kuraray

White Paper

Antimicrobial properties of Isopentyldiol

Summary

In this study, 3-methylbutane-1,3-diol (IPD) known as multi-purpose specialty ingredient in combination with antimicrobial agents in cosmetics were investigated for their antimicrobial activity against bacteria and fungi in cosmetic formulations.

Minimum inhibitory concentration (MIC) tests of IPD showed bactericidal activities at different concentrations.

Furthermore, via analysis of fractional inhibitory concentration (FIC), tests showed a synergistic antibacterial effect of IPD with ethylghexylglycerin (EHG), phenoxyethanol (PE), and caprylylglycol (CG). This has been compared with synergistic effects with EHG, PE and CG of Butyleneglycol (BG) and 2-Methyl-1,3-propanediol (MPD).

IPD was detected as having synergistic effect with other antibacterial molecules. When EHG was mixed with IPD, there were synergistic or additional activity against S. aureus and it was suggested that the combination with CG was useful to control both bacteria and fungi.

This study confirmed the antibacterial synergy of IPD with antimicrobial main raw materials frequently used in cosmetics, thus contributing to prediction of the antibacterial activity of the skin conditioning agents in cosmetic formulations.

Content

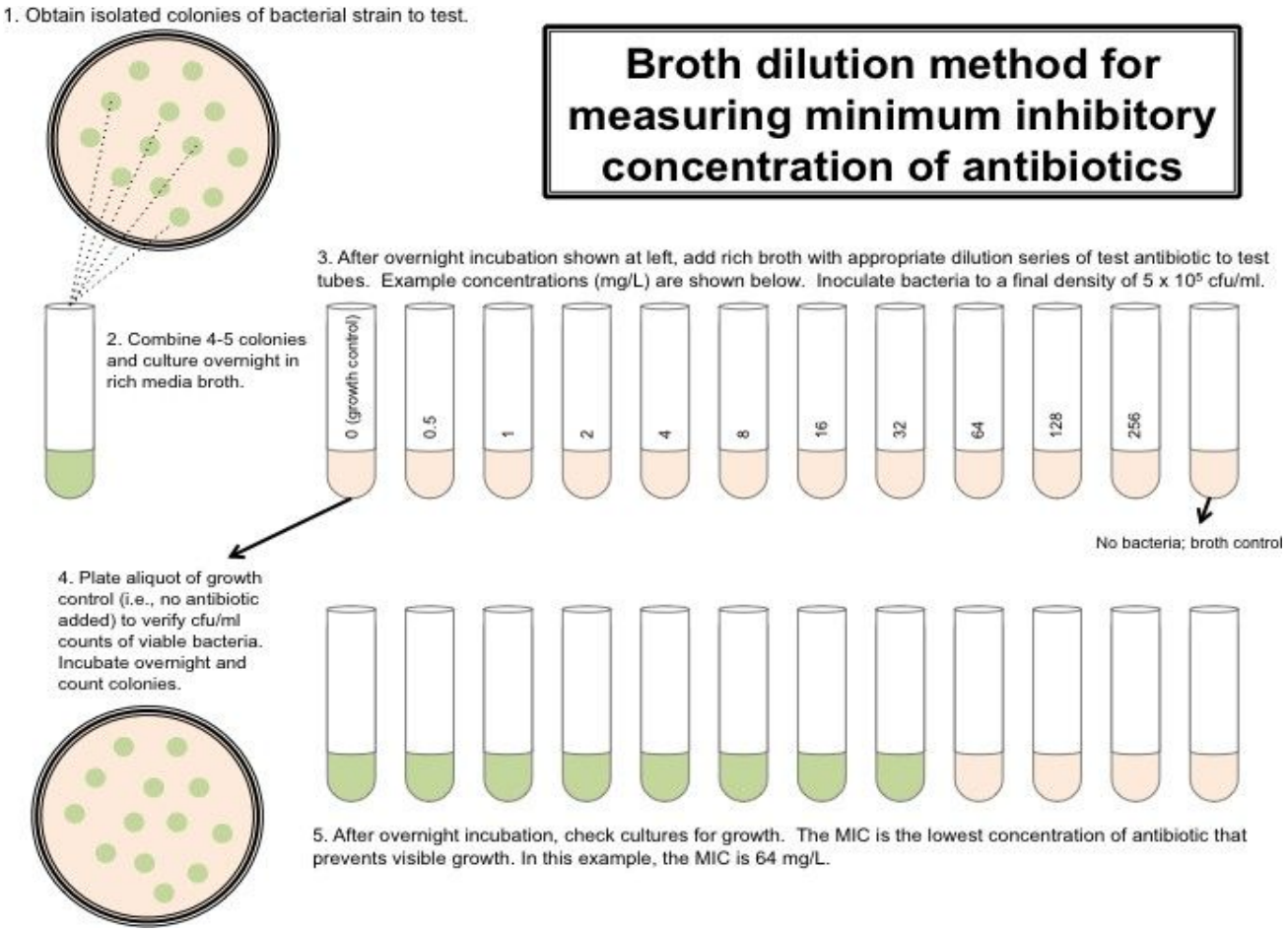
Microorganisms and Culture condition for MIC determination

