Development of the Moisturizing Cleansing Pad Having Sterilizing and Anti-bacterial Effect against Covid-19

In-Young Kim, Ji-Min Noh, Eun-Kyung Seul, and Joo-Youb Lee

***Abstract* — This study is to study the development of a moisturized cleansing pad with sterilization and antibacterial effects in respond to COVID-19. Using MimicLipid MSM-1000 of the nonionic surfactant was developed with the O/W emulsion micelles that are forming multilamellar structures. Chlorine dioxide and chlorhexidine digluconate as antibacterial sanitizer were encapsulated and stabilized in these micelles, so that it could be remained stable even after long-term use. The appearance of formula-1 is a milky white** liquid, **and it has a characteristic odor. The pH of this formulation was 6.1. Also, the specific gravity is 1.014 and it has soft and moist texture. Moreover, there is no stinging or irritation when applied to the skin. The particle size distribution of this emulsion was about 1-3 µm. As a result of quantitative analysis of chlorine dioxide which is enclosed in O/W emulsion after 8 weeks, it was found that 85.59% or more at 45 °C and 93.47% at 25 °C. The skin moisturizing effect of O/W-emulsion increased to 58.9% immediately after the sample was applied, 28.5% after 4 hours and 17.3% after 8 hours, showing 3.3 times better effect than before application, and about 5.4 times better moisturizing power than the control group. The cleansing power of the O/W emulsion was not very high in the case of a simple 60% ethanol solution for makeup residues, lipstick, and fine dust. On the other hand, in Formula-1, all three were found to have excellent cleansing power. As an application product of the cosmetics industry, a pouch-type moisturizing cleansing pad was developed and commercialized, and a product having a sterilization effect and an anti-bacterial effect could be developed without using ethanol.**

***Keywords* — COVID-19, chlorine dioxide, stability, sterilization, anti-bacterial, moisturizing, cleansing.**

I. Introduction

As the COVID-19 pandemic is prolonged, the whole world people use sanitizer with ethanol mostly. In some areas, it was reported that long-term use of ethanol sanitizer not only causes erythema, skin stinging and itching but the side effect of occurring psoriasis more often [1]. Honestly, people have used sanitizer with ethanol only in special cases. Also, it was hard to find examples of using these products in daily life for a long period of time. Since emerging of COVID-19 and its variant virus, the use frequency of ethanol disinfectant has increased. Accordingly, the occurrence of side effects is getting more serious than before. In the cosmetic industry, cleansing products that is rinse-off type using ethanol are commercialized time to time in order to respond to COVID-19. But fundamental solution has not been found. Besides, a lot of hand sanitizers have been commercialized in pandemic world. Especially most of disinfectants are containing high content of ethanol [2]. These products are classified into liquid spray-types and transparent gel types for sanitizing microorganisms including bacteria and virus. Specifically, application of products containing high concentration of ethanol have been main cause of severe stinging, erythema, dry skin, allergies, and accelerated aging [3]-[5]. As the skin of children and infants is sensitive and fragile, there have been occurred the lots of side effects compared to adults. but they cannot stop using the disinfectant. Therefore, it is important to develop a cosmetic product that has a superior cleansing effect while having the sterilization and disinfection ability without ethanol to solve these problems. It has also anti-irritation and moisturizing effect. Generally, the demand of anti-bacterial products for personal hygiene and the prevention of infectious diseases has been soaring. Ethyl alcohol and isopropyl alcohol are used as the main raw materials for disinfectants. As COVID-19 is spreading around the world, people cannot help but to use the ethanol disinfectant wherever they go. Thus, the skin is becoming more and more dehydrated rapidly [6]. Particularly, psoriasis and erythema on hand have been occurred to professional expert such as doctors and nurses who have duty of caring for lots of patients. Even those symptoms are getting worse, it is quite difficult to find a safe sterilizer as an alternative to ethanol [7]. Kim et al. developed a technology for forming hydrophilic liquid crystals using natural botanical surfactants to suppress occurring of skin side effects, even if ethanol is contained [8]. The ethanol of the product developed with this mechanism can sterilize the Bacteria present on the skin while the liquid crystal emulsion protects the skin barrier. it can be considered as the new concept of sanitizer which can protect the skin from being asteatosis [9]. Also, Biobeautech took out a patent for stabilizing the chlorine dioxide by encapsulating it in an O/W emulsion [10]-[13].

