The distribution of particle size is ranged from 50 to 653nm and its mean size is 200nm in the picture. Also, we have observed for six months if MLV liposome vesicle are stable in both 5°C and 45°C of incubators (Fig. 8). As the results, we found that they are stable and inconsistent with its temperature whether wigh or low and also found that particles of microemulsion are stable, not to be split, as the result of observing the size of particles for an hour in 5,000 rpm by using a centrifugal.

3.6 Application of Cosmetics

Our moisturizing cream has been made as the application of phospholipid base, which is figured in Table 2. And we succeeded in enlarging osmotic effect on additive ingredients by adding vitamin-E acetate and vitamin-A palmitate in hydrophobic group and AHAs in hydrophilic group.

FIGURE 5. EFFECT OF AIR PRESSURE CONDITION ON THE FORMATION OF MLV LIPOSOMES.

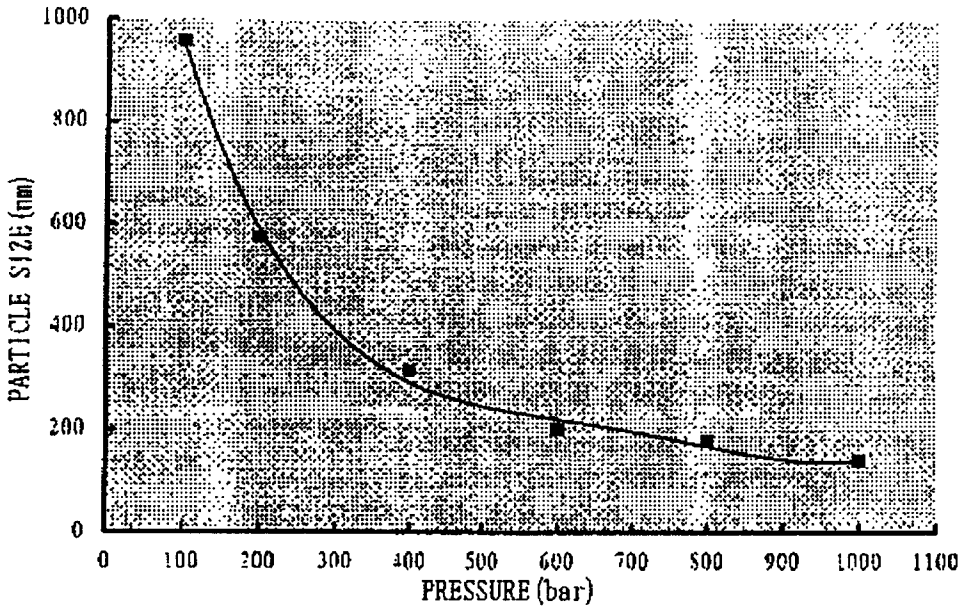

FIGURE 6. THE OPTIMUM CONDITIONS OF TEMPERATURE FOR VESICLES.

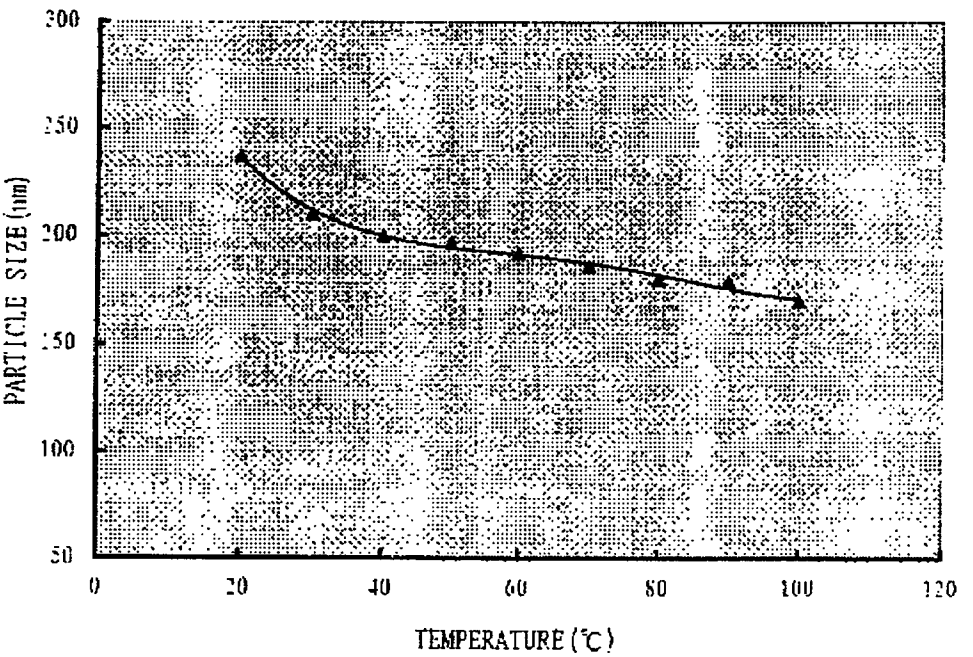

FIGURE 7. PARTICLE SIZE TO NUMBERS OF PASSED FOR MICROFLUIDIZER.

