A STUDY ON THE STABILITY, EFFICACY, AND EFFECT OF COLLOIDAL SILVER EMULSION

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Abstract

A colloid refers to dispersed particles of a solid or liquid having the diameter of about 10^{-5} - 10^{-7} cm. Such colloidal silver is produced by electrolysis. In this paper, colloidal silvers of various concentrations according to charge amount and time are produced, and their anti-microbial activities are measured. And optimum conditions for emulsion are measured by varying the concentration of colloidal silvers. Further, stability of the emulsion is measured with a Zeta potential, chrome meter by applying colloidal silvers to creams (W/Si, O/W, MLV).

1. PREFACE

The recent trend of cosmetics has been progressed to pursue high functionality and high added values. Particularly, silver has not been used widely although its effect has been known from the past. Silver (Ag) is a chemical element having the atomic number of 47 and atomic weight of 107.8682. Ag has been known since BC 1,500, and its oxidation state has been known to be +1 usually, or +2 or +3 rarely. There have been no exact methods of proving antibiotic effects of silver, however, most widely accepted theory is that silver produces a specific enzyme which is used by various types of bacteria, viruses, and fungi for their metabolism. Particularly,

whereas antibiotics kill about 5 - 6 pathogenic organs, silver is able to kill about 650 pathogenic organs, is about non-toxic, and is not resistive. Silver has a superior counteracting effect of poison, and therefore, been used as an antidote. According to Dong-Eui-Bo-Gam (a famous medical book in Korea since old times), it has been effective for curing leucorrhoea, convulsion, epilepsy, etc. It has been known that NASA uses silver filters for drinking water in order to prevent infectious diseases such as dysentery, etc. It has been also known that oral or external application of a small amount of silver increases immunity greatly, and is effective for curing cold, conjunctivitis, gonorrhoea, leprosy, inflammation of lymphatic gland, mole, pimple, and burn.

Colloidal dispersion refers to small particles dispersed in a continuous medium. A large number of small particles form amorphous aggregates, and considerable amount of these aggregates are composed of a single molecule having a large molecular weight. The size of dispersed particles ranges approximately 1 μ m - 1 nm or 1/400,000 inch - 1/4,000,000 inch although it may not be exact (Figure 1, Table 1). Conditions for the colloid system are that a large number of particles should heterogeneous, multi-phasic, and insoluble.

There are five methods of producing the colloidal silver as shown in Table 2. Among them, grind process and electro-colloidal method are used mainly, which are solely allowed by FDA at present. Between the two, electro-colloidal manufacturing process is known to have better effects generally. Large-sized silver particles of about 1/4,000 inch are produced if the grind method is used. These particles may be electrically charged or may not be. Also, since these particles are larger sized compared to electrically charged particles, repulsive force due to this electric charge is not strong enough to cancel gravity acting on these particles, and therefore, particles sink to the bottom by this gravity, and not effective products are made eventually.

In order to solve the above problems, some manufacturing companies increase viscosity of solutions and maintain long-time dispersed state of these particles by adding a stabilizer (usually protein). However, using such stabilizer is unable to stop silver particles from sinking to the bottom eventually, and containers have to be shaken in order to disperse these particles in the solution again. This vicious cycle is then simply continued.

The most critical problem with this solution method is that a stabilizer added to assist dispersion of particles blocks effects of silver particles. In conclusion, products made this way are not very different from those in which water and ordinary metal silver are mixed and shaken. Other manufacturing companies manufacture colloidal silvers by simply mixing the metal and water solution using the grind process. However, such products have significantly low effects and doubtful quality compared to electo-colloidal silvers.

In a proper electrical process, silver particles are separated and outputted from the ingot, where the size of a particle is far smaller than 100 nm. If the size of a silver particle is 1-10 nm and particles have the same charge throughout, it is not necessary to add a stabilizer to the colloidal silver in order to disperse particles. The reason for it is that anti-elasticity due to magnetic field thus formed (Brown Movement) cancels gravity acted on particles, and accordingly, particles maintain the dispersed state in the water solution continuously. That is, stability in dispersion of colloidal silvers depends on the size of particles and the manufacturing process employed.

There are many factors indicating the quality of colloidal silvers. What is most important among them is the color of a product. As the size of each silver particle becomes larger, the color of suspension is changed.

Quality	Best	Better	Normal	Inferior
Color	Yellow	Brown	Red Gray	Black

If the size of a particle is increased, quality of the product is lowered in proportion to that. The color of an electro-produced colloidal silver is remarkably different from that of a grind-produced product. An ideal color of the colloidal silver is almost colorless or very weak yellow. If a strong and thin light passes through the colloidal water solution, the path of the light is turbid and refracted (Faraday-Tyndall Cone Effect). The easiest and best way of observing this Faraday-Tyndall Cone Effect is to illuminate a very bright flash light to the test tube containing the colloidal silver in a dark room.