This study focused on developing a cleansing product that protects skin to be clean and healthy. It is consequently developed a disinfectant to respond to COVID-19 without ethanol by attempting to stabilize a chloride dioxide solution and chlorhexidine digluconate as alternative sanitary

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materials. As a detailed study, it was developed that the hydrophilic micelles using selected botanical surfactants and loaded sterilizing materials in vesicles which make the stable O/W emulsion. the surface chemical behavior and the structure and physical properties of stable micelles are observed. Furthermore, this time is studied the encapsulation mechanism of key components using O/W micelles. In conclusion, our team report the development of multi-functional cleansing pad that was completed quantitative analysis and evaluated *in-vitro* clinical anti-bacterial activity, moisturizing and cleansing properties.

II. Materials and Methods

A. Materials

The reagents used in this study are as follows. MimicLipid-MSM1000 (Biobeautech, Korea), Solubil EWG-1100 (Biobeautech, Korea) and BioPyro-PGI-250 (Biobeautech, Korea) were used as botanical surfactants. As moisturizing agents, glycerin (LG Chemistry, Korea), 1,3-butylene glycol (Oxea, USA), dipropylene glycol (SKC, Korea) and methylpropanediol (Biobeautech, Korea) were used. As a disinfectant, chlorine dioxide 100ppm water solution (Eclogentech, Korea), chlorhexidine digluconate 20% solution (Wako, Japan), 1,2-hexanediol (Biobeautech, Korea), caprylyl glycol (Biobeautech, Korea) was used. Purified water (Biobeautech, Korea) as a solvent and all the other materials as only cosmetic's materials were used in this study.

B. Equipments

The devices used in this study were a homomixer (HY-0001A, Hanyang Machinery, Korea), a disper mixer (Hansung Machinery, Korea), agitator (AG1, Woowon Machinery, Korea), an ultrasonicator (KSU-80, Korea Co-Protec, Korea) was used. For the antibacterial activity test, Petrifilm-AC, Petrifilm-EC medium (3M, USA) and conical tube were used. Quantitative analysis of chlorine dioxide was completed using Q-CL501 (SINSCHE, Korea). An optical microscope (BX-51, Olympus, Japan) was used to observe the emulsified particles, a pH meter device (PHS-38W, Donglim Science, Korea) and a skin clinical diagnostic device (Aramo-TS, Aram Huvis, Korea) was used.

C. Manufacturing of Hydrophilic O/W Emulsion

The composition of the hydrophilic O/W emulsion is shown in Table I and the manufacturing process is shown in Fig. 1. The details of manufacturing method are described as follows. First, the aqueous phase (A) has to be dissolved by heating to 80 °C. After heating and dissolving the oil phase (B) to 80°C and then mix with (A) to emulsify by stirring at 3,500 rpm with a homo-mixer for 5 minutes. Add phase (C) to this and stir for 5 minutes at 3,500 rpm with a homo-mixer to disperse. Add the phase (D) after cooling to 30 °C and stir it slowly at 50-200 rpm in order to encapsulate chlorine dioxide in O/W emulsion. The manufacture process was completed by cooling to 20 °C. MimicLipid MSM-1000 is botanical surfactant that forms hydrogen bonding with the chlorine dioxide to make the stable multi-lamellar and micelles [12].

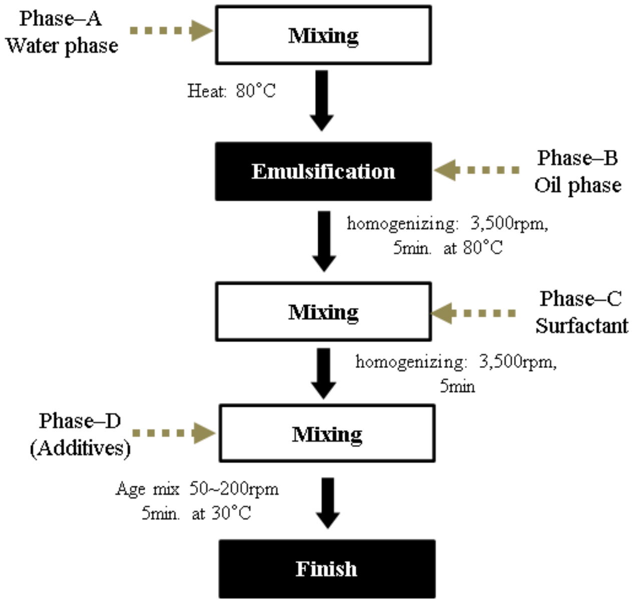


Fig. 1. Manufacturing Process of O/W emulsion containing sterilizing agent.

