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Project title Absorbing and Retaining the Moisture via Humectant System

Student names Mr.Nattapon Chavananon ID 6033812423

Mr.Nattapat Vivattanasan ID 6033813023

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Absorbing and Retaining the Moisture via Humectant System

by
Mr. Nattapon Chavananon
Mr. Nattapat Vivattanasan

In Partial Fulfillment for the Degree of Bachelor of ScienceProgram in Applied Chemistry (International Program)Department of Chemistry, Faculty of Science Chulalongkorn University
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Examination committees

1. Assoc. Prof. Dr. Surachai Pornpakakul Advisor
2. Assoc. Prof. Dr. Worawan Bhanthumnavin Committee
3. Assist. Prof. Dr. Varawut Tangpasulthadol Chair Committee

Endorsed and approved by the Head of Department of Chemistry

Surachai Pornpakakul
……
(Assoc. Prof. Dr. Surachai Pornpakakul)
Advisor

Vp. Aaeen
……
(Associate Professor Voravee Hoven, PhD.)
Head of Department of Chemistry

Date 28 December 2020

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Student Name Mr. Nattapon Chavananon Student ID 6033812423
Mr. Nattapat Vivattanasan Student ID 6033813023
Advisor Name Assoc. Prof. Dr. Surachai Pornpakakul
Department of Chemistry, Faculty of Science, Chulalongkorn University, Academic Year 2020

Abstract

This study had been undertaken to evaluate water-retaining activity of different humectant systems. Six humectants including sodium hyaluronate (HA), glycerol (G), propylene glycol (PG), urea, hydrogel (HG), PEG-400, were examined their water-retaining activity and physical effects on pig skin, and those on human skin. As a single humectant, 1.50% HA showed the best performance. For 2-humectant-based and 3-humectant-based combinations, 1.50%HA-2.00%HG, and 1.5%HA-2.0%HG-6.50%G, were the outstanding formulations. Application of 1.5%HA-2.0%HG -6.50%G onto pig skin did exhibit an impressive result which moisture was maintained on pigskin over 24 h., while the rest of the samples dried up. In addition, 1.5%HA-2.0%HG-6.50%G was examined on human skin of 3 subjects and provided the most agreeable comment, giving smoothness and moisture to the skin without any adverse effect. The outcome was indicative that the humectant formula with the HA-HG-G based combination showed positive water-retaining activity, which can become a promising formulation useful in other applications such as medicine, dermatology and cosmetics.

Acknowledgement

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Table of Content

List of Tables

List of figures

Chapter 1
Introduction

1.1 Introduction to the research problem and significance

Humectants are ingredients that play important roles in keeping moisture in the stratum corneum, or human's outermost layer of the skin. Humectants are hygroscopic organic compounds, which when touched with skin, can function by bonding with water molecules to increase water content in the skin itself. Under hydrated surroundings, the skin will work on itself to repair damaged zones, increase elasticity, and enhance the anti-aging process.[1]One of the most commonly marketed cosmetic products for daily usage are moisturizers. Not only is it demanded in the beauty industry, moisture-locking products are also necessities in skin patients. Prolonged, untreated dryness can lead to cracking, inflammation, and eventually infection. In this case, a typical moisturizing cream may not be sufficient. Multiple humectant composites formulation with enhanced moisturizing effects may be considered. In addition, flawless skin appearance may improve a patient's quality of life, whether it be from self-esteem, social life, or psychological health.[2] Therefore, this study will investigate water absorb and retaining properties of widely known humectants including sodium hyaluronate (HA), glycerin (G), propylene glycol (PG), urea, polyethylene glycol (PEG 400), and hydrogel (HG). The aim of this project was to find the most efficient and effective formulation of moisturizer.

1.2 Research objectives

To find the most effective and efficient humectant-based formulation which is able to retain and attract more moisture.

1.3 Literature Review

Moisturizer

Moisturizers are major ingredients for most of the daily skin care and recommended items in dermatology practice. It is one of the most crucial strategies used by dermatologists to maintain skin health as well as treating dermatitis that arise from skin dehydration. There are four primary types of moisturizers; humectants, emollients, occlusives, and protein rejuvenators, relying upon their system of activity. Most humectants have low molecular weights. They usually contain hygroscopic substances which help the skin to absorb water by trapping moisture from the dermis and the surrounding environment into the epidermis. Emollients are made up of saturated and unsaturated lipids, such as palm oil and coconut oil, that work by strengthening skin barrier function, skin flexibility and smoothness. Occlusives are waxes and oils that form an inert layer on the skin surface that inhibit moisture evaporation. Protein rejuvenators are small molecular weight proteins believed to replenish essential skin proteins, commonly known as collagen and keratin.[3] Due to time limitation, only humectants of various kinds will be explored in the experiment.

Humectant and its Mechanism

Humectant is a hygroscopic substance which means it is able to attract moisture from two sources. Dehydration occurs between the deep down skin layer, dermis, and surface layer, epidermis, where the humectants are drawing the water from the surrounding atmosphere. Humectant pulls in and holds the moisture from the surrounding environment through assimilation, bringing the water vapor into or underneath the skin surface. The molecular structure of humectants consists of hydroxyl group(-OH) (Figure 1), which is favorable for the attraction to water molecules through hydrogen bonding.[4] The most commonly used humectants are hyaluronic acid, urea, propylene glycol, hydrogel, and glycerol.[3]

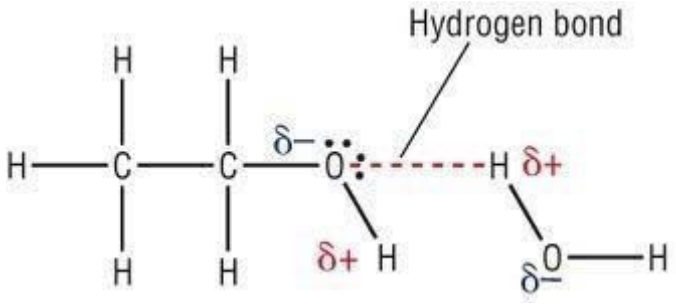


Figure 1 -OH does the hydrogen bonding with surrounding water in the atmosphere

Sodium hyaluronate

Sodium hyaluronate is water-solvent sodium salt type of hyaluronic acid, a characteristic substance found in different connective tissues of people that has a novel ability to hold water.[5] It can be found normally in the liquid that washes joints due to it is important for the lubrication of the tissue surfaces in diarthrodial joints because of its viscoelastic properties.[6]

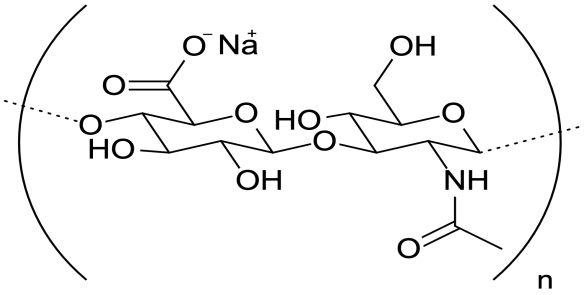


Figure 2 Structure of sodium hyaluronate

The elements of sodium hyaluronate depend on the capacity of its particle to retain and hold water. GAG, Glycosaminoglycans, can be found in the extracellular lattice of all vertebrates just as in certain microorganisms. It gives a very much hydrated pericellular environment. GAGs impact explicit interaction that influence significant cell processes, for example, the development of collagen fibrils, cell-cell connections, development factors authoritative, and cell guideline by affecting actual cycles, for example, water retention.[7] When present in a skin health items, it can assist with combating dryness and decrease indications of maturing by giving high measure of moisture to the skin.

Glycerol

Glycerol is a colorless, scentless fluid with a sweet tasteIt, also known as glycyl alcohol, glycerin or glycerine.[8] Glycerol is seen in natural frameworks as an intermediate in lipid digestion since surplus starch can be changed over into long chain unsaturated fats and esterified with the three hydroxyl gatherings.[9]

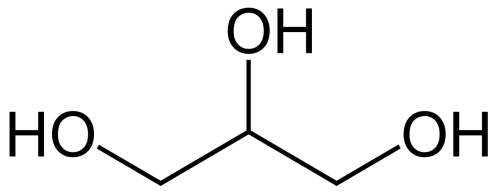


Figure 3 Structure of glycerol

The presence of three hydroxyl groups makes the compound hygroscopic, with a tendency to retain dampness from the air.[10] Since hydroxyl groups are present, hydroxyl groups will do hydrogen bonding with the surrounding water molecule in their atmosphere.[11] This definitely makes it helpful as a humectant in beauty care products and food, holding water and keeping the substance from drying out.

Propylene glycol

Propylene glycol, also known as 1,2-dihydroxypropane, 1,2-propanediol, methyl glycol, and trimethyl glycol, is clear, colorless substance.[12] Propylene glycol does strong intermolecular hydrogen bonding interaction with aerosol droplets in surrounding area to assimilate additional water and keep up moisture in products, such as E-liquid.[13] It might exist in air in the vapor structure, in spite of the fact that propylene glycol must be warmed or energetically shaken to create a vapor.

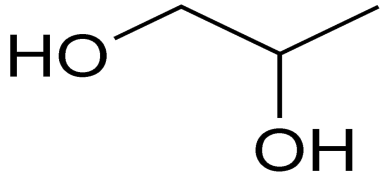


Figure 4 Structure of propylene glycol

As there are 2 hydroxyl groups present which make it to be hygroscopic. Furthermore, it is a good humectant that is fit for holding moisture over a wide scope of relative moistness.[11]

Urea

Urea, or carbamide, is classified to be an organic compound with chemical formula $CO(NH_2)_2$. The characteristics of urea are white or colorless solid, odorless, extremely dissolve in the water and be neutral when dissolved with water. Urea is the substance that has 2 amino acid groups combined with a carbonyl function group. It has been popularly used for producing fertilizers because an important component is nitrogen which is easily found in the urea and also a significant raw material for the chemical business.