Minimum inhibitory concentration (MIC) and of IPD was tested individually on Gram +ve and Gram –ve bacterial strains and fungi. Bacterial and fungi strains were maintained on nutrient agar at 4° C and sub-cultured every month. Culture conditions after test sample injection Bacterias are Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) medium, and yeast and fungi in Potato Dextrose Broth (PDA) and Potato Dextrose Broth (PDB) medium.

- Bacteria: 35 to 37 ° C for 24 to 48 hours
- Yeast, Fungi : 25 to 28 ° C for 48 to 72 hours

Strains	Species
Staphylococcus aureus	Gram (+) bacteria
Pseudomonas aeruginosa	Gram (-) bacteria
Escherichia coli	Gram (-) bacteria
Candida albicans	Yeast
Aspergillus niger	Fungi

Minimum inhibitory concentrations (MIC) and, further on, synergistic effect of IPD against selected microorganisms were determined by standard agar dilution method.



Tryptic Soy Broth (TSB) or Potato Dextrose Broth (PDB) were prepared by adjusting the concentration of each antimicrobial agent. A total of 200 [mu] l of TSB or PDB injected with the antimicrobial material was dispensed into microdilution plate (or 10 ml of TSB or PDB solution in each tube). Each plate (test tube) was inoculated with 1 x 10^5~1 x 10^6 CFU / ml of bacterial inoculum or 1 x 10^4~1 x 10^5 CFU / ml of mold inoculum. Petri dishes are charged with freshly prepared agar solution to which various concentrations of test samples with potential antimicrobial activity

are added. After solidification and drying, the test dishes comprising test compounds at different concentrations are inoculated with 1µL of the respective test microorganism suspensions. Inoculated plates are incubated under varying conditions depending on the type of the test organism (24 ~ 48 hours for bacteria; 48 ~ 72 hours for Yeast and Fungi).

The MIC is the lowest concentration of active compound at which no growth of the microorganism is observed macroscopically.
Minimum Inhibitory Concentration (MIC) of IPD and alternative molecules:

Material	MIC (Minimum Inhibitory Concentration)				
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger
IPD	15.0 %	10.0 %	10.0 %	10.0 %	10.0 %
Butylene glycol	15.0 %	10.0 %	10.0 %	15.0 %	10.0 %
Dipropylene glycol	20.0 %	15.0 %	15.0 %	20.0 %	15.0 %
1,2-Propylene glycol	20.0 %	15.0 %	15.0 %	15.0 %	10.0 %
1,3-Propylene glycol	20.0 %	15.0 %	15.0 %	15.0 %	10.0 %
2-Methyl-1,3-propanediol	10.0 %	10.0 %	10.0 %	10.0 %	10.0 %
Hexyleneglycol	5.0 %	5.0 %	5.0 %	5.0 %	5.0 %
Pentyleneglycol	5.0 %	5.0 %	5.0 %	5.0 %	5.0 %
Glycerin	20.0 %	20.0 %	20.0 %	40.0 %	40.0 %

Minimum Inhibitory Concentration (MIC) of Phenoxyethanol, Ethylhexylglycerin and Caprylylglycol:

Material	Minimum inhibitory concentration (MIC)				
	S. aureus	P. aeruginosa	E. coli	C. albicans	A. niger
Phenoxyethanol	0.30 %	0.25 %	0.25 %	0.20 %	0.15 %
Ethylhexylglycerin	0.15 %	0.20 %	0.15 %	0.15%	0.15 %
Caprylylglycol	0.20 %	0.20 %	0.10 %	0.20 %	0.10%

Synergistic Effect of IPD by FIC (Fractional inhibitory Concentration) and Test sample

From the stock solutions a twofold dilutions of each IPD and test samples (Phenoxyethanol, EHG and Caprylyl glycol) the MIC were distributed into each microfuge tubes to obtain a varying concentrations of 2.5, 5.0, 10, 20, 40, 80, 160, 250 and 500µg/ml of each. IPD was used for checkerboard method. A total volume of 2ml was made in each tube by distributing Muller Hinton broth along with 200µl of the inoculum. The microfuge tubes with one IPD of the combination were placed in rows in ascending concentrations starting at zero MIC and ending at two times the MIC. The other IPD was similarly distributed among the columns. Thus, each of the microfuge tubes was held in a unique combination of concentrations of the two IPD and test samples. The tube is inoculated with $1x10^5$ ~ $1x10^6$ cfu/ml of bacteria and cultured at 35-37°C for 24-48 hours and $1x10^4$ ~ $1x10^5$ cfu/ml of fungus is incubated at 25-28°C for 48-72 hours Cultures, MIC were read as minimal diluent without turbidity. The results were interpreted using FICI (fractional inhibitory concentration index). According to the Clinical Laboratory Standards Institute (2006) guidelines for microbiological dilution, MIC is defined as the lowest concentration of IPD that completely inhibits the growth of the blank. Synergy is to be expressed when the ratio of each IPD to the MIC of IPD is the same for all components of the mixture. The synergy between IPD and the material was tested with the FIC index. Each FIC index was determined using the following equation :

$$\Sigma FIC = FIC\ A + FIC\ B = C_A/MIC_A + C_B/MIC_B$$

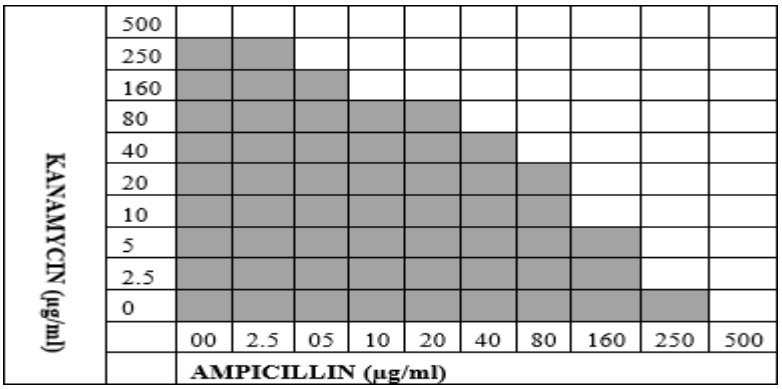
The ΣFIC were calculated as follows: ΣFIC = FIC A + FIC B, where FIC A is the MIC of drug A in the combination/MIC of drug A alone, and FIC B is the MIC of drug B in the combination/MIC of drug B alone.

The combination is considered synergistic when the fractional inhibitory concentration (ΣFIC) index is:

Lower than 0.5 : strong synergy
0.5 to 1.0 : weak synergy
1.0 : additive
1.0 to 2.0 : subadditive
2.0 : indifferent
Higher than 2.0 : antagonistic.

The lower the FIC, the most effective is the synergetic effect of the components.

Example below of assay where the synergistic activity of two materials, A and B was determined.



Same has been done with IPD in combination with the different molecules, tests results are in the 3 next tables.

- IPD (component A) in combination with CG, EHC, PE (component B)
- BG in combination with CG, EHC, PE
- Methyl Propane Diol in combination with CG, EHC, PE

Conclusion of the FIC tests :

The best FIC values (see next pages) come with IPD as component A and CG, EHC, PE as component B vs BG and MPD as component A. Synergetic effect is thus more efficient with IPD as with BG and MPD in combination 2 by 2 with CG,

EHC, PE.