TABLE I: Composition of Oil-In-Water Emulsion Formula

Phase	Ingredient Name	Formula-1	Remarks
Water phase (A)	Butylene Glycol	3.000	Moisturizer
	Glycerin	2.000	Moisturizer
	1,2-Hexanediol	1.200	Conditioning agent
	Methylpropanediol	1.000	Conditioning agent
	Disodium EDTA	0.030	Chelating agent
	Allantoin	0.100	Conditioning agent
	Panthenol	0.100	Conditioning agent
	Water	61.200	Solvent
Oil phase (B)	MimicLipid MSM-1000(1)	1.000	Surfactant
	Caprylic/Capric Triglyceride	3.000	Emollient oil
	Citrus Aurantium Leaf Oil	0.050	Emollient oil
	Phytosqualene	2.000	Emollient oil
	Caprylyl Glycol	0.100	Conditioning agent
Surfactant (C)	Solubil EWG-1100(2)	1.000	Surfactant
	Bio-PhyroPGI-250(3)	1.000	Surfactant
	Dipropylene Glycol	5.000	Moisturizer
	Water	10.000	Solvent
	Chlorine dioxide 100ppm solution	8.200	Sterilizing agent
	Chlorhexidine digluconate 20% soln.	0.020	Sterilizing agent
	Total	100.000	

- (1)MimicLipid MSM-1000: Sucrose Distearate / Sucrose Oleate / Polyglyceryl-10 Dioleate/ Phytosphingosine.
- (2)Solubil EWG-1100: Polyglyceryl-10 Isostearate/ Polyglyceryl-10 Oleate.

- [(3)]Bio-PhyroPGI-250: Glycereth-25 PCA Isostearate.

D. Measurement of Physical Properties

In order to study the physicochemical behavior of the hydrophilic O/W emulsion containing chlorine dioxide, which measured the specific gravity by using a general cosmetic test method and the pH was measured by using a pH meter. Appearance, color, and odor were measured by expert with the naked eye and tactile sense. The particle size distribution and shape of the emulsion were observed with a polarization and optical microscope equipped with an alternative lens and an objective lens [14].

E. Quantitatively Analysis of Chlorine Dioxide

In order to quantitatively analyze the content of chlorine dioxide contained in the hydrophilic O/W emulsion, the chlorine dioxide concentration was measured by using the SINSCHE Q-CL501D chlorine concentration meter according to the DPD spectrophotometry method and the USEPA (20th Edition) U.S. environmental protection agency standard method [15].

F. Antibacterial Test

For the antibacterial activity test, bacteria (*Escherichia coli*), fungi (*Trichophyton rubrum*), *Staphylococcus aureus* and mold (*Malassezia furfur*) are inoculated into the medium. Put it in an incubator with a certain amount of the Formula-1. And then, it is evaluated the disinfection ability [16].

G. In-vivo Clinical Evaluation for Moisturizing Ability

To evaluate the moisturizing property, 10 male and female subjects among 20 s to 50 s were selected. the moisturizing ability was evaluated by measuring the amount of moisture present in the skin before the application of the sample and 8 hours after application. The moisture content was measured by using an Aramo-TS equipped with their own software. The measurement conditions were carried out in an incubation room and it can be possible to control temperature and humidity to minimize measurement errors [17].

H. Evaluation for Cleansing Ability

In order to evaluate *in-vivo* test cleansing ability, 10 males and females as subjects whose age range from 20 s to 50 s were selected. After applying the standardized contaminants on the skin, evaluating the measurement of contaminants residue. The contaminants that used in this study are standardized sample of foundation, lipstick and fine dust.

III. Results and Discussion

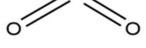
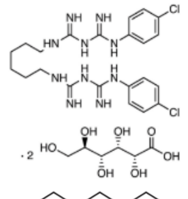
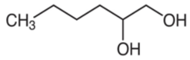
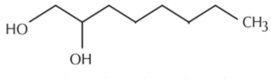
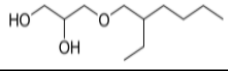
A. The Encapsulating Mechanism of O/W Emulsion and the Characteristics of Antibacterial Agent

1) The mechanism and characteristic of antibacterial agent.