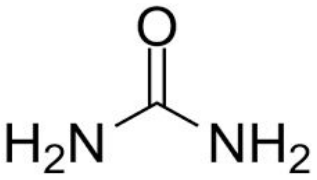


Figure 5 Structure of urea

Urea is a hydrophilic substance which means it has the ability to hold the water molecule that will keep skin plump and moist. At the molecular level, the amino acid and polypeptides in urea were adjusted inside the skin which made for moisturising the delicate tissues. Some studies said that the keratolytic and hydrating effects of specific urea are inferable from breakage of hydrogen bonds in the stratum corneum, releasing epidermal keratin, and expanding water-binding sites.[14]

Hydrogels

Hydrogels are some kind of polymer chains network which are well known as hydrophilic. Hydrogels do not commonly dissolve because of their chemical or physical cross-links and/or chain entanglements. The example of natural polymer networks are collagen and gelatin, it also can be created by synthetic. Generally, water must comprise more than 10% of the total volume for a material to be a hydrogel. Additionally, hydrogels have a level of adaptability closed to the normal tissue because of their significant water content. [15] Hydrogel is widely used in various options, such as scaffolds in tissue engineering, for maintained medicine or drug shipment, contain sensitivity of specific molecules, as biosensors. [16]

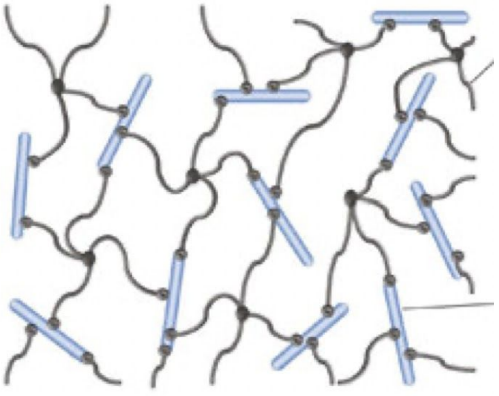


Figure 6: Structure of hydrogel [16]

Hydrogels are three-dimensional network structures which have the ability to act in hydrophilic polymer substances that means it can hold a large amount of water while keeping their structure due to chemical or physical cross-linking properties of individual polymer chains. [16] The water absorption mechanism of hydrogels is the negative charge of the polymer chain that will attach with the H atom in the water which has the positive charge and that makes hydrogen bonding between polymers in hydrogels and water and that is how hydrogels can keep the water.

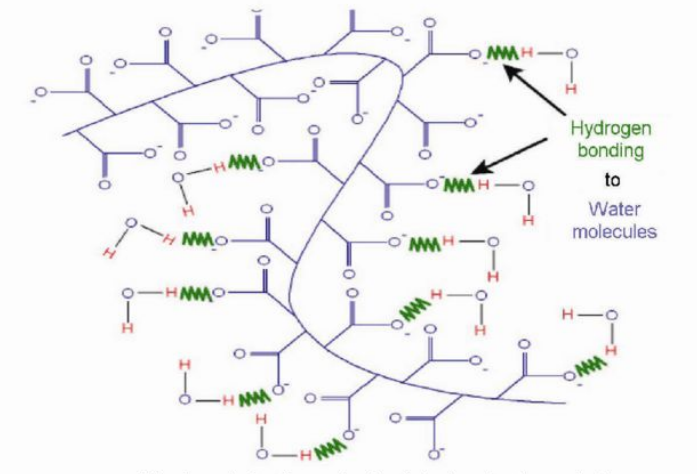


Figure 7 Hydrogel retaining water mechanism[17]

Polyethylene Glycol 400

Polyethylene glycol 400 or PEG 400 has a low molecular weight when compared with polyethylene glycol, also colorless, odorless and in liquid phase. It can be dissolved in various solvents such as acetone, alcohols, glycerin, glycols and water; this made PEG 400 has been widely used in pharmaceuticals and cosmetics.[18]

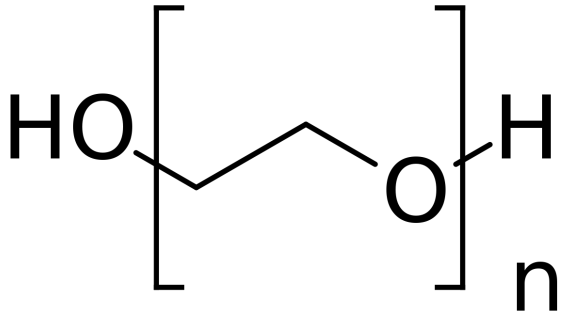


Figure 8 Structure of PEG 400

Polyethylene glycol 400 (PEG 400) is a hydrophilic substance and that means it also be a hygroscopic substance, a substance which has the ability to attract or keep water molecules via the surrounding environment. Polyethylene glycol 400 can dissolve in water and various organic solvent such as aromatic hydrocarbons. Therefore, PEG 400 can attach the water from the -OH group and make hydrogen bonds between water and -OH group from the molecule.[18]

Human Skin

Human skin is the outer part of the body and has the largest area of the integumentary system about 12 to 15 percent from human weight and has 1.5 - 2 m^2 for the surface area.[19]Our skin has 2 main layers which are epidermis and dermis. The basement membrane is used for divining between epidermis and dermis layers. Epidermis is the outermost skin layer, creating the barrier to protect various types of danger from the surrounding environment. The middle layer of skin was named dermis, consisting of collagen and irregular connective tissues; major roles are storing and absorbing the nutrients.[20]

The integumentary system has various functions in terms of balancing the body structure. All body framework systems work in an interconnected way to keep up the inside conditions basic to capacity of our bodies. Skin has a significant occupation to ensure the body and enroll as the body's protection front line to safeguard against contamination, temperature and other difficulties homeostasis.[21]

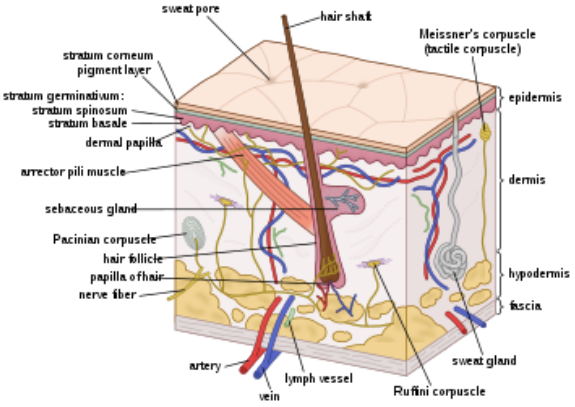


Figure 9 Human skin[22]

Pig skin

Pigskin is very similar to human skin because the ratio of epidermal and dermal thickness are quite close to human skin and also have comparative hair follicle and vein patterns. For biochemical, the dermal collagen and versatile substance is comparative in both human and pig skin. Lastly, both skin have comparative reactions with different growth factors.[23]

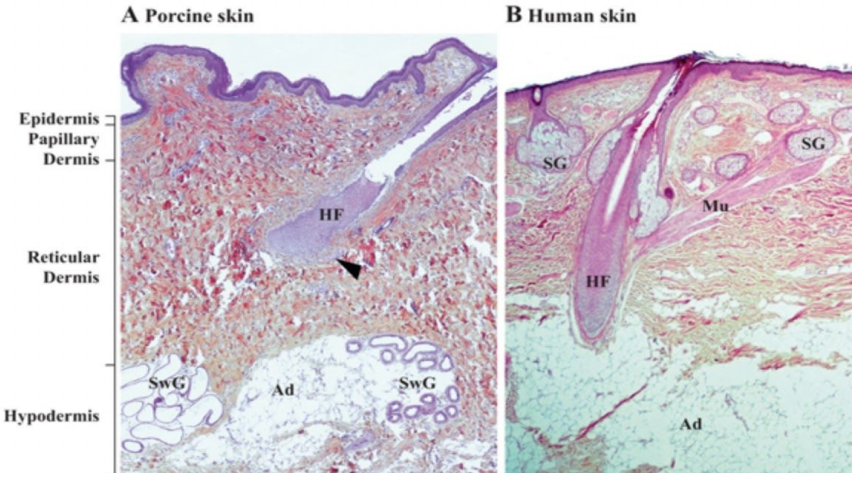


Figure 10 Human skin compared with pigskin [24]

Chapter 2
Experimental

2.1 List of equipment and instrument

(1) 50mL beaker, (2) 25 mL measuring cylinder, (3) parafilm, (4) dropper, (5) Erlenmeyer flask, (6) stirring rod, (7) micropipette, (8) glass petri dish, (9) lab spoon, (10) plastic bag, (11) semi-micro balance.

2.2 List of Chemicals and materials

(1) Sodium hyaluronate, (2) glycerol, (3) propylene glycol, (4) polyethylene glycol (PEG 400), (5) urea, (7) hydrogel. These chemicals were commercially available. Pork skin was purchased from a local fresh market, kindly provided by our advisor.

2.3 Preparing humectants

Each humectant was prepared into 4 different concentrations, which were varied upon the percentage typically used in the market as referred to the Literatures (Table 1).

