The Improvement of Antimicrobial Inorganic Pigments for Cosmetics

Hee-Jung Kim, Chang-Giu Han, Young-Woon Lee

Lamy Dermal Scientific Research Institute, Lamy Cosmetics Co.Ltd.,
240-1, Saeum-Dong, Icheon-Si, Kyunggi-Do, Korea, 467-080

Abstract

Silver-containing antimicrobial inorganic pigments that have been developed so far still have problems, which result from silver's unique metallic color and discoloration. Therefore, those things are used only for make-up cosmetics or just the restricted amount is used. Although the use of white-base pigments or iron oxides has been considered to solve those problems, they virtually fail to serve as a perfect substitute. So it seems difficult to use enough quantity of those materials or to apply them to diverse kinds of products.

The purpose of this study was, accordingly, to attain the complete removal of metallic color and the maintenance of color. Additionally, a rosemary extract was employed to develop a silver-containing inorganic antimicrobial pigment(Ag-AIP-R) that has an improved antimicrobial effect and antioxidative effect.

Introduction

Silver is widely used in various fields including medicine, as one of metallic ions that have an antimicrobial effect. Moreover, silver is largely employed to solve the various kinds of problems of the existing organic preservatives. When organic preservative is used in cosmetics, a phenomenon occurs that activity deteriorates because of pH, solubility, the sort of surfactant, polymer, electrolyte or absorption on pigments or the inner wall of the container.[1] As this phenomenon, the compound concentration must be increased to correct the reduced concentration. Besides, organic preservative gives stimulus to the skin.[2] In recent days, studies has actively been made to apply silver to cosmetics as a possible way to eliminate those problems caused by the use of organic preservative. Since studies

have been made on antimicrobial effect of metal ions[3),4)], being initiated by Nägeli and Crystian's experiment in 1894, it has been known that silver has the best antimicrobial effect, followed by mercury, copper, cadmium, chrome, nickel, plumbum, cobalt, zinc and iron in the order named,[5),6)] although this list of order was not strict. Accordingly, antimicrobial inorganic pigments containing silver have been developed repeatedly.[7), 8)]

The Process of Experiment

Based on a lot of the existing studies, a pigment was produced to search out an antimicrobial metallic ion, through Sol-gel process.[9),10)] This pigment was of three-dimentional lattice, in which silica and zinc oxide were inserted at the ratio of 3:1. Next, the aqueous solution of silver nitrate, in which silver content amounted to 1% of total weight, was inserted into the pigment through aerosol spray and that was processed with doping. At that time, temperature was set first at 300℃, and then increased by stage from 300℃ to 600℃ and from 600℃ to 800℃, to turn silver into minute crystal particles. And then, that was sintered for three hours at 825℃ to become an oxidized metal that had a firm amorphous coating layer like glass, including metallic silver in a transparent lattice. After this was cooled by 100℃, sodium silicate was turn into a solution to occupy 2.5% of the total weight and this solution was put into the pigment through aerosol spray, and that was dried at 150℃. Finally, mixing rosemary extract powder through dry coating completed Ag-AIP-R. The amount of rosemary extract powder mixed in the pigment was 1% of the total weight. This pigment was put to the test of antimicrobial effect, of antioxidative effect and of safety to the skin. In addition, Its usefulness was affirmed by performing a discoloration experiment, MIC test and usability evaluation about diverse kinds of treatments that included no organic preservative.

Ag-AIP-R's Usefulness and Safety

Antimicrobial Effect. To test Ag-AIP-R's antimicrobial effect, a comparison was made between blank, Ag-AIP and Ag-AIP-R. The first thing contained no preservative. The second involved 1.0g of silver, and the third was one that added a rosemary extract to Ag-AIP at the rate of 1.0% over the total weight. The inoculums used in this test were *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans* and *Aspergillus niger*. The result of comparison was shown in Table 1-1, expressing those things' antimicrobial effect simply and easily. It's found in this table that the addition of rosemary extract improved the antimicrobial effect.