Table II shows the main and auxiliary components with antibacterial activity used in this study. Since it is necessary to develop a prescription that can be actually commercialized in the cosmetic industry, an auxiliary antibacterial agent is used so that it can have a superb effect.

This product is developed as a portable pouch form that does not contain ethanol for reducing the irritation while having a moisturizing and cleansing ability. First, as shown in Table II, which used chlorine dioxide solution (ClO_2: 67.45 g/mol) and chlorhexidine digluconate ($C_{22}H_{30}Cl_2N_{10}\cdot 2C_6H_{12}O_7$: 897.77 g/mol) as sterilizing agents. Also, 1,2-hexanediol ($C_7H_{16}O_2$: 132.20074 g/mol), caprylyl glycol ($C_8H_{18}O_2$: 146.2 g/mol) and ethylhexylglycerin ($C_{11}H_{24}O_3$: 204.30646 g/mol) were optionally used for sub antibacterial agents. The reason for using these ingredients is to remove harmful microorganisms from the skin and to have the preservation power of the product itself. In addition, it can have a synergistic effect with the chlorine dioxide solution and increase the stability by forming micelles of O/W emulsion. This is the result of a study on how to maintain the content of chlorine dioxide even after long-term storage. The detailed antibacterial ability and cleansing mechanism are shown in Fig. 2. It can be observed disinfection effect that removes germs on the skin when applied to the skin. In addition, this sample can get rid of makeup residues including foundation, lipstick and fine dust on the skin, the active ingredients and humectants are absorbed into the skin so as to provide nutrition and hydration.

TABLE II: Summary of Sterilizing Agent and Anti-Bacterial Functions

INCI name	Chemical structures	Other Name & Functions
Cholrine dioxide (ClO_2 : 67.45 *g/mol)*		*Chlorosyloxidanyl* Sterilizing & anti-bacterial effect
Chlorhexidine digluconate MW: ($C_{22}H_{30}Cl_2N_{10}\cdot 2C_6H_{12}O_7$: 897.77 *g/mol)*		1,6-Bis(N5-[p-chlorophenyl]-N1-biguanido) hexane Sterilizing & anti-bacterial effect
1,2-Hexanediol (*$C_7H_{16}O_2$;132.20074 g/mol)*		*(2S)-2-methylhexane-**1,2**-diol* *Co-preservative*
Caprylyl glycol ($C_8H_{18}O_2$:146.2 *g/mol)*		Octane-1,2-diol *Co-preservative*
Ethylhexylglycerin ($C_{11}H_{24}O_3$: *204.30646 g/mol)*		3-[2-(ethylhexyl)oxyl]-1,2-propandiol *Co-preservative*

Our group was made up the Formula-1 composition of EWG (Environmental Working Group, US) green grade material in order to be suitable for sensitive and dry skin having no irritation.

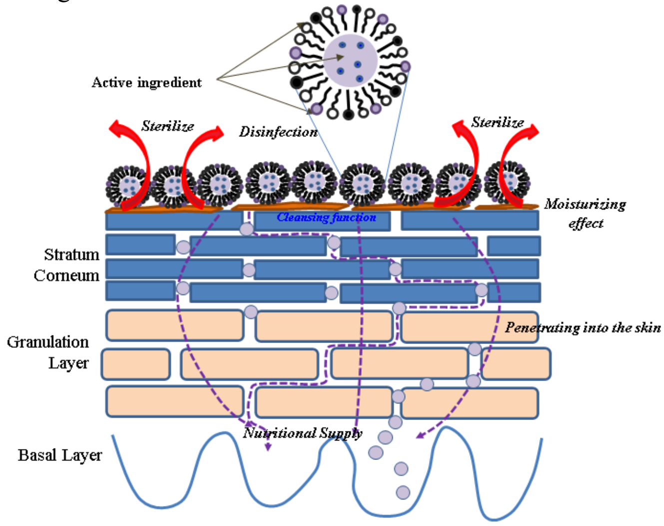


Fig. 2. The skin adsorption mechanism of skin model formulated by Formula-1 in o/w emulsion micelles containing active ingredients and anti-bacterial effect.

In conclusion, it explains that O/W emulsion equipped with active ingredients removes dirty contaminants from the skin surface, kills various harmful bacteria, and absorbs active ingredients to the base layer to exhibit various effects.