Lowest average treatment rates of IPD as component A and component B, is when CG is component B. To have significant effect on S. aureus, a higher amount of IPD is needed. To further reduce such concentration, as third component can support the antibacterial activity of IPD/CG.

Further investigations (see next test results) in a lotion and in a emulsion will show other combination that also use 3 components to reach an effective synergetic action on the tested germs.

A	B	Microorganism	MIC (%) A / B	Minimum ΣFIC Index	Concentration for Observing Minimum ΣFIC	Synergesticity
IPD	Phenoxyethanol	S. aureus	IPD : 15.0 % / PE : 0.3 %	0.5	IPD : 3.75 % / PE : 0.075 %	Weak Synergy
		P. aeruginosa	IPD : 10.0 % / PE : 0.25 %	1.5	IPD : 5.00 % / PE : 0.25 %	Subadditive
		E. coli	IPD : 10.0 % / PE : 0.25 %	0.75	IPD : 2.50 % / PE : 0.125 %	Weak Synergy
		C. albicans	IPD : 10.0 % / PE : 0.20 %	0.53	IPD : 0.31 % / PE : 0.1%	Weak Synergy
		A. niger	IPD : 10.0 % / PE : 0.15 %	0.53	IPD : 5.00 % / PE : 0.005%	Weak Synergy
	Ethylhexylglycerin	S. aureus	IPD : 15.0 % / EHG : 0.15 %	0.53	IPD : 7.50 % / EHG : 0.005%	Weak Synergy
		P. aeruginosa	IPD : 10.0 % / EHG : 0.20 %	0.75	IPD : 5.00 % / EHG : 0.05 %	Weak Synergy
		E. coli	IPD : 10.0 % / EHG : 0.15 %	1.0	IPD : 5.0 % / EHG : 0.075 %	Additive
		C. albicans	IPD : 10.0 % / EHG : 0.15 %	0.75	IPD : 5.00 % / EHG : 0.038 %	Weak Synergy
		A. niger	IPD : 10.0 % / EHG : 0.15 %	1.0	IPD : 5.00 % / EHG : 0.075 %	Additive
	Caprylylglycol	S. aureus	IPD : 15.0 % / CG : 0.2 %	0.56	IPD : 7.50 % / CG : 0.006 %	Weak Synergy
		P. aeruginosa	IPD : 10.0 % / CG : 0.2 %	0.56	IPD : 0.625 % / CG : 0.05 %	Weak Synergy
		E. coli	IPD : 10.0 % / CG : 0.1 %	0.62	IPD : 5.00 % / CG : 0.013 %	Weak Synergy
		C. albicans	IPD : 10.0 % / CG : 0.2 %	0.51	IPD : 0.313 % / CG : 0.05 %	Weak Synergy
		A. niger	IPD : 10.0 % / CG : 0.1 %	0.53	IPD : 5.00 % / CG : 0.0027 %	Weak Synergy

A	B	Microorganism	MIC (%) A / B	Minimum ΣFIC Index	Concentration for Observing Minimum ΣFIC	Synergesticity

Butyleneglycol	Phenoxyethanol	S.aureus	BG : 15.0 % / PE : 0.30 %	1.03	BG : 15.0 % PE : 0.009 %	subadditive
		P. aeruginosa	BG : 10.0 % / PE : 0.25 %	1.13	BG : 1.25 % PE : 0.25 %	subadditive
		E. coli	BG : 10.0 % / PE : 0.25 %	1.03	BG : 10.0 % / PE : 0.008 %	subadditive
		C. albicans	BG : 15.0 % / PE : 0.20 %	1.25	BG : 15.0 % / PE : 0.05 %	subadditive
		A. niger	BG : 10.0 % / PE : 0.15 %	1.03	BG : 10.0 % / PE : 0.005 %	subadditive
	Ethylhexylglycerin	S.aureus	BG : 15.0 % / EHG : 0.15 %	1.03	BG : 15.0 % / EHG : 0.005 %	subadditive
		P. aeruginosa	BG : 10.0 % / EHG : 0.20 %	1.13	BG : 10.0 % / EHG : 0.025 %	subadditive
		E. coli	BG : 10.0 % / EHG : 0.15 %	1.00	BG : 5.0 % / EHG : 0.075 %	additive
		C. albicans	BG : 15.0 % / EHG : 0.15 %	1.13	BG : 7.5 % / EHG : 0.019 %	subadditive
		A. niger	BG : 10.0 % / EHG : 0.15 %	1.03	BG : 10.0 % / EHG : 0.005 %	subadditive
	Caprylylglycol	S.aureus	BG : 15.0 % / CG : 0.2 %	1.00	BG : 7.5 % / CG : 0.05 %	additive
		P. aeruginosa	BG: 10.0 % / CG : 0.2 %	1.00	BG : 5.0 % / CG : 0.05 %	additive
		E. coli	BG : 10.0 % / CG : 0.1 %	1.00	BG : 5.0 % / CG : 0.05 %	additive
		C. albicans	BG : 15.0 % / CG : 0.2 %	1.5	BG : 15.0 % / CG : 0.05 %	subadditive
		A. niger	BG : 10.0 % / CG : 0.1 %	1.03	BG : 10. % / CG : 0.002 %	subadditive