Table 1-1 Cell Counts in the 24hrs of the Ag-AIP-R

Inoculum	Preservative	Start	After 1hr	After 10hr	After 24hr
E.coli	Non	$5.4*10^2$	$6.9*10^2$	$9.4*10^4$	8.7*108
	Ag-AIP	$5.4*10^2$	3.2*10	1.0*10	<10
	Ag-AIP-R	$5.4*10^2$	2.7*10	<10	<10
P.aerug-inosa	Non	$7.6*10^2$	$1.6*10^3$	$8.4*10^6$	8.1*108
	Ag-AIP	$7.6*10^2$	4.1*10	<10	<10
	Ag-AIP-R	$7.6*10^2$	2.7*10	<10	<10
S.aureus	Non	$8.4*10^2$	$1.8*10^3$	$7.9*10^5$	1.9*108
	Ag-AIP	$8.4*10^2$	3.6*10	<10	<10
	Ag-AIP-R	$8.4*10^2$	1.1*10	<10	<10
C.albicans	Non	$7.3*10^2$	$8.1*10^2$	$6.2*10^5$	8.6*107
	Ag-AIP	$7.3*10^2$	4.1*10	1.2*10	<10
	Ag-AIP-R	$7.3*10^2$	1.6*10	<10	<10
A.niger	Non	$1.7*10^3$	$1.8*10^3$	$1.6*10^5$	2.0*107
	Ag-AIP	$1.7*10^3$	8.5*10	2.1*10	<10
	Ag-AIP-R	$1.7*10^3$	5.2*10	<10	<10

Antioxidative Effect. This study intended to give more excellent performance to this cosmetic material, by using a rosemary extract that was known to have an antimicrobial effect and a strong antioxidative effect. The antioxidative effect was tested by free radical scavenging activity method. The free radical scavenging activity method was generated by improving the method of Fujita, et al., and by using safe 1,1-Diphenyl- 2-picrylhydrazyl radical(DPPH). DPPH dissolved in methanol, representing OD 0.6 at 513nm. After DPPH 2ml was mixed with sample 1% and solution 1ml and then was incubated at room temperature for an hour, the value of OD at 513nm was checked. Table 1-2, which compared Ag-AIP-R with the various kinds of antioxidants, showed that Ag-AIP-R had an antioxidative effect.

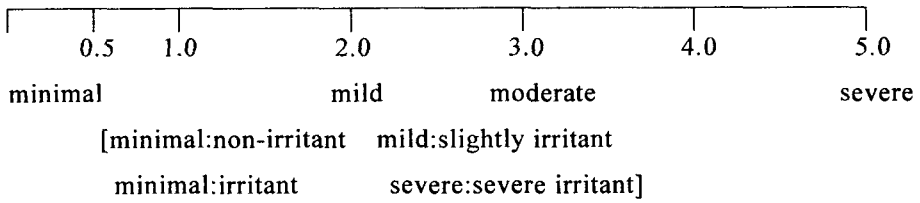
Table 1-2. Free Radical Scavenging Activity(FRS %) of Ag-AIP-R and Antioxidants

	OD at 513nm	FRS %
Ag-AIP-R	0.8831	20
γ - Tocopherol	1.0860	2
Tocopheryl acetate	0.9948	10
dl-α -Tocopherol	0.0295	97
L-Ascorbic acid	0.0382	97

Safety. How much Ag-AIP-R was safe to the skin was tested with in vitro method of SKINTEX UMA . After applying sample 100mg to blank and activated cuvette separately, those materials were

incubated for three hours at 25℃. Next, the sample was removed from the materials, and the value of the solution's OD included in low chamber was tested at 470nm by spectrophotometer(Jenway 6060 colorimeter). As a result, PDII was found to be 0.23, and SKINTEX UMA, which was conducted as below, showed that Ag-AIP-R was safe.

SKINTEX scoring



Evaluation in Different Treatments

Antidiscoloration. Facial cream containing 5% of Ag-AIP-R was used to confirm this material's effect on metallic color and discoloration, which was the major purpose of this study. After it's ascertained, by using Chromameter(MINOLTA CR-200), that the color of facial cream used in this study was white, it was placed respectively for 30 days at temperature of -4℃ and 40℃ in 24-hour turn, using a cycling tester(YAMATO program incubator IN-SI) and below sunlight. Then the color of facial cream was checked, and the results were compared. The source of light was D_{65}. After the sample 0.1g was spread on a white board evenly, the board was covered with slide glass. The test was made repeatedly ten times to get the mean value. According to Table 2-1, the color of facial cream including Ag-AIP-R was nearly white, and the color didn't alter even in a condition that sunlight or temperature changed.