2) The mechanism of stabilizing encapsulated Hydrophilic O/W emulsion

As a method of stabilizing chlorine dioxide and chlorhexidine digluconate, which used botanical surfactants for forming the O/W emulsion micelles. And the active ingredient was encapsulated in the micelles to keep stable even after long-term use. This mechanism is explained in Fig. 3. First, it is formed the micelles by using MimicLipid MSM-1000 as shown in Formula-1 of Table I so that the sterilizing and antibacterial agents can be encapsulated agents into micelles. This surfactant has a characteristic that makes a multilamellar structure stable in order to encapsulate the active ingredients, humectants and various additives. Fig. 3(a) is a structural diagram of O/W emulsion micelles that were formed when 1 wt% of MimicLipid MSM-1000 was used, and (b) is a schematic diagram in which the active ingredients are encapsulated in the micelles and bonded to the periphery to form a stable structure. It was designed to form the most stable structure, as the emulsion was made in this way.

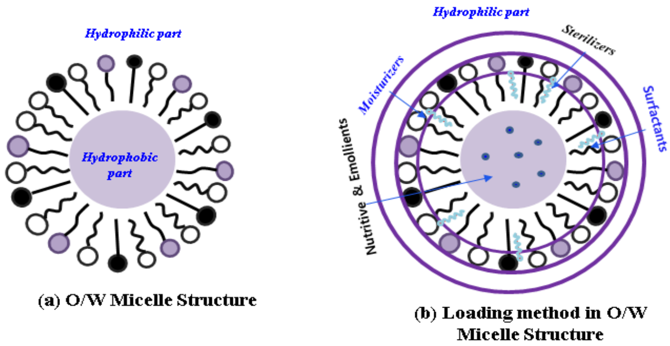


Fig. 3. Molecular structures of O/W emulsion containing chlorine dioxide and chlorhexidine digluconate.

This has the advantage of stably encapsulating unstable chlorine dioxide and forming a stable micelle to maintain the active ingredient for a long period of time. In addition, there is a big difference in providing efficacy where necessary step by step from the surface of the skin to the inside of the skin.

B. The Results of Physical Properties

The formulation of the cleansing pad was shown in Formula-1 of Table I, and the physical properties of sample were measured. The appearance of sample is a milky white liquid and it has a characteristic odor. The potential of Hydrogen was measured as 6.1 and the specific gravity is 1.014. it has soft and moist texture. there was no stinging or irritation when applied to the skin. Fig. 4. (a) is a picture of 60% ethanol solution which is observed under a polarized microscope as a control and Fig. 4. (b) is a microscopic photograph in which O/W emulsion micelles are formed. It can be seen as a stable emulsion phase which consists of encapsulated disinfectant in the multilamellar structure. Thus, it was observed that the distribution of particle size level from 1 to 3 μm.

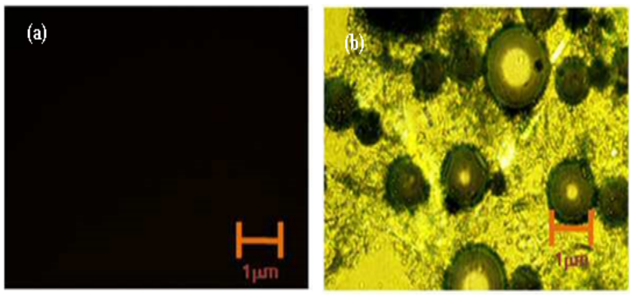


Fig. 4. Molecular structures of O/W emulsion containing chlorine dioxide and chlorhexidine digluconate. (a): conventional emulsion, (b): O/W emulsion of Formula-1.

C. The Results of Chlorine Dioxide Quantitative Analysis

Put the Formula-1 of Table I in an incubator at cold temperature (4 °C), room temperature (25 °C), and constant temperature (45 °C). After 8 weeks, the content of chlorine dioxide in this sample was measured and shown in Fig. 5 as graph. The content of chlorine dioxide is measured using SINSCHE Q-CL501D Device. Each sample was measured three times and the mean value was shown with standard deviation. As a control group, the content of chlorine dioxide was measured by simply mixing a sample with purified water under the same condition. All samples were developed at 8.12 ppm as shown in Fig. 5. In the case of the control sample, it decreased by more than 46.55% after a week and decreased by more than 76.23% at 45°C. It was also decreased by 95.56% after 8 weeks, confirming that the stability of chlorine dioxide was not good. On the other hand, under the condition of 45 ºC of Formula-1 stabilized with O/W emulsion, 99.13% was maintained after a week and 85.59% was remained even after 8 weeks. At 25 ºC, 93.47% of chlorine dioxide remained after 8 weeks. From these results, it was found that the formulation stabilized by forming an O/W emulsion has significantly superior stability. This could be considered that the antibacterial ingredients were packed in the O/W emulsion micelles. According to this mechanism, it could be determined that we can develop it to be available in commercial cosmetic market.