Table 1 Formulation of each substance in percentage

Humectant :	Concentrations suggested in Literature	Concentrations used in this experiment			
Sodium hyaluronate	2.00%[25]	0.50%	1.00%	1.50%	2.50%
Glycerol	5.00%[26]	3.00%	4.50%	6.50%	8.00%
Propylene glycol	8.00%[27]	1.00%	2.00%	5.00%	10.00%
Urea	10.00%[28]	1.00%	2.00%	5.00%	10.00%
Hydrogel	Proper amount [16]	1.00%	2.00%	5.00%	10.00%
Polyethylene glycol 400	Proper amount [29]	2.00%	3.50%	4.50%	6.00%

In the preparation of humectants, each chemical was weighed using a semi-micro balance and distilled water was added to make a solution (10.00 g). The weighings were repeated to produce solutions of different concentrations. Each mixture was stirred to become homogeneous, tightly covered with parafilm to prevent water evaporation, and let sit to be ready for further experiments. An example of the preparation for making sodium hyaluronate solutions is shown in Table 2.

Table 2 Formulation ratio of sodium hyaluronate solutions

Concentration required	Sodium hyaluronate	Water	Total
0.50%	0.05 g	9.95 g	10.00 g
1.00%	0.10 g	9.90 g	10.00 g
1.50%	0.15 g	9.85 g	10.00 g
2.50%	0.25 g	9.75 g	10.00 g

2.4 Determining water-retaining ability of the single humectants at various concentrations

Rate of evaporation was used to determine water-retaining ability of each humectant. Firstly, 0.250 mL of the solution was drawn out using a micropipette and placed onto a 5 x 5 cm piece of plastic on a glass petri dish (Figure 11), then it was weighed and recorded. The solutions were let stand at room temperature for 2 h, 4 h, and 24 h. After each time period, the solution was re-weighed and recorded. The percentage weight loss would suggest water loss and thus imply water-retaining ability of the solution. The process was repeated 3 times, and with other concentrations. Pure water was used as a control factor during the experiment. The percentage water loss of each humectant was calculated by : Percentage water loss = (Initial weight) - (Weight after 2 h / 4 h / 24 h) ÷ (Initial weight) * 100

Figure 11 Experimental diagram for Section 2.4

2.5 Determining water-retaining activity of 2-humectant-based composites

The only humectant at the concentration that provided the best result was used to create a new formulation with other humectants at their most competent concentrations (Table 3) according to results from Section 2.4. In the preparation of 2-humectant-based composites, the solutions were simply mixed and stirred well until the mixture became homogeneous. The experiment to determine water-retaining activity was repeated in accordance to Section 2.4.

Table 3 2-humectant-based formulation matrix

The best humectant at best concentration	Humectant B	Humectant C	Humectant D	Humectant E
Humectant A x.xx%	x.xx%			
		x.xx%		
			x.xx%	
				x.xx%

2.6 Determining water-retaining activity of 3-humectant-based composites

Furthermore, two humectants at the concentrations that provided the best result were used to create a new formulation with other humectants at their most competent concentrations (Table 4) according to results from Section 2.5. In the preparation of 3-humectant-based composites, the solutions were simply mixed and stirred well until the mixture became homogeneous. Again, the experiment to determine water-retaining activity was repeated in accordance to Section 2.4. The best humectant formulation was determined.

Table 4 3-humectant-based formulation matrix

The Best Humectant at Best Concentration (A)	The Best Humectant at Best Concentration (B)	Humectant C	Humectant D	Humectant E
Base		Substance		
x.xx%	x.xx%	x.xx%		
			x.xx%	
				x.xx%

2.7 Evaluating water-retaining ability through physical appearance analysis on pig skin

Pig skin has very similar morphological and functional characteristics to human skin, and has been often used as a substitute in numerous studies.[30] In order to further evaluate water-retaining ability of the 3-humectant-based composites, the products were applied directly on to pig skin (Figure 12). For preparation, pig skin was washed thoroughly with water, let dry before cutting into 5x5 cm samples, and placed on a glass petri dish. Each 3-humectant-based composite was gently applied on the pig skin sample using rubber gloves to reduce the contamination. The weight of pig skin was measured and recorded before and after application. Also, for physical observation, pig skin samples were photographed and recorded.

Figure 12 Pig skin sample before cutting into 5x5 cm^2 pieces

2.8 Evaluating water-retaining ability through physical appearance analysis on human skin

In order to evaluate further the water-retaining ability of each 3-humectant-based composites, physical analysis on human skin was experimented. Three healthy human male volunteers from 21 to 22 years old took part in this experiment. A 5x5 cm square was drawn on the arms as the area for application. Each volunteer applied different 3-humectant-based composites from Section 2.5 for 5 consecutive days on different skin areas. Each day after 2 h, 4 h, and 6 hours, the volunteer's skin was photographed and recorded. Qualitative information was noted, such as the touch and feels each composites gave to the skin.

Chapter 3
Results and discussion

3.1 Water-retaining ability of different single humectants at various concentrations

The average percentage loss for each sodium hyaluronate was determined, and graphs relative to control were shown in Figure 13. (For data of HA, G, PG, Urea, HG, and PEG400 indicated that the highest water retaining of G, PG, Urea, HA was obtained at varying concentrations and percentages were shown respectively in Appendix A.

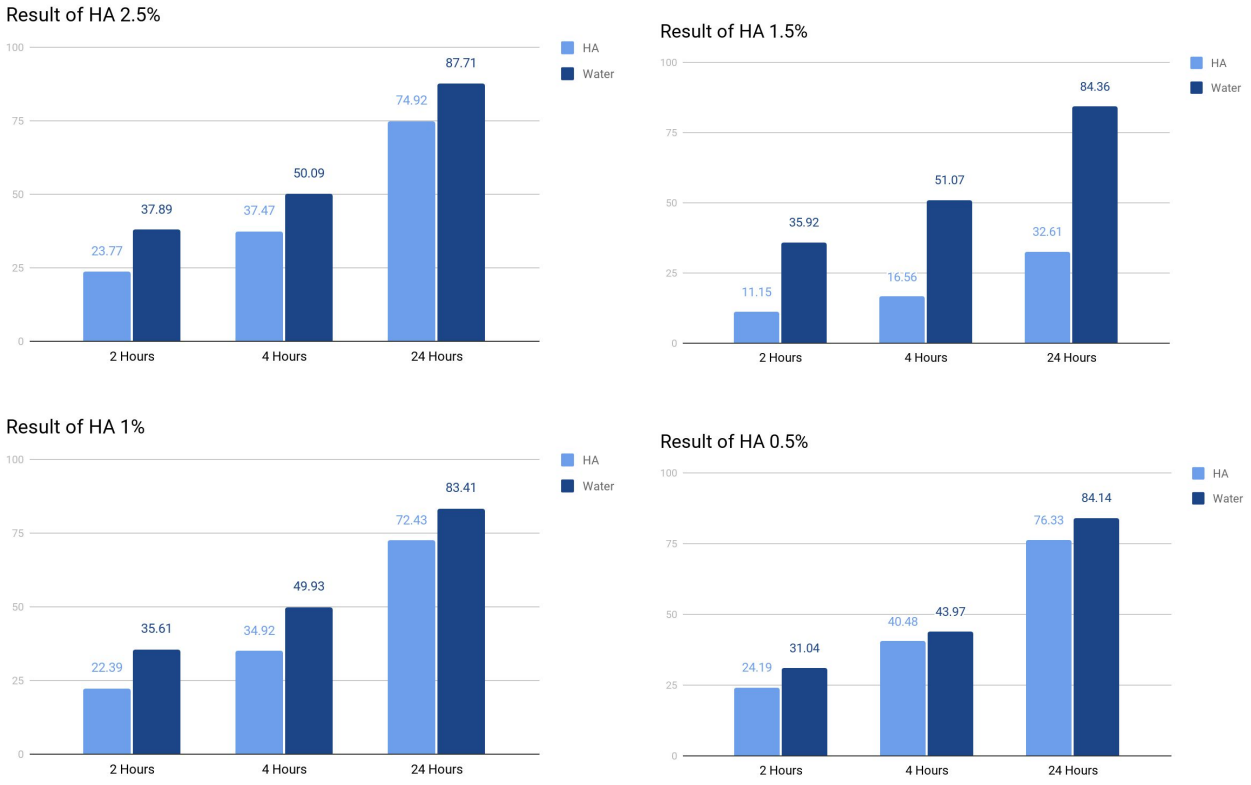


Figure 13 Percentage water loss of sodium hyaluronate solutions at different concentrations after 2 hours, 4 hours and 24 hours relative to control.

From Figure 13, it showed that the percentage loss of control did not vary much from each experiment. However, the percentage water loss of 1.50% sodium hyaluronate were significantly low compared to the other concentrations, indicating best water-retaining activity. Most outstanding results from each humectant were plotted on a graph to determine two best humectants for further experiment.

Figure 14 illustrates the percentage water loss of each humectant at its best-performing concentration, The resulting percentage water loss after 24 h for each was 32.61%, 54.63%, 52.51%, 58.67%, 39.09%, and 41.53% respectively. It can be seen that 1.50% HA solution outperformed the other humectants, indicating best water-retaining activity.

Since the experiment aimed to achieve a practical formulation, our advisor suggested that in the real-world cosmetic industry, hydrogel is one of the crucial ingredients needed in a cream formulation, as it is a thickening agent to provide the cream with appropriate consistency and texture. Therefore, it was suggested that 5.00% HG were to be used in the formulation of 2-humectant-based composite instead of 1.50% HA in the next experiment. However, the consistency of 5.00% HG was practically unfeasible since it was too viscous. As a result, 2.00% HG was used in formulating 2-humectant-based composite in the later experiment instead.