Recently many manufacturing companies of colloidal silver have appeared, with which consumers have been confused. The best method of determining whether the product is a real colloid of silver is to confirm existence of additives. If there are stabilizers or trace elements except for water and silver in the colloidal silver, the product is not a real colloidal silver. And if a product has to be freezed for its preservation, this product probably includes other additives that can be contaminated at room temperature.

The best colloidal silver is one manufactured by the electro-colloidal / non-chemical method. This is the only method of making a real homogeneous (evenly distributed) solution. A solution made by this method has entirely colloidal silver particles and water, and is suspended by the electromagnetic field which is generated by electric current which is the result of combining water and silver particles. The size of a particle in the solution is superfine of which diameter is in the range of 0.005 - 0.015 μm, and pure silver particles are suspended in the solution with no chemical compound, stabilizer, dye (some manufacturers use dyes in order to maintain a fine color), or additive is added to it. Silver particles of this type are neither accumulated in the solution to the degree of enabling visual observation nor precipitated to the bottom of a container. Based on the above, the manufacturing method of an emulsion containing silver as well as efficacy and effect of the silver colloid are discussed in the present study.

2. EXPERIMENT

2.1 Experimental materials

Ag used in this experiment was Aldrich Silver, foil, 0.1 mm thick, and 99.99%. The raw materials used for lipid bases were cholesterol, cholesteryl ester, lecithin, etc. PEG-5-soyasterol, polyglyceryl-3-methyl glucose disterate, dimethicone copolyol / cyclomethicones were used as surfactants. Purified water from an ion exchange column was used as water.

2.2 Experimental equipment and instruments

An equipment for making the colloidal silver is shown in Figure 2. The equipment was put into a water bath in order to maintain the temperature constantly, and Ag plates are placed at anode and cathode through which a voltage of 9 V was flowed. For the manufacture of a fine emulsion, T.K Auto Homo Mixer of Tokushu Kika Kogyo Company in Japan was used, and change in the size distribution was measured by using the Laser Light Scattering System (Malvern, UK, Model PCS 4700) which is a measuring instrument of size distribution in order to evaluate stability of an emulsion. Zeta potential was measured in order to find electrostatic effects of the silver colloid (Malvern Instrument, U.K.). The changes in color at 25℃ and 45℃ and under UV were observed by using a Chromameter (Japan, Minolta, CM 100R). AAS (Atomic Absorption Spectrometer 3300, Perkin Elmer) was used for quantitative analysis of Ag under the following conditions:

Wave length	338.3 nm
Slit	0.7 nm
Relative Noise	1.0
Sensitivity	0.11 mg/L
Sensitivity Check	5.00 mg/l
Flame Air	Acetylene oxidizing
Sample Pre-treatment	1.5% (V/V) HNO_3

A Microfluidizer (Microfluidics Corp., U.S.A.) was used in order to make spheres of MLV. Viscosity was measured by using a Viscometer (RVT) of Brookfield.

2.3 Experimental method

2.3.1 Manufacture of creams of various types (Table 3)

2.3.2 Measurement of changes in viscosity due to Ag colloid in W/Si, O/W, MLV creams

Changes in viscosity of O/W cream without adding the silver colloid and those with 4% of 25 ppm and 150 ppm silver colloids were measured. Changes in viscosity of W/Si, O/W, and MLV creams with 150 ppm silver colloid added to make various concentrations from 0% to 10% were also measured.

2.3.3 Measurement of changes in color due to heat and UV in W/Si, O/W, and MLV creams

Changes in color of each cream which has been kept at 25℃ and 45℃ and under UV for two months were measured using a Chromameter.

2.3.4 Measurement of changes in content of Ag colloid of O/W cream

How the contents of Ag colloid of O/W cream containing 20% of 150 ppm silver colloid at 25℃ and 45℃ and under UV have been changed was analyzed quantitatively by using an AAS.

2.3.5 Measurement of electrical affects of Ag colloid in MLV cream

Zeta potential of MLV cream was measured while varying the concentration of 150 ppm Ag colloid from 0 to 20%.