A		B	Microorganism	MIC (%) A / B	Minimum ΣFIC Index	Concentration for Observing Minimum ΣFIC	Synergesticity
2		Phenoxyethanol	S.aureus	MPD : 10.0 % / PE : 0.30 %	1.06	MPD : 10.0 % / PE : 0.019 %	subadditive
			P. aeruginosa	MPD : 10.0 % / PE : 0.25 %	1.03	MPD : 10.0 % / PE : 0.008 %	subadditive
			E. coli	MPD : 10.0 % / PE : 0.25 %	1.06	MPD : 5.0 % / PE : 0.063 %	subadditive

			C. albicans	MPD : 10.0 % / PE : 0.15 %	1.13	MPD : 1.25 % / PE : 0.2 %	subadditive
			A. niger	MPD : 10.0 % / PE : 0.13 %	1.25	MPD : 2.5 % / PE : 0.15 %	subadditive
		Ethylhexylglycerin	S.aureus	MPD : 10.0 % / EHG : 0.15 %	1.06	MPD : 10.0 % / EHG : 0.009 %	subadditive
			P. aeruginosa	MPD : 10.0 % / EHG : 0.20 %	1.00	MPD : 5.0 % / EHG : 0.1 %	additive
			E. coli	MPD : 10.0 % / EHG : 0.15 %	1.00	MPD : 5.0 % / EHG : 0.075 %	additive
			C. albicans	MPD : 10.0 % / EHG : 0.15 %	1.25	MPD : 10.0 % / EHG : 0.038 %	subadditive
			A. niger	MPD : 10.0 % / EHG : 0.15 %	1.03	MPD : 0.31 % / EHG : 0.15 %	subadditive
		Caprylylglycol	S.aureus	MPD : 10.0 % / CG : 0.20 %	1.06	MPD : 5.0 % / CG : 0.03 %	subadditive
			P. aeruginosa	MPD : 10.0 % /CG : 0.20 %	1.03	MPD : 5.0 % / CG : 0.05 %	subadditive
			E. coli	MPD : 10.0 % / CG : 0.10 %	1.00	MPD : 5.0 % / CG : 0.05 %	additive
			C. albicans	MPD : 10.0 % / CG : 0.20 %	1.00	MPD : 5.0 % / CG : 0.05 %	Additive
			A. niger	PD : 10.0 % / CG : 0.1 %	1.25	MPD : 10.0 % / CG : 0.013 %	Subadditive

Preservative Efficacy tests in cosmetic formulations

To evaluate preservative efficacy, a standard challenge test has been used according to the evaluation of the antimicrobial protection of a cosmetic product (ISO 11930 (2012)). Cosmetic emulsions and hydrous lotions were separately inoculated with a single culture of standard microorganisms, Inoculation Amount : 1×10^5 ~ $1x10^6$ cfu/ml for bacteria and 1×10^4 ~ $1x10^5$ cfu/ml for yeast and fungi (cfu = Colony Forming Units). The microorganisms used included the Gram-positive bacterium Staphylococcus aureus; Gram-negative bacteria Escherichia coli and Pseudomonas aeruginosa; yeast Candida albicans; and mold Aspergillus niger.
The inoculated emulsions were then stored over a period of 28 days and changes in the germ counts were measured at days 2, 7, 14 and 28. The preservative system is considered to be effective if a 3 log reduction in germ count can be achieved for bacteria after two days and if a 3 log reduction for yeast and mold can be achieved after seven days. In addition to complete kill of bacteria, complete kill of yeast and mold should be achieved within 28 days.