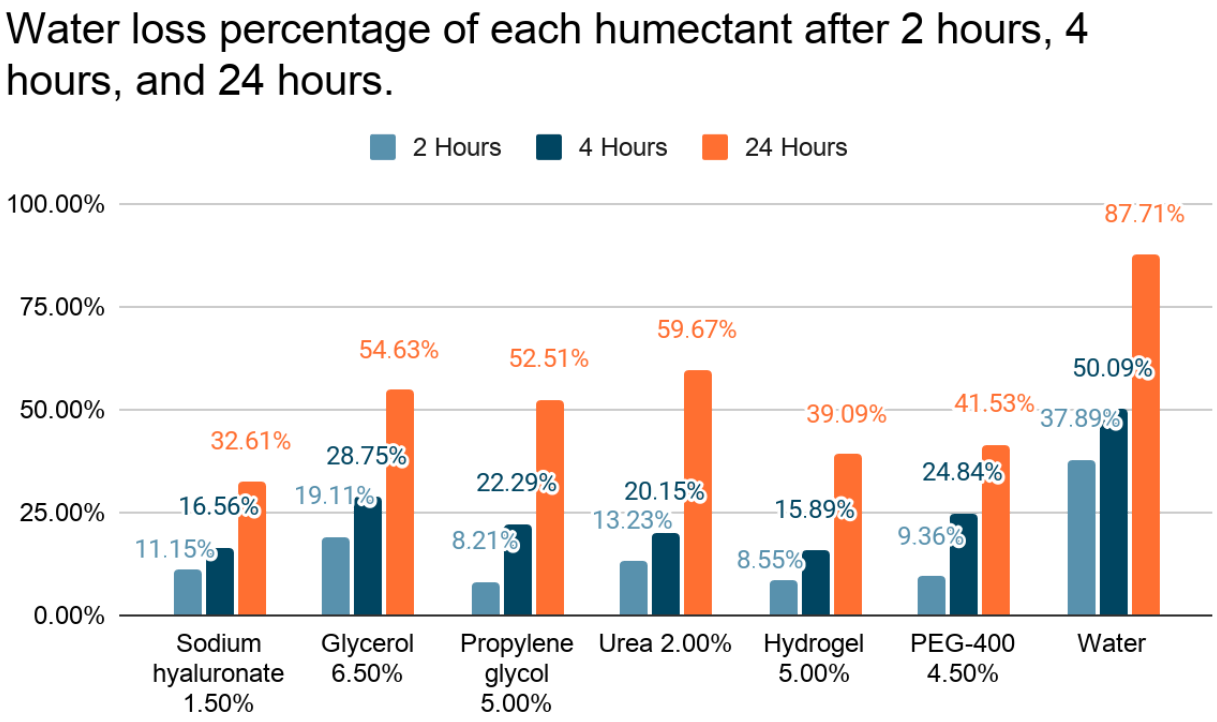


Figure 14 Water loss percentage of each humectant after 2, 4, and 24 hours.

3.2 Water-retaining ability of different 2-humectant-based formulations

According to 3.1, 2.00% HG was used as a base for formulating 2-humectant composition as shown in Table 5. Also, 2.00% HG solutions were used as control.

Table 5 Formulating 2.00% HG with other humectants at their best-performing concentration, with water as control.

Hydrogel	HA	PG	G	UREA	Water
2.00%	1.50%				
		5.00%			
			6.50%		
				2.00%	
					Control

Result (Figure 15), shown that the percentage water loss of each HG-based composite for 24 hours was 52.84% for HA-HG, 64.80% for PG-HG, 64.70% for G-HG, 70.67% for Urea-HG, 71.21% for Water-HG and, and 84.16% for control. It can be seen that HA-HG composite provided the lowest percentage of water loss, indicating best-performing water-retaining activity. Therefore, 1.50% HA and 2.00% HG formulation were used in further experiment.

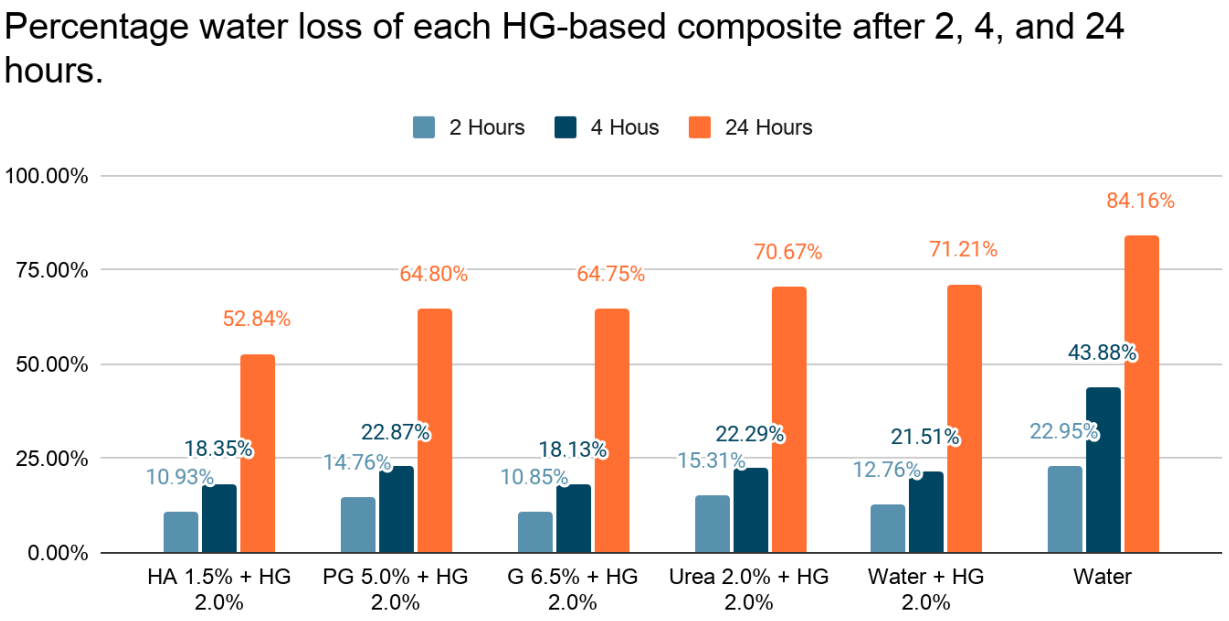


Figure 15 Percentage water loss of each HG-based composite after 2, 4, and 24 hours.

3.3 Water-retaining ability of different 3-humectant-based formulations

The best-performing humectants from Section 3.2, sodium hyaluronate 1.50% with hydrogel 2.00%, was mixed with the best-performing concentration of the other humectants and formulated as shown in Table 6.

Hydrogel	Sodium hyaluronate	G	PG	UREA	Water
Base		Substance			
2.00%	1.50%	6.50%			
			5.00%		
				2.00%	
					Control

Table 6 3-humectant-based formulation table with 2.00% H and 1.50% HA as a base.

Result (Figure 16) showed that the percentage water loss of 3-humectant-based formulations for 24 hours was 54.00% for HA-HG-G, 66.51% for HA-HG-PG, 67.83% for HA-HG-Urea, 59.48% for HA-HG-Water, and 89.05% for control. It can be seen that HA-HG-G solution outperformed the

the best water-retaining activity. However, the consistency of the formulation with 6.50% G was practically unfeasible since it was extremely oily. Therefore, 5.00% glycerol composite, which was the more feasible concentration, was included in the later experiment. Also, our advisor suggested 10.00% Urea composite was to be included in the next experiment as well.

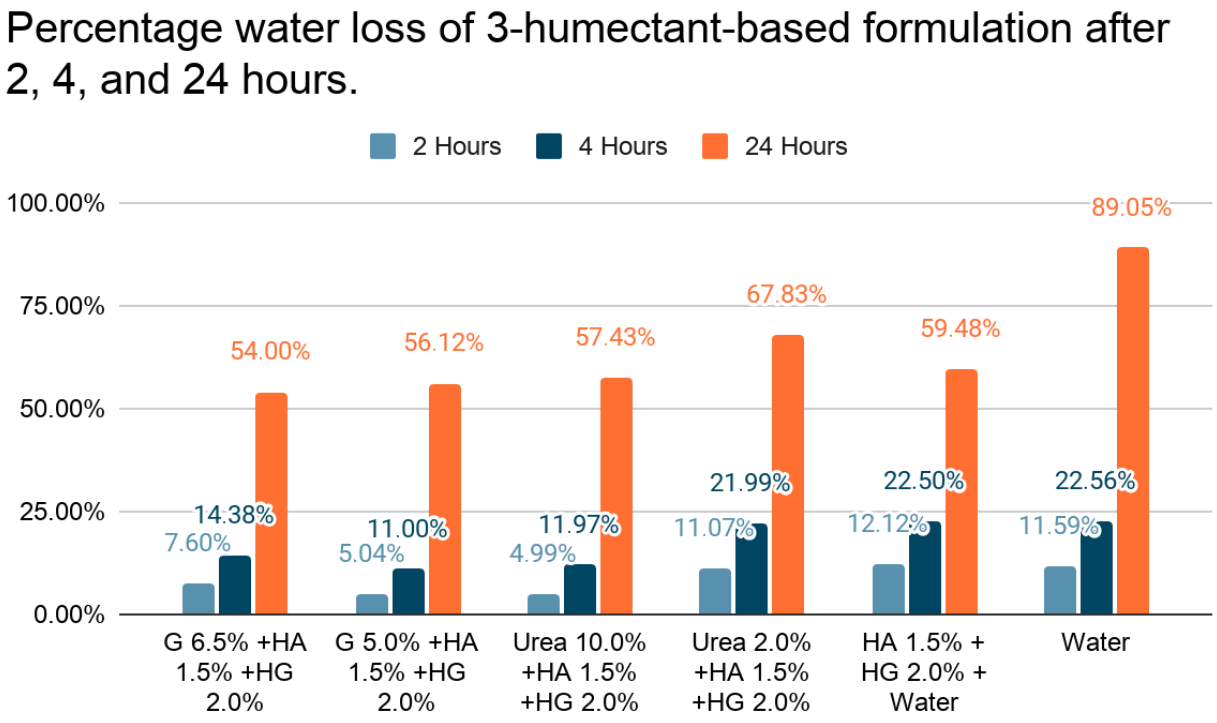


Figure 16 Percentage water loss of each 3-humectant-based formulation after 2, 4, and 24 hours.