2.3.6 Measurement of cell toxicity and anti-microbial activities

In order to measure cell toxicity, the Transformed Mouse Fibroblast L929 cell which was collected by trypsinization was suspended into DMEM containing BCS of 2%, 100 ml of cell suspension (2500 - 3000 cell/well) was inoculated into each well of 96 Well Tissue Culter Plate, which was then cultured at 37℃ under

5% CO_2 for two days. After that, it was replaced with Serum Free Medium 90 μl, to which 10 μl of the sample substance was processed and cultured for two days. After culture is completed, 100 μl of Neutral Red Solution (50 μg/ml) was added to each cell and reacted for three hours. After Neutral Red passed completely the plasma membrane and concentrated in lysosome of living cells or not damaged cells, it is fixed with 100 μl of 1.0% formalin/1.0% $CaCl_2$, and extracted from the cell by using 1.0% acetic acid/50% ethanol solution. Then cell toxicity of extracted Neutral Red was measured by using an Elisa Reader at 540 nm for each concentration of 25 ppm and 150 ppm silver colloids. Staphylococcus aureus ATCC 6538, Bacillus subtilis ATCC 6633, Pseudomonas aeruginosa ATCC 9027, Escherichia coli ATCC 8739, Candida albicans ATCC 10231, and Aspergillus niger ATCC 16404 were used as testing strains for the measurement of anti-microbial activities. These testing strains were cultured in Tryptic Soy Broth and Sabourand Dextrose Broth at 35℃ and 25℃ for 24 hours and 5 days, and used as inoculation solutions. An inoculation solution has a concentration of 10^6 cfu/ml, and the degree of death of germs was measured by performing APC after 1 day and 3 days after it is inoculated into the sample. Anti-microbial activities in an emulsion was measured in the same way as the above by using Staphylococcus aureau ATCC 6538 and Pseudomonas auruginosa ATCC 9027 as testing strains. The sample used for the measurement of anti-microbial activities was O/W cream. 0.2% N-M, 0.15% N-P, and general purified water were used for Sample I; no preservatives but general purified water was used for Sample II; and 150 ppm Ag colloid instead of no preservatives but general purified water for Sample III.

3. RESULT AND REVIEW

3.1 Changes in viscosity due to silver colloid in W/Si, O/W, and MLV creams

Changes in viscosity of W/Si, O/W, and MLV creams containing 0 - 10% of 150 ppm silver colloid are shown in Figure 4. As shown in Figure 4, viscosity is rapidly decreased in O/W due to the affect of Ag colloid, while it does not have any affects in W/Si and MLV. Changes in viscosity when 4% of 25 ppm and 150 ppm

silver colloids are put into O/W cream are shown in Figure 5. It is seen that the viscosity is decreased as the concentration of the silver colloid is increased.

3.2 Measurement of changes in color due to heat and UV in W/Si, OW, and MLV creams

Changes in color for each cream kept at 25℃ and 45℃ and under UV are shown in Figure 6. As shown in Figure 6, the degree of changes in color of a cream by the silver colloid according to time was 2.5 times more stable at 45℃ and twice more stable under UV in MLV cream than in other creams. This is because MLV forms a stable multi-lamella structure and stabilizes the silver colloid.

3.3 Measurement of changes in content of Ag colloid of O/W cream

Analysis of the contents of Ag colloid at 25℃ and 45℃ and under UV is shown in Figure 7. It is seen that the content of Ag colloid in creams kept at 45℃ and under UV is more decreased compared to that in a cream kept at 25℃. The reason for it seems to be that the silver colloid is converted to another substance by heat and UV.

3.4 Measurement of electrical affects of Ag colloid in MLV cream

The Zeta potential of MLV cream with the concentration of 150 ppm Ag colloid changed by 0 - 20% is shown in Figure 8. It is seen from the result shown in the figure that the best dispersibility of an emulsion is obtained when the content of Ag colloid is 4 W/W %.

3.5 Measurement of cell toxicity and anti-microbial activities

Cell toxicity of 25 ppm and 150 ppm silver colloids is shown in Figure 9. It is seen that the silver

colloid has a very small cell toxicity, and has anti-microbial activities against Gram(+) and Gram(-) bacteria, yeast, and mold (Table 4). The results of measuring anti-microbial activities in an emulsion are also shown in Table 5. It is seen from the results that using Ag colloidal solution instead of a general purified water used for cosmetics shows very superior antiseptic effects.

4. CONCLUSION

The present study is conducted in order to obtain the most stable type of a base and efficacy and effect of Ag colloid in W/Si, O/W, and MLV creams containing Ag colloid. The results of the study are as follows:

1. It is seen that MLV cream forming liposome using a lipid base and micro-fluidizer from W/Si, O/W, and MLV creams is very stable against changes of viscosity and color.