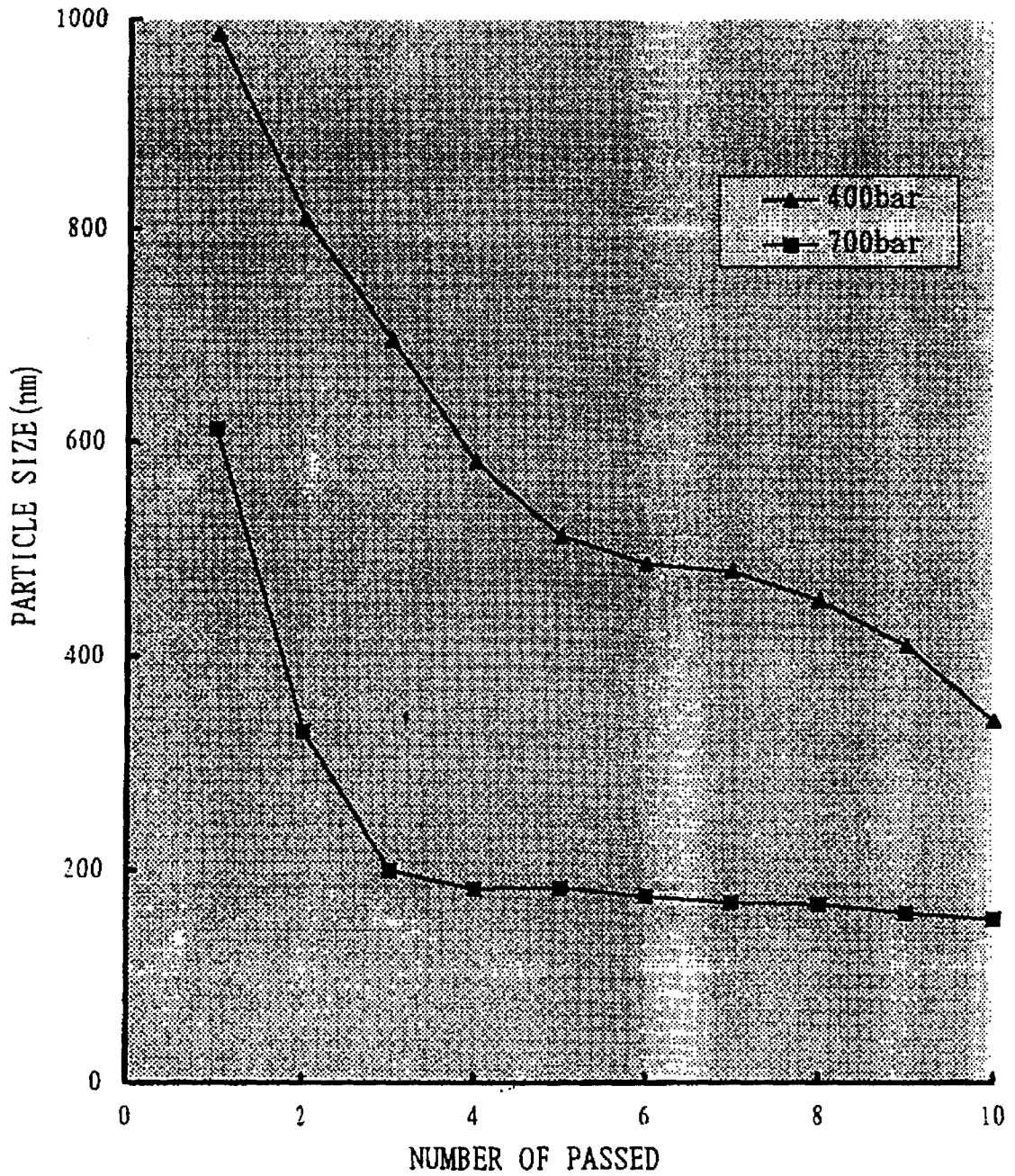

FIGURE 8. FREEZE-FRACTURE ELECTRON MICROSCOPY PHOTOS OF MLV LIPOSOMES;MICROEMULSIFIED LIPOSOME PREPARATION 3 TIMES PASSED USING THE MICROFLUIDIZER(mag.X16,000)

FIGURE 9. PARTICLE SIZE DISTRIBUTION OF MLV LIPOSOMES.