The following formula show 3 alternative preservation systems for 2 approaches, a hydrous lotion, and a W/O emulsion.

Fig. 4 and Fig. 5 graphs show the outcome of these comparative tests.

Conclusion of efficacy tests on formulated lotion and emulsion:

In each case, the reduction of Germs is quick, and comparable for the 3 alternative preservation systems.
In other words, the combination of IPD, EHC and Hexane diol will be competitive against parabens/PE system.

Hydrous Lotion Formula

Ingredient	1	2	3
IPD	-	5.0%	5.0%
Ethylhexylglycerin	-	-	0.4%
1,2-Hexandiol	-	-	1.5%
Methyl paraben	0.2%	0.2%	-
Propyl paraben	0.1%	0.1%	-
Phenoxyethanol	0.5%	0.5%	-
HCO-40	1.0%	1.0%	1.0%
Glycerin	2.0%	2.0%	2.0%
Butyleneglycol	5.0%	-	-
Xanthan gum	0.2%	0.2%	0.2%
Sodium Hyaluronate	0.1%	0.1%	0.1%
Water qsp	100%	100%	100%

W/O Emulsion formula

Ingredient	1	2	3
IPD	-	5.0%	5.0%
Ethylhexylglycerine	-	-	0.4%
Caprylylglycol	-	-	0.2%
Methyl paraben	0.2%	0.2%	-
Propyl paraben	0.1%	0.1%	-
Phenoxyethanol	0.5%	0.5%	-
Polysorbate 60	2.0%	2.0%	2.0%
Sorbitan oleate	0.5%	0.5%	0.5%
Glyceryl stearate	0.5%	0.5%	0.5%
Cyclomethicone	4.0%	4.0%	4.0%
Capric/Caprylic/triglyceride	3.0%	3.0%	3.0%
Phyotosqualane	5.0%	5.0%	5.0%
Stearic acid	0.5%	0.5%	0.5%
Glycerin	5.0%	5.0%	5.0%
Butylene glycol	5.0%	-	-
Xanthan gum	0.1%	0.1%	0.1%
Carbomer	0.2%	0.2%	0.2%
Sodium hyaluronate	0.3%	0.3%	0.3%
Potassium hydroxide	0.1%	0.1%	0.1%
Fragrance	0.2%	0.2%	0.2%
EDTA-2Na	0.05%	0.05%	0.05%
Water qsp	100%	100%	100%

Isopentyldiol(3-methylbutane-1,3-diol, IPD) (Kuraray, Japan), Butylene glycol (Oxea, USA), Dipropylene glycol (BASF, Germany), 1,2-Propylene glycol (SHELL Chemicals, Nederland), 1,3-Propylene glycol (DuPont Tate & lyle BioProducts, UK), Hexylene glycol (Miwon specialty Chemical, Korea), 2-Methyl-1,3-propanediol (STEARINERE DUBOIS, France), Pentylene glycol (Chemland, Korea), Glycerin (KLK OLEO, Malaysia)

Fig. 4. Preservative effect of IPD in Hydrous Lotion formula.