2. The result of measuring the change in content of Ag colloid in O/W cream shows that Ag colloid reacts with heat and UV sensitively and causes reduction of the content.

3. The result of measuring Zeta potential in order to find electrical affect of Ag colloid in MLV cream shows that 4 W/W % has the best dispersibility.

4. It is seen that Ag colloid has very superior anti-microbial activities and almost no cell toxicity.

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0.1n	1n	10n	100n	1 μ	10 μ	100 μ	1mm
Ultramicroscopic region				Microscopic region			
Particles show Brownian movement				No visible Brownian movement			
Particles pass through ordinary filter paper				Particles retained by filter paper			
Paticles show increased solubility				Particles have ordinary solubility			
True solutions	Colloidal solutions			Emulsions and suspensions			
0.1n	1n	10n	100n	1 μ	10 μ	100 μ	1mm

Table 1. Particle distribution

Group	Method	Date
Grind	Ball Mill	1938
	Colloid Mill	1920
	Disk/China Mill	1924
	Aerodispersion Mill	1927
Wave	Ultrasonic	1921
	Radiant Energy	1910
Liquid	Homogenizers	1930
	Prolonged boiling in water	1910
	Mercury vapor condensed on water	1920
Chemical	Chemical Action	1860
Electrical	Electrical Arts	1924
	Cathode atomization	1926
	Vacuum evaporation	1927
	Electrosputtering	1898

Table 2. Manufacturing methods of colloidal silver

Table 3. Formulalas of sample O/W, W/Si, MLV creams

TYPE	INGREDIENTS		PRODUTION METHOD
O/W (1)	(A) POLYGLYCERYL-3-METHYL/ GLUCOSE DISTEARATE	3.00	(A) → HEAT/DISSOLVE ↓ EMULSIFICATION ←(B) ↓ COLLING ↓ O/W
	GLYCERYL STEARATE	2.80	
	STEARYL ALCOHOL	1.20	
	DECYL OLEATE	7.00	
	OCTYL STEARATE	10.00	
	CETYL DIMETHICONE	1.00	
	(B) GLYCERIN	3.00	
	PURE WATER	q.s.	
	COLLOIDAL SILVER	q.s.	
O/W (2)	(A) POLYGLYCERYL-3-METHYL/ GLUCOSE DISTEARATE	3.00	(A) → HEAT/DISSOLVE ↓ EMULSIFICATION ←(B)+(C) ↓ NEUTRALIZATION ←(D) ↓ COLLING ↓ O/W
	GLYCERYL STEARATE	2.00	
	MACADAMIA NUT OIL	10.00	
	STEARYL ALCOHOL	1.00	
	CAPRYLIC/CAPRIC TRIGLYCERIDE	4.00	
	(B) GLYCERIN	3.00	
	PURE WATER	q.s.	
	COLLOIDAL SILVER	q.s.	
	(C) CARBOMER	0.40	
	(D) TRIETHANOLAMINE	0.45	

TYPE	INGREDIENTS		PRODUTION METHOD
W/Si	(A) DIMETHICONE COPOLYOL/ CYCLOMETHICONES	3.00	(A) → DISSOLVE ↓ EMULSIFICATION ←(B) ↓ W/Si
	CYCLOMETHICONE	10.00	
	DIMETHICONE	10.00	
	(B) GLYCERIN	4.00	
	SODIUM PCA	2.00	
	PURE WATER	q.s.	
	COLLOIDAL SILVER	q.s.	
MLV	(A) LIPID BASE	10.00	Figure 3.
	(B) PROPYLENEGLCOL	5.00	
	CETYL PHOSPHATE	0.50	
	PURE WATER	q.s.	
	COLLOIDAL SILVER	q.s.	
	(C) HONEY EXT.	5.00	
	(D) MACADAMIA NUT OIL	10.00	
	SUN FLOWER OIL	5.00	
	(E) POLYACRYLAMIDE/ ISOPARAFFIN/LAURETH-7	3.00	

Test Strain	Result		
	0h.	1d.	3d.
Staphylococcus aureus ATCC 6538	1.0×10^6/mℓ	<10/mℓ	<10/mℓ
Bacillus subtilis ATCC 6633		<10/mℓ	<10/mℓ
Pseudomonas aeruginosa ATCC 9027		<10/mℓ	<10/mℓ
Escherichia coli ATCC 8739		<10/mℓ	<10/mℓ
Candida albicans ATCC 10231		<10/mℓ	<10/mℓ
Aspergillus niger ATCC 16404		<10/mℓ	<10/mℓ