3.4 Evaluating water-retaining ability through physical analysis on pig skin

To evaluate the water-retaining activity of 3-humectant-based composites from Section 3.3, physical analysis on pig skin was experimented. The images of pig skin after application of 0, 2, 4, and 24 h. The results from each composite were relatively similar. After 24 h, every formulation left the pig skin completely crisp and dry except the pig skin sample with HA-HG-G composite looked hydrated and moist, which implied excellent water-retaining capability.

Percentage remaining weight of pig skin before and after application was also recorded according to the percentage remaining weight of each pig skin sample was 94.76% for HA-HG-G6.50%, 96.17% for HA-HG-G5%, 94.16% for HA-HG-Urea10%, 94.68% for HA-HG-Urea2%, 93.53% for HA-HG-PG5% and 95.47% for control. It can be seen that HA-HG-G5.00% solution outperformed the other humectants, supporting the image from Figure 17 that it was an excellent water-retaining formula.

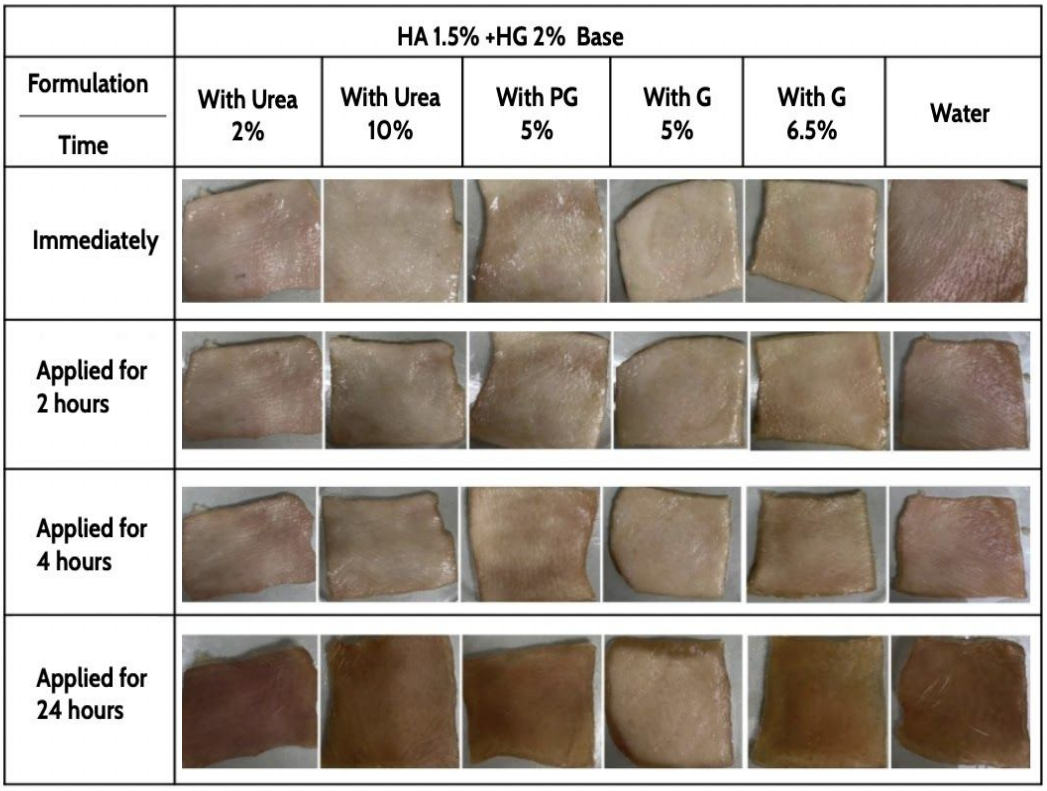


Figure 17 Pig skin after application with each composite.

Table 7 Percentage remaining weight of pig skin after 2 h, 4 h and 24 h of application

Type / Time	G 6.5% +HA 1.5% +HG 2%	G 5% +HA 1.5% +HG 2%	Urea 10% +HA 1.5% +HG 2%	Urea 2% +HA 1.5% +HG 2%	PG 5% +HA 1.5% +HG 2%	Water
Weights of solutions in the pig skin	(0.1078 g) (0.1117 g) (0.0998 g)	(0.2879 g) (0.2419 g) (0.0960 g)	(0.1409 g) (0.1867 g) (0.0213 g)	(0.1689 g) (0.1530 g) (0.1365 g)	(0.1863 g) (0.1882 g) (0.0706 g)	(0.0832 g) (0.0381 g) (0.0589 g)
2 Hours	99.12% 99.18% 98.94% AVG=99.08%	98.87% 99.41% 99.24% AVG=99.17%	99.06% 99.43% 98.83% AVG=99.11%	99.12% 99.06% 99.08% AVG=99.10%	99.12% 98.67% 98.87% AVG=98.89%	99.22% 98.77% 99.39% AVG=99.13%
4 Hours	98.39% 98.59% 98.17% AVG=98.38%	97.99% 98.92% 99.14% AVG=98.68%	98.31% 98.14% 98.00% AVG= 98.15%	98.44% 98.31% 98.39% AVG=98.38%	98.43% 97.66% 98.04% AVG=98.04%	98.71% 97.97% 98.95% AVG=98.54%
24 Hours	94.43% 95.47% 94.38% AVG=94.76%	93.75% 97.18% 97.59% AVG=96.17%	94.31% 93.77% 94.41% AVG=94.16%	95.40% 94.19% 94.46% AVG=94.68%	94.76% 92.24% 93.63% AVG=93.54%	95.65% 94.22% 96.53% AVG=95.47%

3.5 Evaluating water-retaining ability through physical analysis on human skin

The same humectant composites from Section 3.4 were re-evaluated on human skin by direct application on the back of volunteers' arms. The result was examined through naked eyes and volunteers' comments describing the instant 'feels' each formulation, the skin condition after application, and their preference.

Table 8 Evaluating 1.50% HA with 2.00% HG formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	1 (33.3%)	2 (66.7%)	-	-
Adverse Effect				
Redness	3 (100%)	-	-	-
Itching	3 (100%)	-	-	-
Leave a stain	3 (100%)	-	-	-

Table 9 Observation of Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

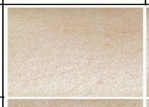
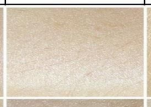
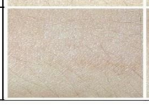
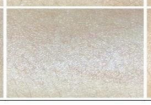
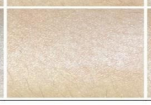
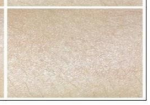
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 1.50% HA with 2.00% HG formulation according to Table 8, the skin was slightly smoother and moister than the area where it was not applied. The effect disappeared after 1-2 h and no irritation occurred. After using it for 5 continuous days, there was not any visible change to the skin, but the texture of skin became softer than the area of skin without application.

Table 10 Evaluating 2.00% urea with HA-HG base formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	2 (66.7%)	1 (33.3%)	-	-
Adverse Effect				
Redness	-	2 (66.7%)	1 (33.3%)	-
Itching	-	3 (100%)	-	-
Leave a stain	-	3 (100%)	-	-

Table 11 Observation of Urea 2.00% with Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

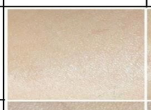
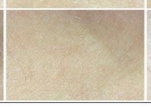
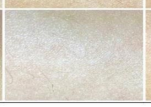
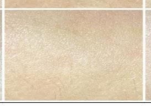
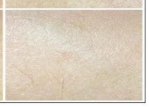
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 2.00% urea with HA-HG base formulation according to Table 9, after 10 minutes of application, all 3 volunteers said that it gave red rashes, and some irritations. It did not give much moisture as the other samples. White stain was left over the skin after application, but disappeared after 10-15 minutes. After using it for 5 continuous days, the area where it is applied on always has red rashes and irritation and also does not have an obvious visible change.

Table 12 Evaluating 10.00% urea with HA-HG base formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	2 (66.7%)	1 (33.3%)	-	-
Adverse Effect				
Redness	-	1 (33.3%)	2 (66.7%)	-
Itching	-	1 (33.3%)	2 (66.7%)	-
Leave a stain	-	3 (100%)	-	-

Table 13 Observation of Urea 10.00% with Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

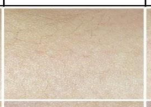
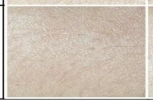
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 10.00% urea with HA-HG base formulation according to Table 10, the outcome was not as good compared to Urea 2%. There was irritation and red rashes that lasted from more than 10 minutes, the skin felt uncomfortable and again, white stains were left. The negative effect disappeared after 30 minutes. After using it for 5 continuous days, the skin had red rashes and irritation. Also, it did not have an obvious visible change, however, the skin felt smoother compared to 2.00% urea.