The reason seemed that silver ion was completely reduced and got stabilized. What mechanism worked here will be studied continuously in more detail.

Table 2-1. Measuring color of Cream with Ag-AIP-R 5%

		Day 0	Day 30
Cream with Ag-AIP-R 5%	L	80.23	80.17
at solar	a	-0.66	-0.61
	b	+3.47	+3.51
Cream with Ag-AIP-R 5%	L	80.23	80.17
at changed temperature	a	-0.66	-0.61
	b	+3.47	+3.51

(cf. Calibration white board : L 97.18 a -0.50 b +2.84)

Formula 2-1. Cream with Ag-AIP-R 5%

Ingredients	wt.%
Powder phase	
Ag-AIP-R	5.00
Oil phase	
Stearic acid	0.50
Cetostearyl alcohol	2.80
Glycerylstearate	1.50
Sorbitan sesquioleate	0.20
Glycerylstearate/PEG-100 stearate	0.50
Mineral oil	5.00
Squalane	6.00
Macademia nut oil	6.00
Tocopheryl acetate	2.00
Dimethicone	0.30
Water phase	
Propylene glycol	5.00
Glycerin	5.00
Xanthan gum	0.10
Triethanolamine	0.25
PEG 40 stearate	0.60
Carboxyvinyl polymer	0.16
Purified water (aqua)	qs 100.00

Preservative Power(MIC Test). To evaluate Ag-AIP-R treatment's preservative power, a challenge test was made over skin-care materials, including Formula 2-1, non-preservative, and organic preservative Paraben 0.3% consisted of Methylparaben 0.2% and Propylparaben 0.1%. The inoculums used in this test were bacteria, including *Escherichia coli, Pseudomanas aeruginosa, Staphylococcus aureus* and *fungi,* including *Candida albicans, Aspergillus niger.* And each of inoculums went through overnight culture, they were inserted respectively by 100μ l in buffer solution. The total number of bacterias inserted in buffer solution was $6*10^2$/ml, and the total number of fungus was $1.1*10^3$/ml. While culturing them at 30℃, the number of colonies that were alive for 4 weeks was counted. The result was shown in Table 2-2. The facial cream containing 5% of Ag-AIP-R was found to have preservative power as similar as cream including organic preservative.

For make-up products, MIC test was made more fully at 3%, 5% and 7% over liquid foundation and cake foundation. According to Table 2-3 and Table 2-4, which showed the outcome, liquid foundation got stabilized at 5% and cake foundation did at 7%.

Therefore, Ag-AIP-R doesn't need any organic preservative, and can be used for the various kinds of cosmetics, like skin-care product including cream and make-up one including liquid foundation or cake foundation.

Table 2-2. Cell counts of the cream

After	Non-preservative		Organic preservative		Ag-AIP-R	
time	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
0hr	$2.5*10^3$	$1.5*10^2$	$2.5*10^3$	$1.5*10^2$	$2.5*10^3$	$1.5*10^2$
1hr	$3.4*10^3$	2.8*10	$2.5*10^3$	9.7*10	2.3*103	$1.2*10^2$
3hr	$4.8*10^6$	countless	$1.7*10^2$	7.4*10	$1.1*10^2$	8.9*10
12hr	countless	countless	6.6*10	1.5*10	5.0*10	3.2*10
24hr	countless	countless	1.1*10	<10	<10	1.2*10
7day	countless	countless	<10	<10	<10	<10
14day	countless	countless	<10	<10	<10	<10
21day	countless	Countless	<10	<10	<10	<10
28day	countless	Countless	<10	<10	<10	<10

Table 2-3 Cell counts of the liquid foundation

Bacteria

After Time	Non-preservative	Organic preservative	Ag-AIP-R 3%	Ag-AIP-R 5%	Ag-AIP-R 7%
0hr	$2.8*10^3$	$2.8*10^3$	$2.8*10^3$	$2.8*10^3$	$2.8*10^3$
1hr	$2.8*10^3$	$1.7*10^3$	$3.0*10^3$	$9.9*10^2$	$1.0*10^3$
3hr	$8.8*10^5$	$5.5*10^2$	$9.0*10^2$	$3.2*10^2$	$2.1*10^2$
12hr	countless	1.8*10	$6.8*10^2$	<10	<10
24hr	countless	<10	9.8*10	<10	<10
7day	countless	<10	4.3*10	<10	<10
14day	countless	<10	3.4*10	<10	<10
21day	countless	<10	2.1*10	<10	<10
28day	countless	<10	1.8*10	<10	<10