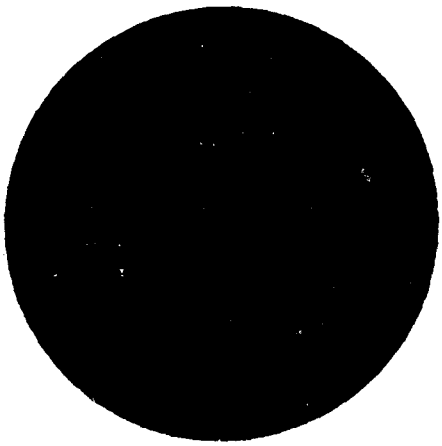
Table 4. Antimicrobial activity of collidal silver

Sample	Test Strain	Result			
		0h.	1d.	3d.	7d.
I	*S. aureus* ATCC 6538	1.0×10^6/mℓ	TNTC	TNTC	TNTC
	Pseudomonas aeruginosa ATCC 9027		TNTC	TNTC	TNTC
II	*S. aureus* ATCC 6538		TNTC	TNTC	TNTC
	Pseudomonas aeruginosa ATCC 9027		TNTC	TNTC	TNTC
III	*S. aureus* ATCC 6538		<10/mℓ	<10/mℓ	<10/mℓ
	Pseudomonas aeruginosa ATCC 9027		<10/mℓ	<10/mℓ	<10/mℓ

Table 5. Antimicrobial activity of collidal silver in O/W cream

Linear Magnification 1:10,000

A.



B.

A. Human Red Blood corpuscle (diameter 7.5 μ, thickness 1.6 μ)

B. Particle of colloidal silver solutions (0.006-0.015 μ)

Fig.1. Particle of colloidal silver solutions.

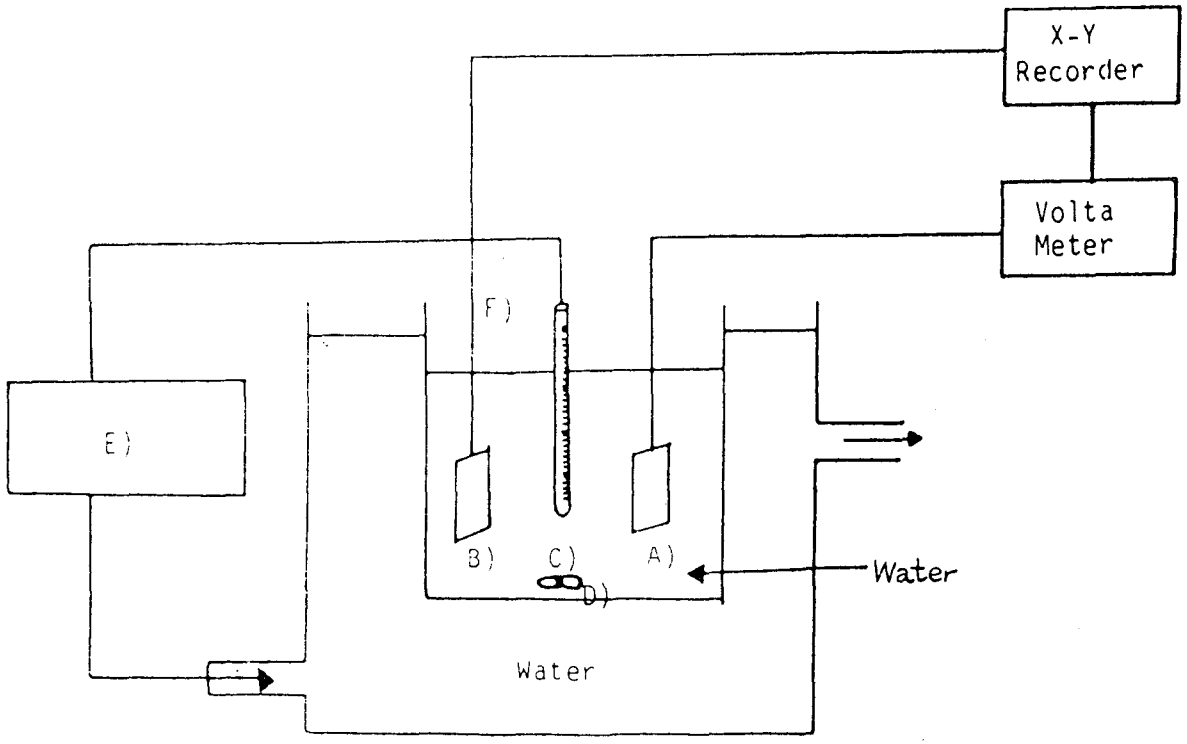


Fig.2. Experimental apparatus.