Table 14 Evaluating 5.00% PG with HA-HG base formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	-	3 (100%)	-	-
Adverse Effect				
Redness	3 (100%)	-	-	-
Itching	3 (100%)	-	-	-
Leave a stain	3 (100%)	-	-	-

Table 15 Observation of Propylene glycol 5.00% with Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

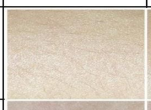
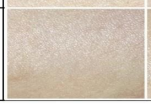
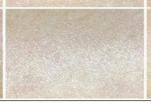
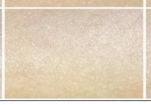
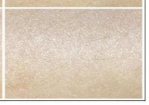
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 5.00% PG with HA-HG base formulation according to Table 11, the skin was quite softer and moist than 1.50% HA with 2.00% HG, and did not have side effects on the skin. The soft skin effect disappeared after 2 hours. After using it for 5 continuous days, there was no obvious visible change, but the texture of skin did turn softer than before application.

Table 16 Evaluating 5.00% glycerol with HA-HG base formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	-	-	3 (100%)	-
Adverse Effect				
Redness	3 (100%)	-	-	-
Itching	3 (100%)	-	-	-
Leave a stain	3 (100%)	-	-	-

Table 17 Observation of Glycerol 5.00% with Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

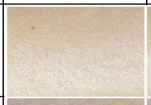
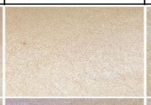
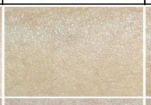
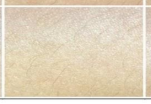
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 5.00% G with HA-HG base formulation according to Table 12, it gave smooth and soothing skin, without irritation or stain. The effect still remained even after 4-6 hours. After using it for 5 continuous days, it did not give an obvious visible change but the skin did feel softer and moister than the first day. All three volunteers agreed that this formula was the best from the rest.

Table 18 Evaluating 6.50% glycerol with HA-HG base formulation on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	-	1 (33.3%)	2 (66.7%)	-
Adverse Effect				
Redness	3 (100%)	-	-	-
Itching	3 (100%)	-	-	-
Leave a stain	3 (100%)	-	-	-

Table 19 Observation of Glycerol 6.50% with Sodium hyaluronate 1.50% and Hydrogel 2.00% effect on human skin

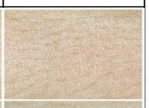
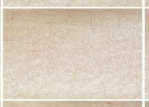
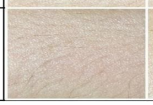
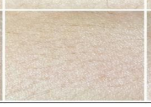
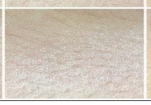
	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with 6.5.00% G with HA-HG base formulation according to Table 13, the skin felt a little bit drier when compared with 5.00% G after applied for 6 hours. After using it for 5 continuous days, the skin does not have an obvious visible change but the skin feels moist and soft when compared to the beginning.

Table 20 Evaluating distilled water on human skin.

	No. (%) of volunteers			
Humectants Effect	**Not at all**	**A Little**	**Moderately**	**Extremely**
Positive Effect				
Smooth and Moist	3 (100%)	-	-	-
Adverse Effect				
Redness	3 (100%)	-	-	-
Itching	3 (100%)	-	-	-
Leave a stain	3 (100%)	-	-	-

Table 21 Observation of distilled water effect on human skin

	BEFORE APPLICATION	AFTER APPLICATION	AFTER 2HRS	AFTER 4HRS	AFTER 6HRS
DAY1					
DAY2					
DAY3					
DAY4					
DAY5					

From testing with distilled water according to Table 14, water evaporated from the skin after approximately 2-3 minutes, so it did not retain moisture from the skin at all. This had no difference to the skin feeling before application. After using it for 5 continuous days, the skin did not change from the beginning.

Chapter 4
Conclusion

In the experiment in determining the water-retaining activity of a single humectant, 1.50% HA solution showed most outstanding water-retaining activity, which was to be used further in the 2-humectant-based formulation. However, since the experiment intended to achieve a practical formulation, our advisor suggested that actually, hydrogel is a requisite ingredient in the formulation of any cream, as it provides the cream with appropriate consistency and texture. Therefore, it was suggested that 5.00% HG were to be used in the formulation of 2-humectant-based composite instead of 1.50% HA in the next experiment. However, the consistency of 5.00% HG was practically unfeasible since it was extremely glutinous. As a result, 2.00% HG, the second best, was chosen to be the base in formulating 2-humectant-based composite in the later experiment instead. Consequently, in the investigation of 2-humectant-based formulation, 1.50% HA and 2.00% HG composite outperformed the other formulations, and therefore was used in the next experiment.

In the evaluation for the best 3-humectant-based composite, HA-HG base with 6.50% G solution outperformed the other humectants. However, its consistency was unfeasible since it was extremely oily. Therefore, 5.00% glycerol composite, was included in the later experiment instead.

Through application on pig skin, the results from each composite were relatively similar. After 24 h, every formulation left the pig skin completely dried up. However, only the pig skin sample with HA-HG base with 5.00% glycerol composite was still fully hydrated and moist, which indicated excellent water-retaining capability. Additional experiments on human skin also suggested positive results with the 5.00% glycerol composite. It can be concluded that the humectant formulation with 1.50% HA, 2.00% HG and 5.00% glycerol can be further studied and potentially be used in various applications such as cosmetics and dermatology.

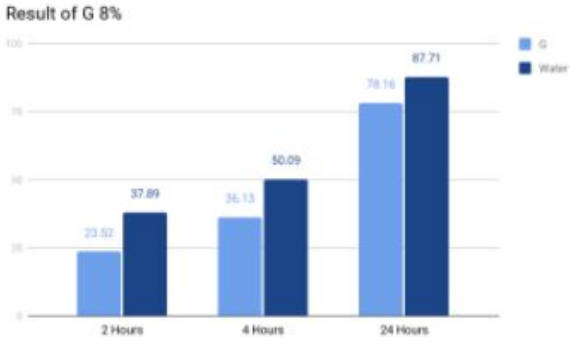
References

1. Verdier-Sévrain S.; Bonté F. Skin hydration: a review on its molecular mechanisms. J *Cosmet Dermatol.* **2007**, *6*, 75-82.
2. Ginsburg, I. H. The Psychosocial Impact of Skin Disease. *Dermatologic Clinics.* **1996**, *14*, 473–484.
3. Purnamawati, S.; Indrastuti, Ni.; Danarti, R.; Saefudin T. The Role of Moisturizers in Addressing Various Kinds of Dermatitis. *Clinical Medicine & Research.* **2017**, *15*, 75-87.
4. Kataoka, Y.; Kitadai, N.; Hisatomi, O.; Nakashima, S. Nature of Hydrogen Bonding of Water Molecules in Aqueous Solutions of Glycerol by Attenuated Total Reflection (ATR) Infrared Spectroscopy. Appl. *Spectrosc.* **2011**, *65* (4), 436–441.
5. Krause, W. E.; Bellomo, E. G.; Colby, R. H. Rheology of Sodium Hyaluronate under Physiological Conditions. *Biomacromolecules* **2001**, *2*, 65–69.
6. Swann, D. A.; Radin, E. L.; Nazimiec, M.; Weisser, P. A.; Curran, N.; Lewinnek, G. Role of Hyaluronic Acid in Joint Lubrication. *Annals of the Rheumatic Diseases.* **1974**, *33*, 318–326.
7. Tosun, H. B.; Gümüştaş, S. A.; Kom, M.; Uludağ, A.; Serbest, S.; Eröksüz, Y. The Effect of Sodium Hyaluronate plus Sodium Chondroitin Sulfate Solution on Peritendinous Adhesion and Tendon Healing: An Experimental Study. *Balkan Med. J.* **2016**, *33* (3), 258–266.
8. Donkin, S. S. Glycerol from Biodiesel Production: The New Corn for Dairy Cattle. *Revista Brasileira de Zootecnia.* **2008**, *37*, 280–286.
9. Abrahamse, E.; Minekus, M.; van Aken, G. A.; van de Heijning, B.; Knol, J.; Bartke, N.; Oozeer, R.; van der Beek, E. M.; Ludwig, T. Development of the Digestive System-Experimental Challenges and Approaches of Infant Lipid Digestion. *Food Digestion.* **2012**, *3*, 63–77.
10. Shi, R.; Liu, Q.; Ding, T.; Han, Y.; Zhang, L.; Chen, D.; Tian, W. Ageing of Soft Thermoplastic Starch with High Glycerol Content. *Journal of Applied Polymer Science.* **2007**, *103*, 574–586.
11. Anderson, J. H., jr.; Wickersheim, K. A. Near Infrared Characterization of Water and Hydroxyl Groups on Silica Surfaces. *Surface Science.* **1964**, *2*, 252–260.
12. Catanzaro, J. M.; Smith, J. G., Jr. Propylene Glycol Dermatitis. *Journal of the American Academy of Dermatology.* **1991**, 24, 90–95.
13. Ooi, B. G.; Dutta, D.; Kazipeta, K.; Chong, N. S. Influence of the E-Cigarette Emission Profile by the Ratio of Glycerol to Propylene Glycol in E-Liquid Composition. *ACS Omega.* **2019**, *4*, 13338–13348.
14. Cornelia I. C. The formulation and evaluation of urea containing products. *Potchefstroom*. **2003**, 12-13.
15. Peppas, N. A.; Slaughter, B. V.; Kanzelberger, M. A. Hydrogels. In *Polymer Science: A Comprehensive Reference*; Matyjaszewski, K., Möller, M., Eds.; Elsevier, **2012**, 385–395.
16. Bahram, M.; Mohseni, N.; Moghtader, M. An Introduction to Hydrogels and Some Recent Applications. In *Emerging Concepts in Analysis and Applications of Hydrogels*; Majee, S. B., Ed.; InTech: London, England, 2016.
17. Bhatnagar, A.; Kumar, R.; Singh, V. P.; Pandey, D. S. Hydrogels:A Boon for Increasing Agricultural Productivity in Water-Stressed Environment. *Curr. Sci.* **2016**, *111* (11), 1773.
18. Ma, T. Y.; Hollander, D.; Krugliak, P.; Katz, K. PEG 400, a Hydrophilic Molecular Probe for Measuring Intestinal Permeability. *Gastroenterology* **1990**, *98* (1), 39–46.
19. Frederic, M.; Judi, N. Fundamentals of Anatomy & Physiology. *Medicine and Health.* **2009**, *8*, 158.