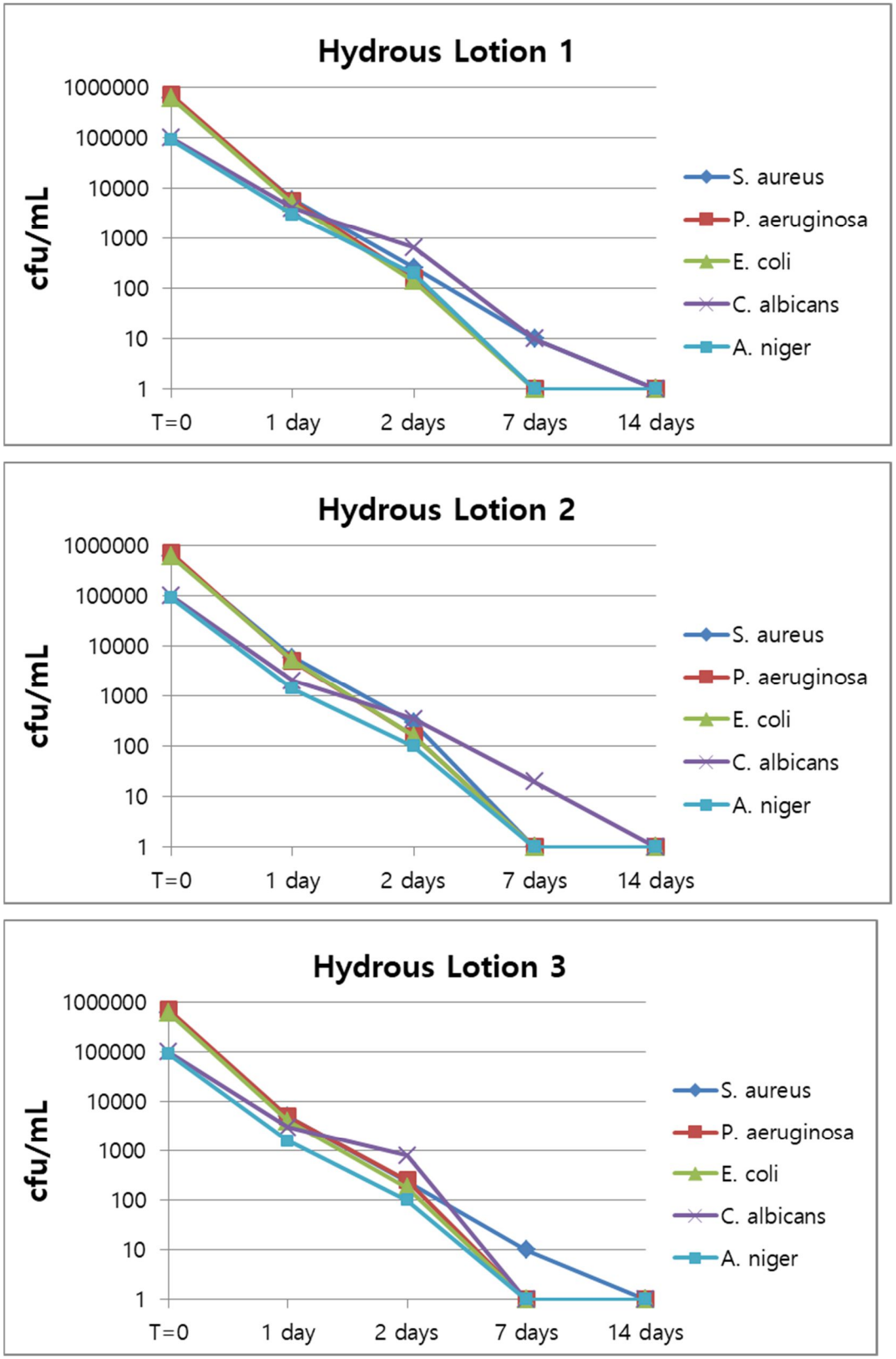
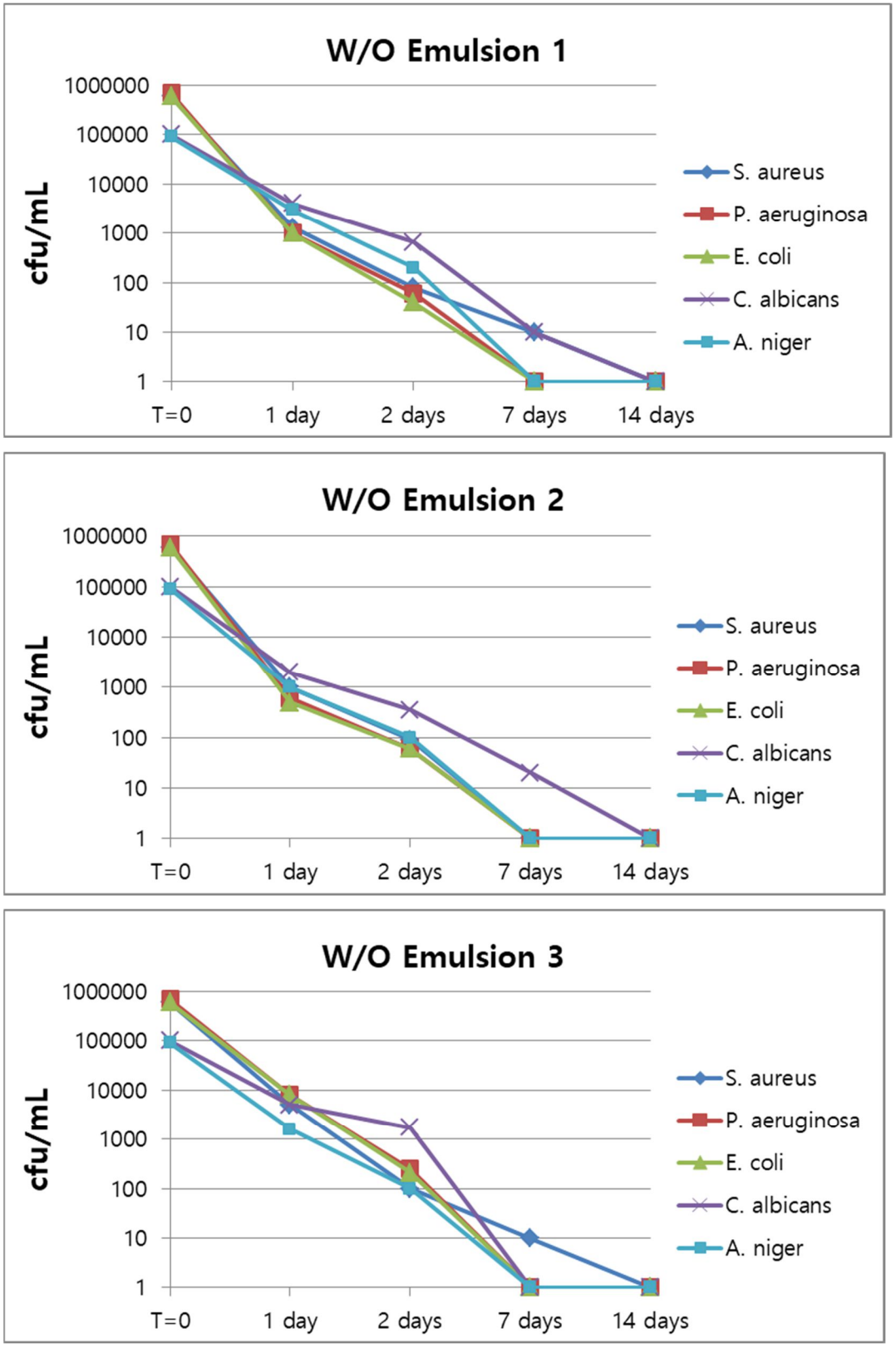