Fungi

After time	Non-preservative	Organic preservative	Ag-AIP-R 3%	Ag-AIP-R 5%	Ag-AIP-R 7%
0hr	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$
1hr	$1.5*10^2$	5.6*10	8.8*10	6.1*10	3.7*10
3hr	$1.6*10^3$	2.5*10	6.4*10	4.3*10	1.8*10
12hr	countless	<10	1.9*10	<10	<10
24hr	countless	<10	<10	<10	<10
7day	countless	<10	<10	<10	<10
14day	countless	<10	<10	<10	<10
21day	countless	<10	<10	<10	<10
28day	countless	<10	<10	<10	<10

Table 2-4 Cell counts of the cake foundation

Bacteria

After time	Non-preservative	Organic preservative	Ag-AIP-R 3%	Ag-AIP-R 5%	Ag-AIP-R 7%
0hr	$2.4*10^3$	$2.4*10^3$	$2.4*10^3$	$2.4*10^3$	$2.4*10^3$
1hr	$4.8*10^6$	$1.2*10^3$	$2.4*10^3$	$1.9*10^3$	$1.1*10^3$
3hr	countless	$3.4*10^2$	$3.2*10^2$	$6.7*10^2$	$4.1*10^2$
12hr	countless	2.6*10	$2.8*10^2$	3.4*10	1.8*10
24hr	countless	<10	$1.0*10^2$	<10	<10
7day	countless	<10	8.9*10	<10	<10
14day	countless	<10	7.8*10	<10	<10
21day	countless	<10	4.1*10	<10	<10
28day	countless	<10	3.6*10	<10	<10

Fungi

After time	Non-preservative	Organic preservative	Ag-AIP-R 3%	Ag-AIP-R 5%	Ag-AIP-R 7%
0hr	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$	$1.4*10^2$
1hr	$1.5*10^2$	8.2*10	8.8*10	4.7*10	2.0*10
3hr	$1.6*10^3$	3.5*10	6.4*10	<10	<10
12hr	countless	<10	1.9*10	<10	<10
24hr	countless	<10	<10	<10	<10
7day	countless	<10	<10	<10	<10
14day	countless	<10	<10	<10	<10
21day	countless	<10	<10	<10	<10
28day	countless	<10	<10	<10	<10

Formula 2-2. Liquid foundation for MIC test

Ingredients	Non-preser -vative	Organic pr -eservative	Ag-AIP-R 3%	AG-AIP-R 5%	Ag-AIP-R 7%
Powder phase					
Mica	2.00	2.00	2.00	2.00	2.00
Iron oxide (yellow)	0.66	0.66	0.66	0.66	0.66
Iron oxide (red)	0.28	0.28	0.28	0.28	0.28
Iron oxide (black)	0.06	0.06	0.06	0.06	0.66
Ag-AIP-R	-	-	3.00	5.00	7.00
Oil phase					
Stearic acid	2.40	2.40	2.40	2.40	2.40
Propylene glycol stearate SE	1.50	1.50	1.50	1.50	1.50
Cetearyl alcohol	1.20	1.20	1.20	1.20	1.20
Isopropyl lanolate	2.50	2.50	2.50	2.50	2.50
Sorbitan sesquioleate	0.30	0.30	0.30	0.30	0.30
Polyoxyethylene sorbitan monostearate	1.00	1.00	1.00	1.00	1.00
Squalane	8.00	8.00	8.00	8.00	8.00
Propyl paraben	-	0.10	-	-	-
Water phase					
Glycerin	5.40	5.40	5.40	5.40	5.40
Propylene glycol	4.10	4.10	4.10	4.10	4.10
Sodium magnesim silicate	0.30	0.30	0.30	0.30	0.30
Xanthan gum	0.10	0.10	0.10	0.10	0.10
Triethanolamine	1.20	1.20	1.20	1.20	1.20
Methyl paraben	-	0.20	-	-	-
Purified water(aqua)	qs100.00	qs100.00	qs100.00	qs100.00	qs100.00