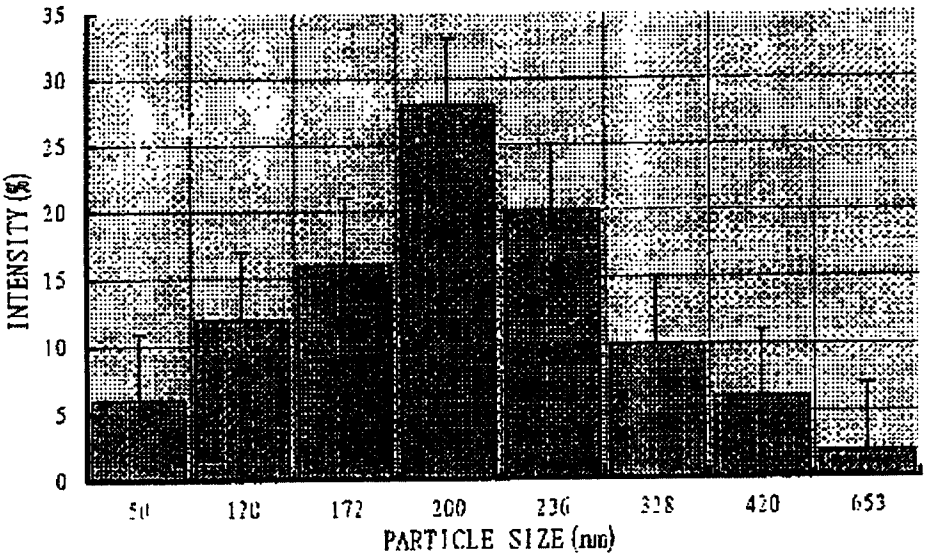

FIGURE 10. STABILITY OF MLV LIPOSOMES.

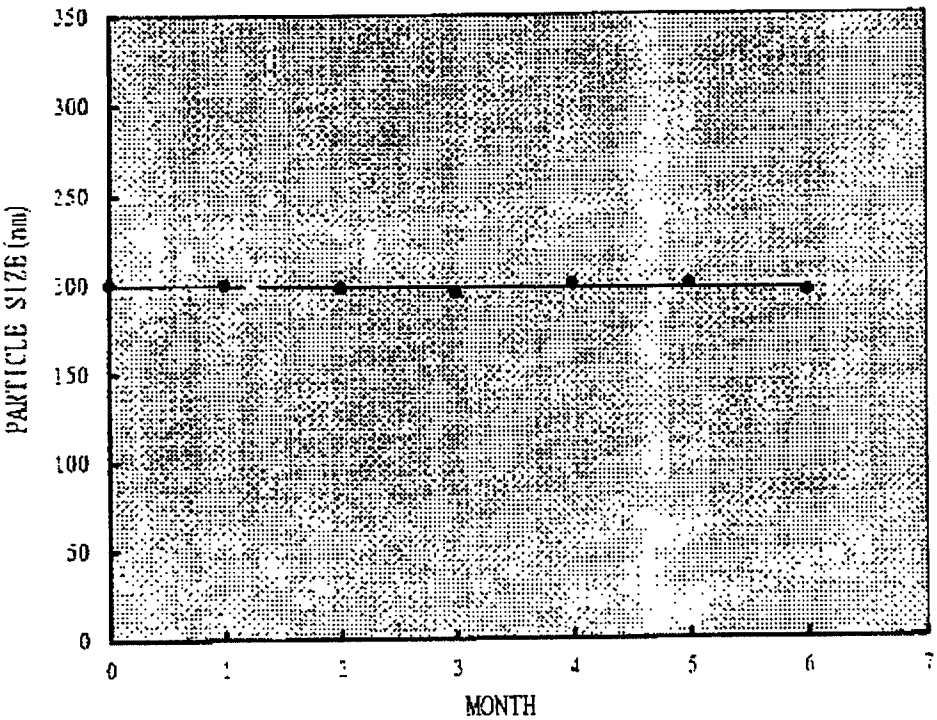

Table 2.

Composition of moisturizing cream for the liposomes.

Phase	Ingredients	Contents(wt,%)
(A)	Phospholipid base	20.00
(B)	Glycerin	
	Pirified water	
(C)	Malic acid	0.50
	Tartaric acid	0.30
	Lactic acid	1.00
	Citric acid	0.10
	Glycolic acid	
(D)	Vitamin-E acetate	2.00
	Vitamin-A palmitate	1.00
	T O T A L	100.00

4. Conclusion

In this research, we have created new phospholipid base for MLV liposomes and used Microfluidizer for more stable vesicles with optimum conditions.

1) The phospholipid base is made from ceramides, cholesterol, cholesteryl ester, lecithin, lanolin ester, and b-sitosterol, and surfactants are made by using (PEG)n-sitosterol(n=5) and K-cetyl phosphate.

2) In order to make MLV liposomes, we created lipid membrane films which a mixture of phospholipid base and polyol group was reacted above Tc(≥95°C) by gelation for 3 hours.

3) We made vesicles stable by passing samples through Microfluidizer and created multilamellar vesicles to make MLV liposomes similar to the structure of men's skin.

4) As the optimum conditions of Microfluidizer, we figured out 700bar for the passing pressure of samples, 40°C for its temperature, and 3 times of frequency to pass through samples.

5) Our MLV liposomes is stable on conditions of a low temperature(5°C) and a high temperature (45°C), which is not to be split in a large range.

6) We produced our own moisturizing cream which has a good affinity to skin by means of this system.

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