D. Antibacterial Performance Evaluation Result

For the evaluation of the antibacterial activity of the Formula-1 in Table I, bacteria (*Escherichia coli*), fungus (*Trichophyton rubrum*), *Staphylococcus aureus*, and mold (*Malassezia furfur*) were inoculated into the medium with Formula-1 for the antimicrobial activity test. We used 60% ethanol solution as a control group and a Formula-1 was put in an incubator. The results are shown in Table III which is measured the decrement of a microorganism after a week.

In the case of the 60% ethanol solution used as a control group, it showed an excellent disinfection power on bacteria and fungi. But a small amount of *Staphylococcus aureus* and mold bacteria had been survived. On the other hand, Formula-1 which is developed in this study, all of the microorganisms (bacteria, fungi, staphylococcus, and mold) were sterilized. Therefore, as a result of comprehensive study, it was interpreted as having a superior killing effect in the sample than in the control group.

E. Evaluation Result of Moisturizing Effect

In the case of moisturizing ability, the moist in the stratum corneum of the skin was measured using *in-vivo* test and it is shown as the graph in Fig. 4. 10 males and females as subjects whose age range from 20 s to 50 s were selected in order to measure the moist value before application to 8 hours after applicated. The device of Aramo-TS equipped with its own software was used to evaluate moist value. Scale error was minimized by using incubation that is able to control temperature and humidity. The moisture content before applying the sample to the skin was about 5.6%. In 60% ethanol solution, it showed a decreased tendency to 4.5% after 2 hours and to 3.2% after 8 hours. While in Formula-1, it increased to 58.9% immediately after application of the sample. It is also appeared 28.5% after 4 hours and 17.3% after 8 hours, which was 3.3 times higher than before application. Therefore, it can be seen that it has about 5.4 times superior moisturizing power than the control group. It can be predicted that glycerin and 1,3-butylene glycol are encapsulated in the O/W emulsion micelles. Formula-1 is expected to be useful in the Omikron Pandemic situation because it can have superior moisturizing power compared to the placebo group and has cleansing and antibacterial power.

F. The Result of Evaluated Cleansing Ability

In order to evaluate *in-vivo* test cleansing ability, 10 male and female subjects whose ages range from the 20s to 50s were selected. After applying the standardized contaminants on the skin, evaluate the measurement of contaminants' residue contents. The contaminants that are used in this study are standardized foundation, lipstick, and fine dust. It is shown in Fig. 8 by charting the result. In the control group of the 60% ethanol solution, we could observe that the 57.2% for foundation, 73.3% for lipstick, and 67.8% for fine dust were removed. While in Formula-1, it was found that cleansing ability was about 93.7% for foundation, 98.5% for lipstick and 92.5% for fine dust. Consequently, it is reported that all of the contaminants were removed perfectly in the Formula-1 sample. This is the reason why the nonionic surfactants of Solubil EWG-1100 and Bio-PhyroPGI-250 are contained.

TABLE III: SUMMARY OF STERILIZING AGENT AND ANTI-BACTERIAL FUNCTION

Section	A kind of bacteria	Control		Sample		Remarks
		Placebo	Ethanol 60% Sol.	Placebo	Formula-1	
Germ	*Escherichia coli*	X	◎	Δ	◎	
Fungus	*Trichophyton rubrum*	X	◎	X	◎	
Staphylocoocus	*Staphylococcus aureus*	X	○	X	◎	
Fungal bacteria	*Malassezia furfur*	X	○	X	◎	
Total results		X	○	X	◎	

X: bad, Δ: weak, ○: moderate, ◎: excellent.