A) Cathode Ag plate B) Anode Ag plate

C) Thermostat D) Magentic stirrer

E) Thermo regulater & water supplier F) Cu wire

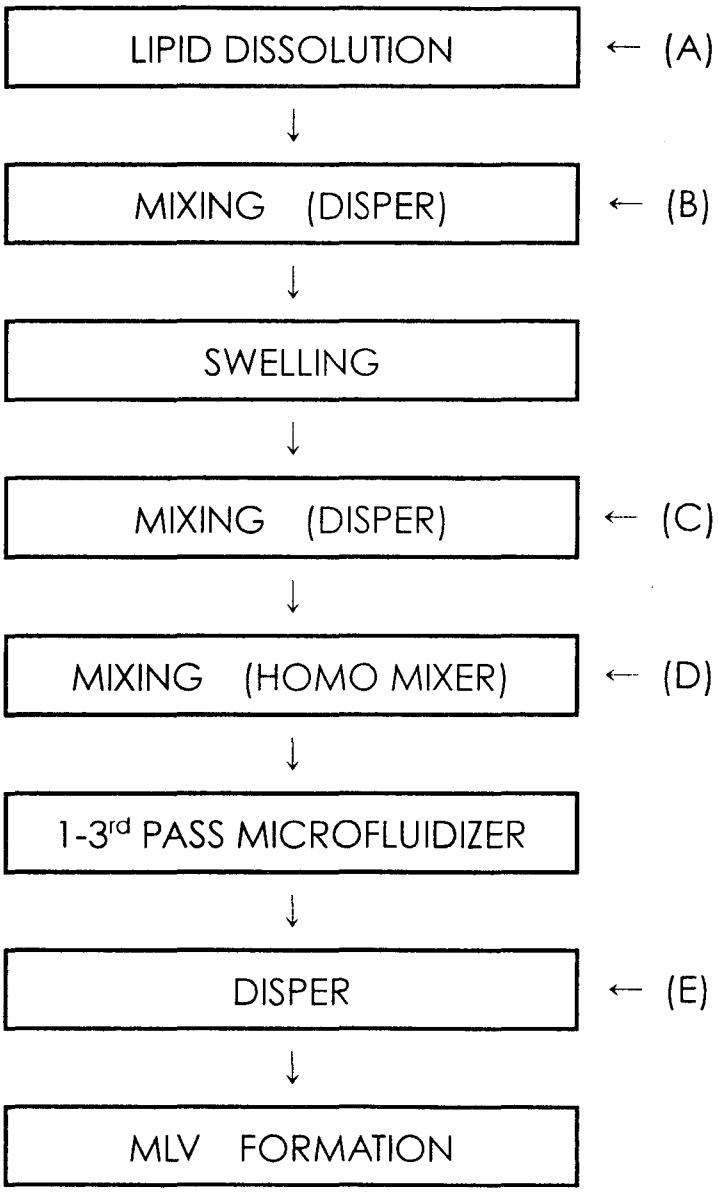


Fig. 3. Method of manufacture

Fig. 4. Viscosity diagram of colloidal silver in W/Si, O/W, MLV creams