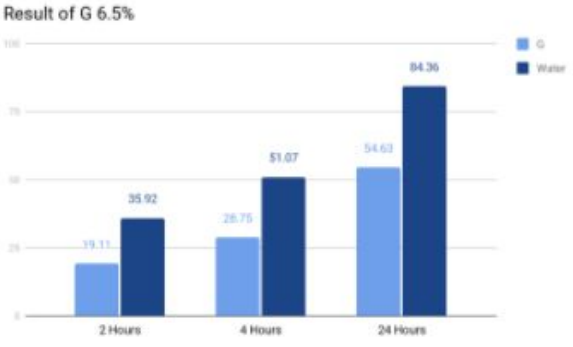
20. McGrath, J.A.; Eady, R.A.; Pope, F.M. (2004). Rook's Textbook of Dermatology. *Medicine, Nursing and dentistry.* **2004**, *7*, 3.1-3.6.
21. Swindle, M. M.; Makin, A.; Herron, A. J.; Clubb, F. J., Jr; Frazier, K. S. Swine as Models in Biomedical Research and Toxicology Testing. *Vet. Pathol.* **2012**, *49*, 344–356.
22. Ng, K. W.; Lau, W. M. Skin Deep: The Basics of Human Skin Structure and Drug Penetration. In *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement*; Springer Berlin Heidelberg: Berlin, Heidelberg, **2015**, 3–11.
23. Moon, S. Y.; Kim, D. B.; Gweon, B.; Choe, W.; Song, H. P.; Jo, C. Feasibility Study of the Sterilization of Pork and Human Skin Surfaces by Atmospheric Pressure Plasmas. *Thin Solid Films.* **2009**, *517*, 4272–4275.
24. Summerfield, A., Meurens, F. and Ricklin, M.E. 'The immunology of the porcine skin and its value as a model for human skin', Molecular Immunology, **2015**, *66*(1), pp. 14-21.
25. Becker, L. C.; Bergfeld, W. F.; Belsito, D. V.; Klaassen, C. D.; Marks, J. G., Jr; Shank, R. C.; Slaga, T. J.; Snyder, P. W.; Cosmetic Ingredient Review Expert Panel; Andersen, F. A. Final Report of the Safety Assessment of Hyaluronic Acid, Potassium Hyaluronate, and Sodium Hyaluronate. *International Journal of Toxicology.* **2009**, *28*, 5–67.
26. Escabi, R. S.; DeKay, H. G. New Developments in Calamine Lotion. *Journal of the American Pharmacists Association.* **1956**, *17*, 30–49.
27. Shah, S. N. H.; Tahir, M. A.; Safdar, A.; Riaz, R.; Shahzad, Y.; Rabbani, M.; Karim, S.; Murtaza, G. Effect of Permeation Enhancers on the Release Behavior and Permeation Kinetics of Novel Tramadol Lotions. *Tropical Journal of Pharmaceutical Research.* **2013**, *12*, 27–32.
28. Celleno, L. Topical Urea in Skincare: A Review. *Dermatologic and Therapy.* 2018, 31.
29. Brisaert, M.; Plaizier-Vercammen, J. A. Investigation on the Photostability of Tretinoin in Creams. *Int. J. Pharm.* **2007**, *334*, 56–61.
30. Kong, R.; Bhargava, R. Characterization of Porcine Skin as a Model for Human Skin Studies Using Infrared Spectroscopic Imaging. *Analyst.* **2011**, *136*, 2359–2366.

Appendix A

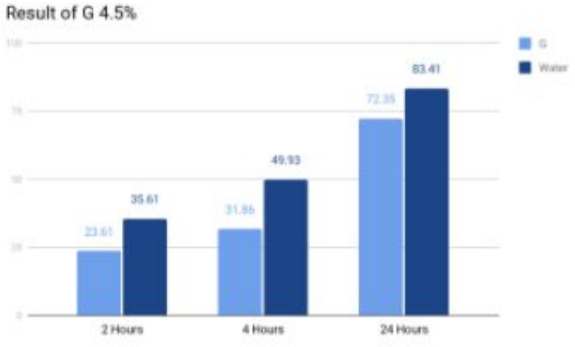
Appendix A.1 : Results for Glycerol



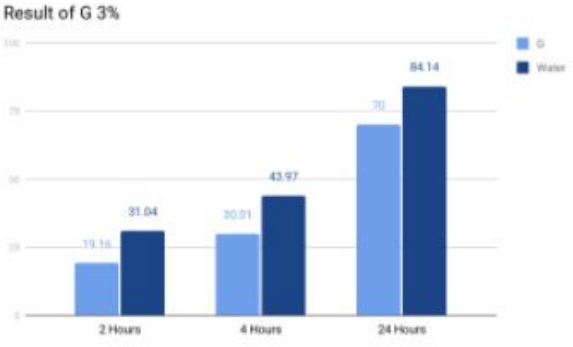
Graph: Result of Glycerol 8.00% vs water



Graph: Result of Glycerol 6.50% vs water

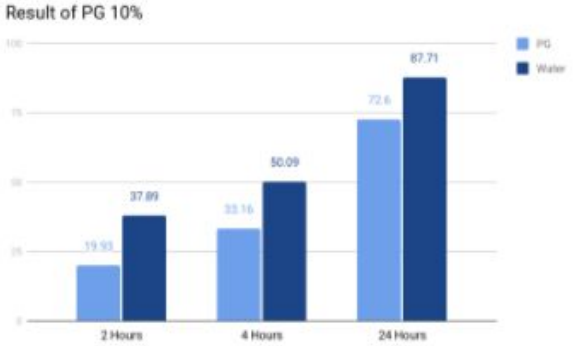


Graph: Result of Glycerol 4.50% vs water

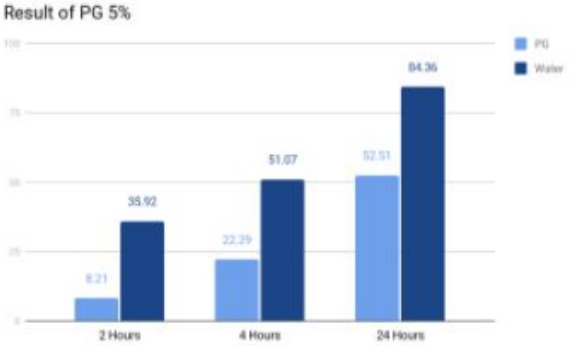


Graph: Result of Glycerol 3.00% vs water

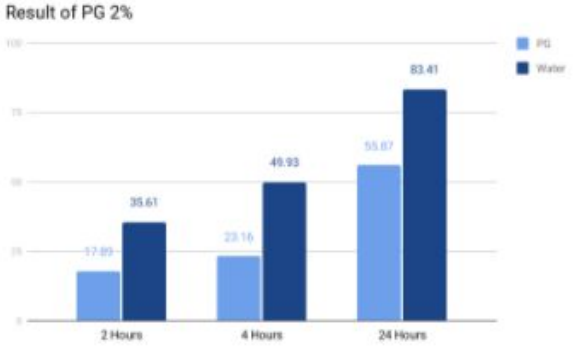
Appendix A.2 : Results for Propylene glycol



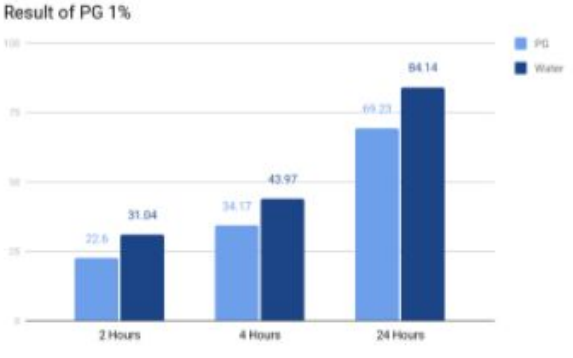
Graph: Result of Propylene glycol 10.00% vs water