Formula 2-3.Cake foundation for MIC test

Ingredients	Non	Organic	Ag-AIP-R 3%	AG-AIP-R 5%	Ag-AIP-R 7%
Silicone treated talc	to100.00	to100.00	to100.00	to100.00	qs100.00
Silicone treated sericite	20.00	20.00	20.00	20.00	20.00
Silicone treated titanated mica	20.00	20.00	20.00	20.00	20.00
Nylon	5.00	5.00	5.00	5.00	5.00
Boron nitride	5.00	5.00	5.00	5.00	5.00
Iron oxide (yellow)	3.20	3.20	3.20	3.20	3.20
Iron oxide (red)	0.60	0.60	0.60	0.60	0.60
Iron oxide (black)	0.30	0.30	0.30	0.30	0.30
Dimethicone	8.40	8.40	8.40	8.40	8.40
Butyl paraben	-	0.10	-	-	-
Propyl paraben	-	0.20	-	-	-
Ag-AIP-R	-	-	3.00	5.00	7.00

Usability and Safety. The most fundamental property that cosmetics must have is usability. This study tried to give a smooth spread ability and a soft touch to Ag-AIP-R-using products. To evaluate its spread ability and touch, 30 women who were in the late twenties and late thirties were asked to use liquid foundation to which MIC 5% was applied. And those women were asked to answer the following questions, and their reply was shown in Table 2-4. According to this table, 83% or more of the subjects expressed satisfaction at a soft touch, color expression or durability, which represented those features were fairly excellent. But 47% were dissatisfied at spread ability, which showed it needed to improve. This was because Ag-AIP-R was dry and hard. Accordingly, it needs to make a continuing effort to get over this problem.

Table 2-5. Usability of liquid foundation with Ag-AIP-R 5%

	Satisfaction		Not Satisfaction	
Spread ability	16 persons	(53%)	14 persons	(47%)
Texture	25	(83%)	5	(17%)
Expression of color	28	(93%)	2	(7%)
Durability of make up	26	(87%)	4	(13%)

Skin safety is also one of the basic properties needed by cosmetics. To test this property, patch test was carried out over 30 male and female adults. Liquid foundation containing 5% of Ag-AIP-R was compared with one involving organic preservative Formula 2-2. The subjects put two pieces of patch on their upper inner aspects of the arms separately for 4 hours. The two pieces of patch were separately painted with the above-mentioned two samples. After the patches were removed from their arms, Draize test was made to find out how many red spots appeared on their arms. According to Table 2-5 showed the result, Ag-AIP-R, the antimicrobial inorganic pigment, became known to be safer than organic preservative.

Table 2-6. Safety comparison with organic preservative in liquid foundation (Human patch test)

	Organic Preservative		Ag-AIP-R 5%	
No	visual	score	visual	score
1	-	0	-	0
2	-	0	-	0
3	-	0	-	0
4	+/-	1	-	0
5	-	0	-	0
6	-	0	-	0
7	+/-	1	-	0
8	-	0	-	0
9	+/-	1	-	0
10	-	0	-	0
11	-	0	-	0
12	+/-	1	-	0
13	-	0	-	0
14	-	0	-	0
15	-	0	-	0
mean		0.27		0

Conclusion

A more improved antimicrobial inorganic pigment was developed in this study, by solving the problems that occurred as the existing silver-containing antimicrobial pigment was used for cosmetics. Moreover, its function gets better as one of cosmetics materials.

The compound pigments, which is composed of silica, zinc oxide through Sol-gel process and metal silver which attains to be reduced perfectly, have antimicrobial effect without giving impact on the color of cosmetics or causing the change of color.

The rosemary extract that is mixed with the pigment at the ratio of 1% over the total weight serves to enhance an antimicrobial effect and to add an antioxidative effect.

The facial cream, liquid foundation and cake foundation, which use this pigment(Ag-AIP-R), don't need any sort of organic preservative. Their MIC is 5%, 5% and 7% respectively.

Furthermore, this antimicrobial inorganic pigment is expected to be applied diversely, because it is not only useful, as one of cosmetic materials, but also safe to the skin.

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