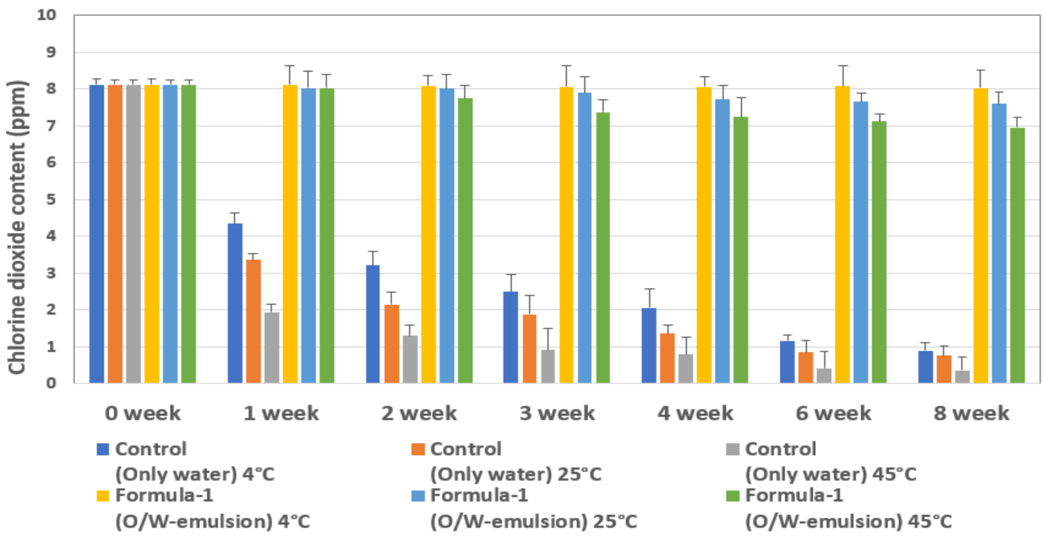


Fig. 5. Determination of chlorine dioxide content with stabilizing test result of Formula-1 comparing control sample. Stability test condition: incubated in 4 °C, 25 °C, 45 °C for 8 weeks, respectively.

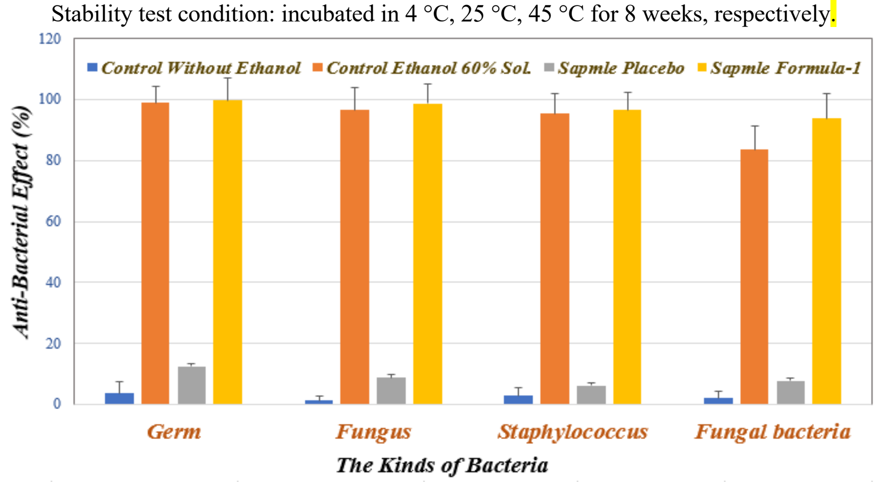


Fig. 6. Anti-bacterial activity of Formula-1 compared with ethanol 60% solution incubated for a week. Control: without ethanol and ethanol 60% solution, sample: placebo and Formula-1.

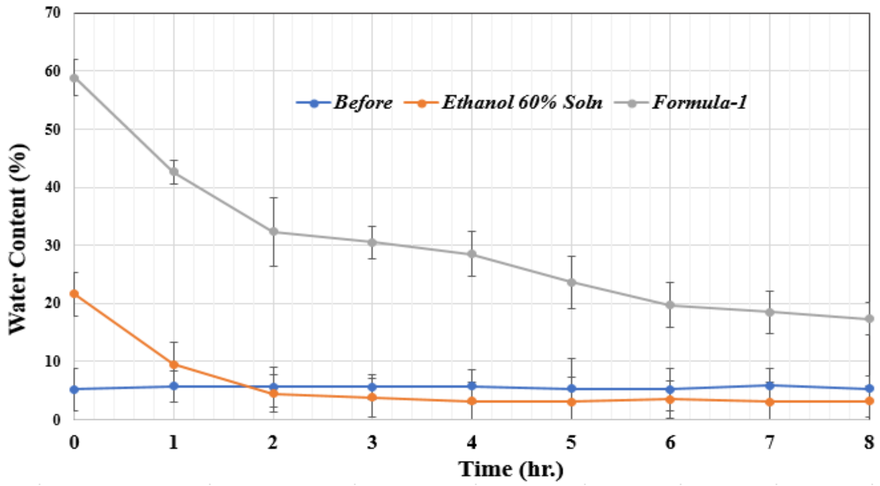


Fig. 7. Stability test results of Formula-1 comparing control sample. Stability test condition: incubated in 4 °C, 25 °C, 45 °C for 8 weeks.