Graph: Result of Propylene glycol 5.00% vs water

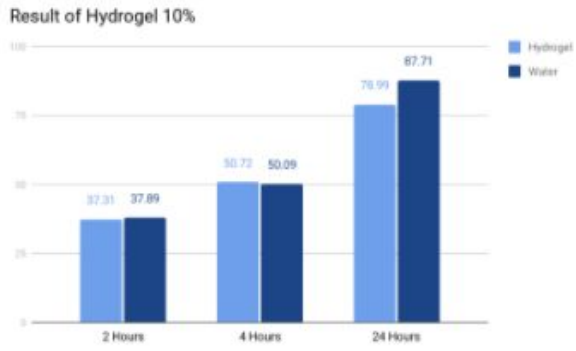


Graph: Result of Propylene glycol 2.00% vs water

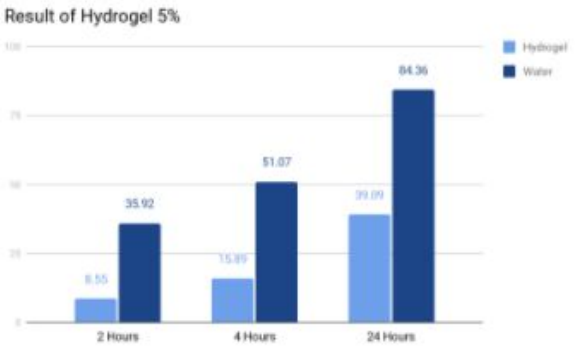


Graph: Result of Propylene glycol 1.00% vs water

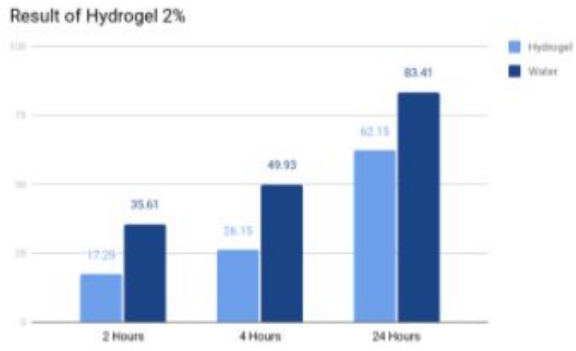
Appendix A.3 : Results for Hydrogel



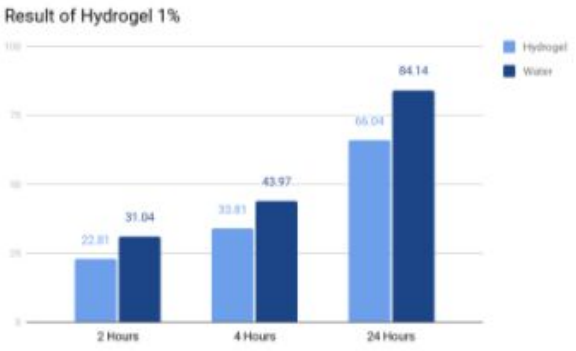
Graph: Result of Hydrogel 10.00% vs water



Graph: Result of Hydrogel 5.00% vs water

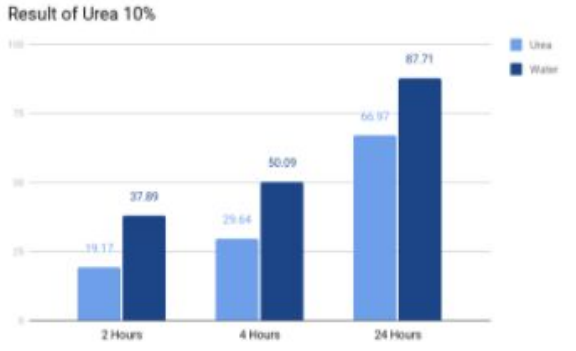


Graph: Result of Hydrogel 2.00% vs water

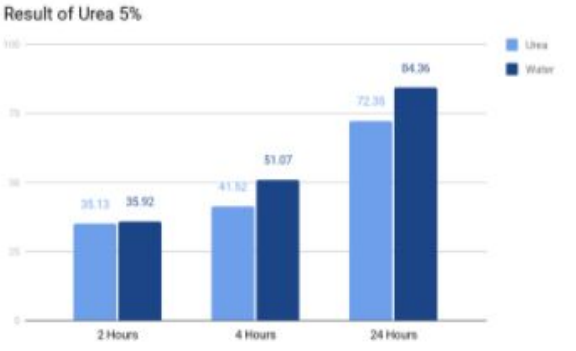


Graph: Result of Hydrogel 1.00% vs water

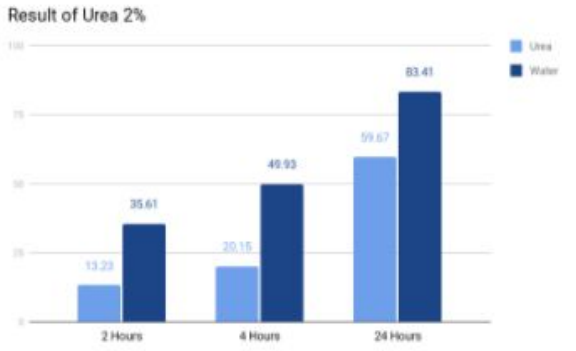
Appendix A.4 : Results for Urea



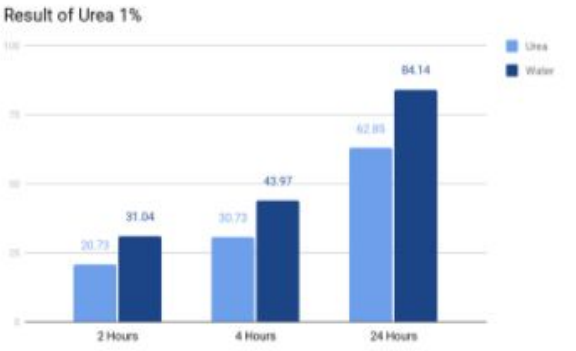
Graph: Result of Urea 10.00% vs water



Graph: Result of Ureal 5.00% vs water

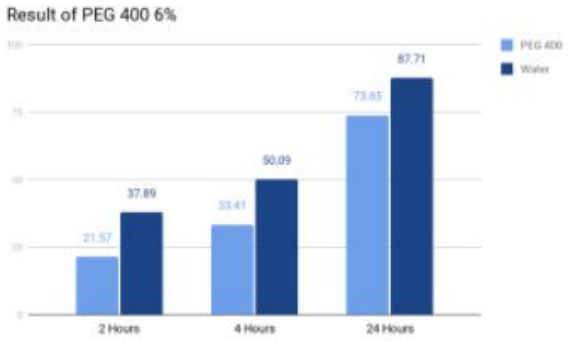


Graph: Result of Urea 2.00% vs water

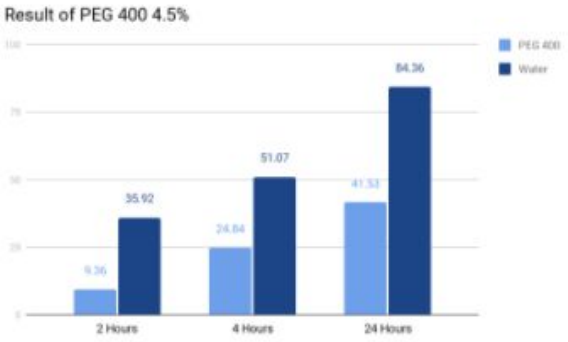


Graph: Result of Urea 1.00% vs water

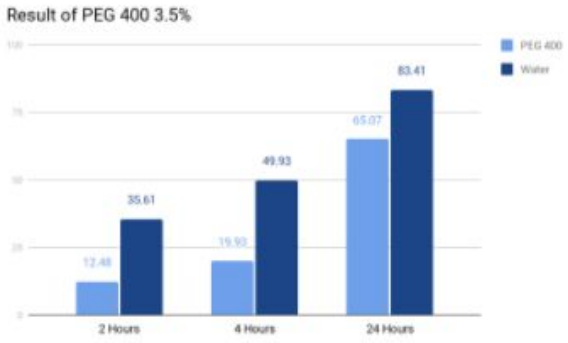
Appendix A.5 : Results for PEG-400



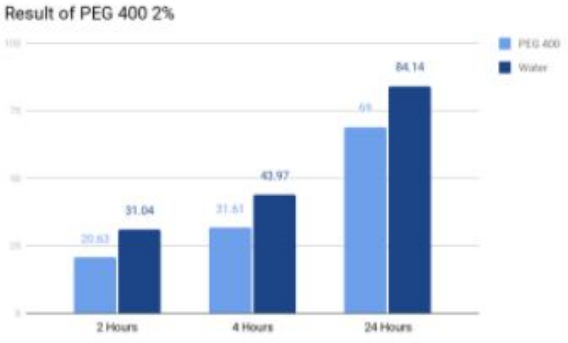
Graph: Result of PEG- 400 6.00% vs water



Graph: Result of PEG-400 4.50% vs water



Graph: Result of PEG- 400 3.50% vs water



Graph: Result of PEG-400 2.00% vs water

Biography

Mister Nattapon Chavananon was born on the 13th of January, 1999 in Bangkok, Thailand. He graduated from Assumption College class of 2017 and continued studying bachelor degree at Chulalongkorn University, Faculty of Science, Bachelor of Science in Applied Chemistry (BSAC), Bangkok, Thailand. The current address is 346/591 Moo Baan Suanthon, Soi Ratchadapisek 36, Chatuchak, Bangkok, Thailand 10900

Contact information : nattaponbsac@gmail.com

Mister Nattapat Vivattansan was born on the 30th of November, 1998 in Bangkok, Thailand. He graduated from Thai-Chinese International School class of 2017 and continued studying bachelor degree at Chulalongkorn University, Faculty of Science, Bachelor of Science in Applied Chemistry (BSAC), Bangkok, Thailand. The current address is 15 Soi Bangna-trad 13, Bangna, Bangna, Bangkok, Thailand 10260

Contact information : nattapatbsac@gmail.com