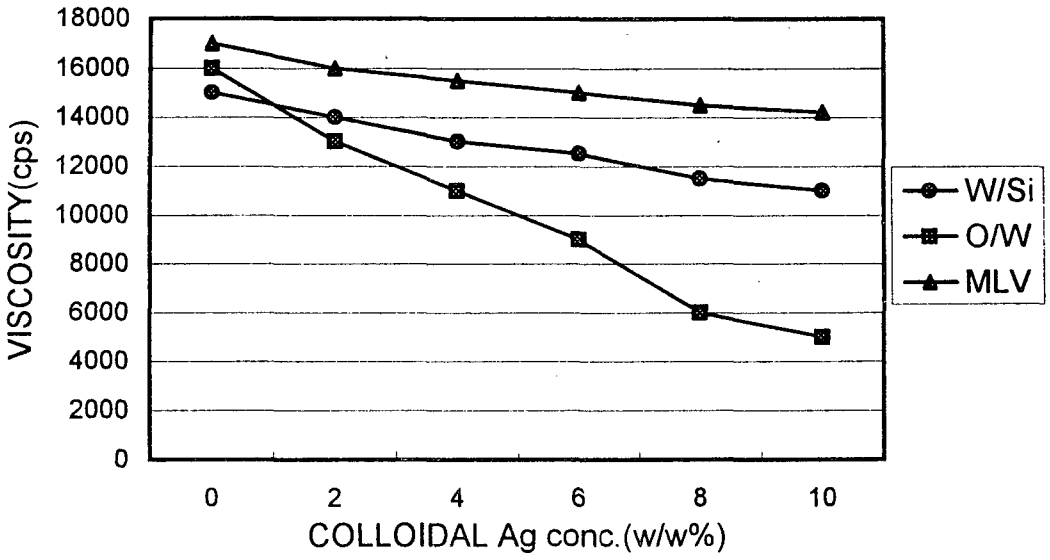


Fig 5. Viscosity diagram of colloidal silver in O/W cream

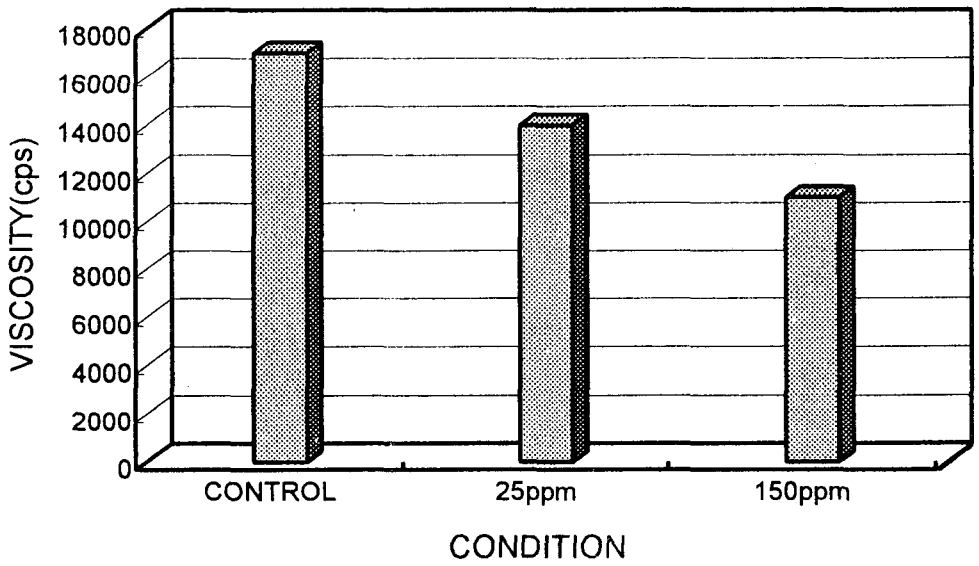
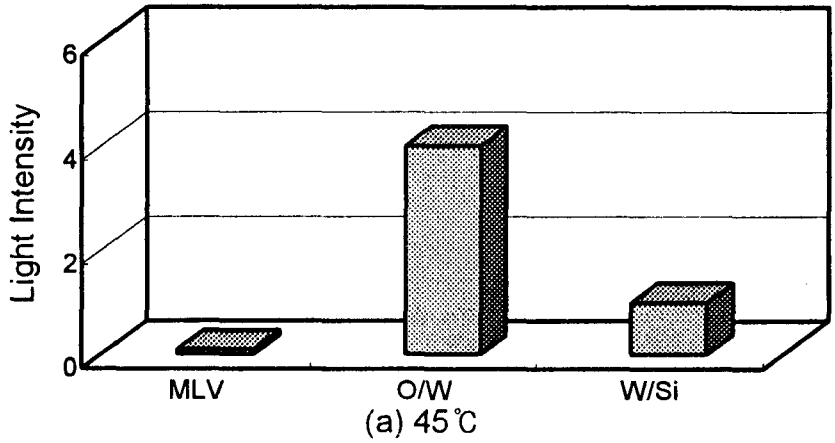
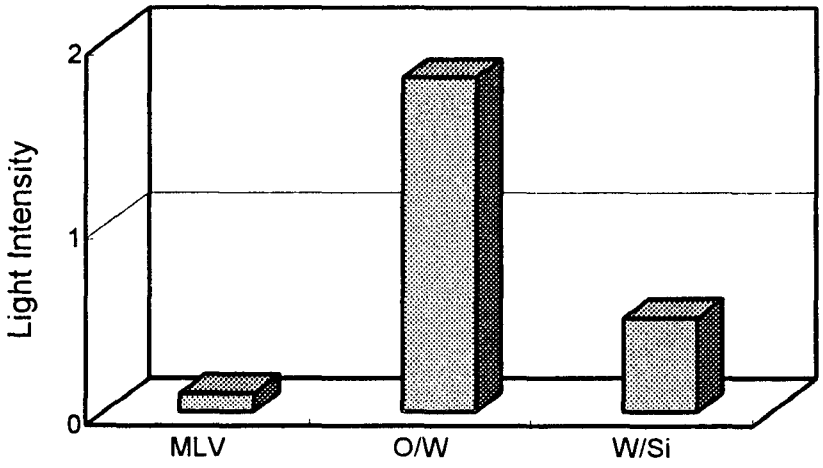