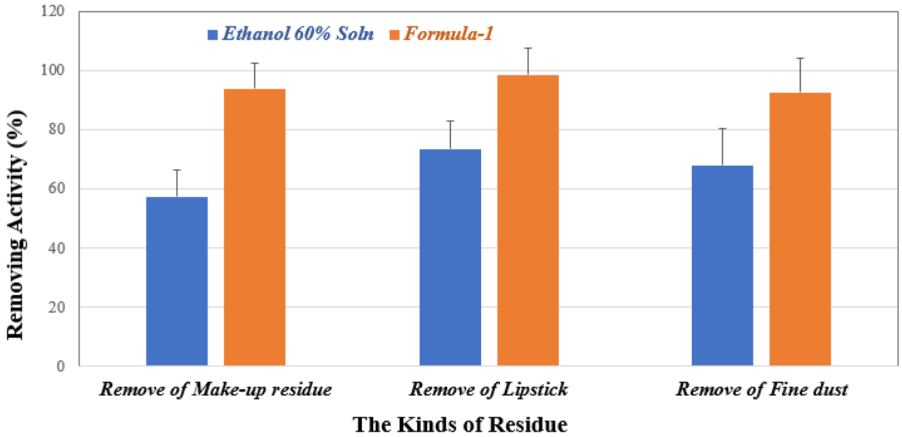


Fig. 8. Cleansing effect of Formula-1 comparing control sample. Stability test condition: incubated at 4 °C, 25 °C, and 45 °C for 8 weeks.

IV. Conclusion

The followings were obtained as a result of the study about the development of a moisturizing cleansing pad with an antibacterial effect in response to COVID-19. it was possible to make O/W emulsion micelles that form multilamellar structures by using MimicLipid MSM-1000 as nonionic surfactant. Chlorine dioxide and chlorhexidine digluconate were encapsulated and stabilized in micelles. Thus, these could be remained stable even after long-term use. The appearance of this formulation was a milky white liquid and has a characteristic odor. The potential of hydrogen was 6.1 and the specific gravity was 1.014. The texture was soft and moist and there was no stimulus stinging or irritation when applied to the skin. The particle size distribution of the emulsion ranges from 1 to 3 μm. As a result of observing the stability of chloride dioxide encapsulated in the O/W emulsion after 8 weeks, it was found that 85.59% or more was remained at 45 °C. Also, 93.47% has remained at 25 °C. For the manufacturing process of O/W emulsion, bacteria (*Escherichia coli),* fungi (*Trichophyton rubrum*), *Staphylococcus aureus,* and mold (*Malassezia furfur*) were inoculated into the medium and applied together with the sample. And it was observed that Formula-1 disinfected all 4 kinds of microorganisms. The moisturizing ability of O/W emulsion is increased to 58.9% immediately after applicated. Also, it was increased to 28.5% after 4 hours and 17.3% after 8 hours showing a 3.3 times higher effect than before application and about 5.4 times superior to the control group. Thus, it can be reported that it has excellent moisturizing power. As a result of the cleansing ability of the O/W emulsion, a 60% ethanol solution cannot remove makeup residues and fine dust clearly. On the other hand, the Formula-1 sample cleansed all three kinds of contaminants perfectly. It was considered that nonionic surfactants, Solubil EWG-1100 and Bio-PhyroPGI-250, affected the result. A pouch-type moisturizing cleansing pad was developed and commercialized. The pouch-type moisturizing cleansing pad was developed and commercialized as a brand-new item for the cosmetic industry. Especially, it is significant that a moisturizing product with sterilization and antibacterial effects could be developed without ethanol. Furthermore, it is expected that this technology will be applied to various products in the future.

Acknowledgements

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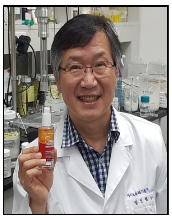

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