Fig. 5. Preservative effect of IPD in W/O Emulsion formula.



General conclusion

3-methylbutane-1,3-diol (IPD) known as multi-purpose ingredient for cosmetics and antimicrobial agents were investigated together for their antimicrobial activity against bacteria and fungi in cosmetic formulations.

Minimum inhibitory concentration (MIC) tests of IPD showed bactericidal activities at 15%, 10% and 10% for S. aureus, P. auruginosa and E. coli, respectively.

The analysis of fractional inhibitory concentration (FIC) values revealed a synergistic antibacterial effect of IPD with antibiotics. The checkerboard assay was conducted to confirm the antibacterial synergy of Isopentyldiol (IPD), with ethylghexylglycerin (EHG), phenoxyethanol (PE), and caprylylglycol (CG), and compared with synergistic effects with EHG, PE and CG of Butyleneglycol (BG) and 2-Methyl-1,3-propanediol (MPD).

These systems were then challenged in cosmetic formulations, a lotion and an emulsion.

As outcome to these tests, IPD confirms its synergetic effect with usual raw materials frequently used for antibacterial effect to protect the cosmetic formulations. The different combinations with IPD showed an efficient and quick antibacterial activity, thus proposing an alternative to parabens/PE systems.

Additionally, IPD treatment rate showing the best cost effective solution at 5% is the recommended concentration suggested to benefit other features of IPD such as moisturizing agent, for its unique emollience, for hair repair and color protection, for color cosmetic formulation, as active solubilizer and vector, and for many more characteristics.