Fig. 6. The color stability of W/Si, O/W, MLV creams after 2 months storage at 45℃, UV



(a) 45℃



(b) UV

Fig. 7. Percent remaining of colloidal silver in O/W creams during 2 months storage at 25℃, 45℃, UV condition

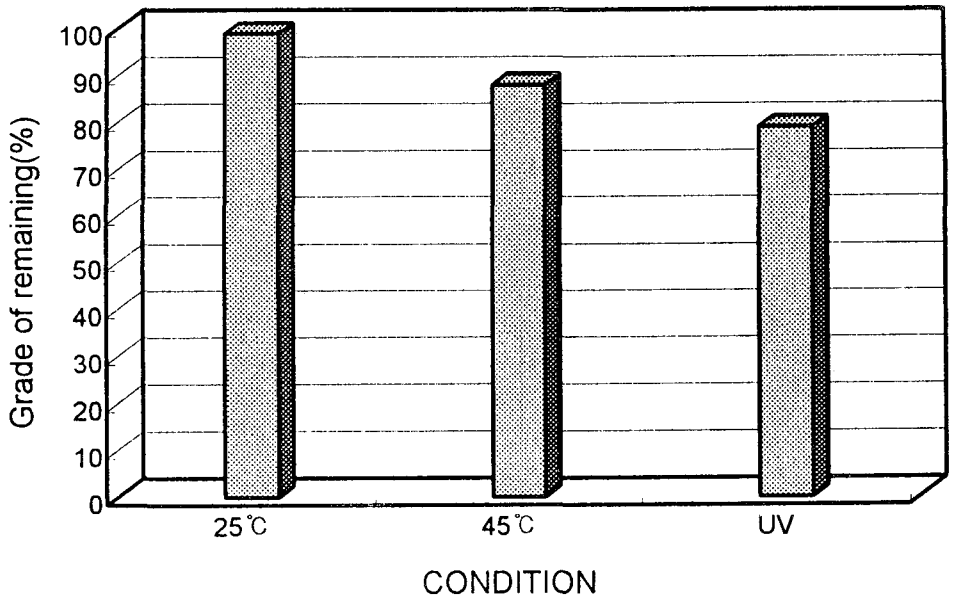


Fig. 8. Changes in Zeta potential of the MLV as a function of colloidal silver

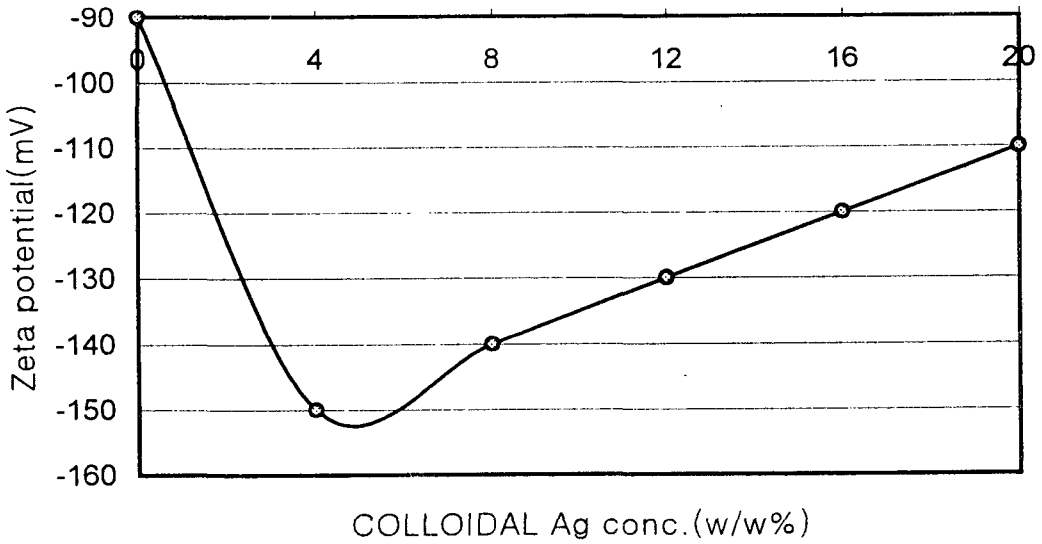


Fig. 9. Cytotoxicity measument of colloidal siver at 25ppm, 150ppm