VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY
UNIVERSITY OF SCIENCE AND TECHNOLOGY OF HANOI

FINAL TECHNICAL REPORT
USTH-20 PROGRAM

TITLE:

In English: Natural cosmetic mask in the form of gel beads with Spirulina Platensis as the main

In Vietnamese: Sản xuất mặt nạ viên dạng gel từ Spirulina Platensis

Group leader: Phạm Hải Nam

Class: B3

Major: Pharmacological, Medical and Agronomical Biotechnology

Hanoi, December 15th, 2019

UNIVERSITY OF SCIENCE AND TECHNOLOGY OF HANOI
Direction of Research, Innovation and Technology Transfer

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I. General information

Department	Pharmacological, Medical and Agronomical Biotechnology
Title	In English: Natural cosmetic mask in the form of gel beads with Spirulina platensis as the main. In Vietnamese: Sản xuất mặt nạ viên dạng gel từ Spirulina Platensis.
Project code	USTH20/PB.01/2019
Implementation period	From April 2019 To December 2019
Group leader	Phạm Hải Nam
Approved budget	20.000.000 VND

II. Research outcomes

2.1 A brief description on the approved objectives

(Please briefly describe in this section the main research objectives/ work tasks that have been described in details in your accepted proposal. The description is for purpose to help the evaluation committee quickly appreciates whether the proposed tasks have been done during the project implementation)

The high protein level of various microalgal species is one of the main reasons to consider them an unconventional source of these compounds. *Spirulina platensis* stands out for being one of the richest protein sources of microbial origin (460–630 g kg−1, dry matter basis), having similar protein levels when compared to meat and soybeans. When it was dried, valuation of Spirulina platensis has 5% water, 24% carbohydrates, 8% fat, and about 60% (51–71%) protein. (Campanella, Russo, & Avino, 2002). The use of *S. platensis* in food can bring benefits to human health owing to its chemical composition, since it has high levels of vitamins, minerals, phenolics, essential fatty acids, amino acids and pigments and a vast spectrum of antioxidants. Furthemore, using this alga as a natural cosmetic ingredient is much more attracted in home-made cosmetic suplier in today market because of the above-interesting composition.

This proposal is to investigate in outstanding commercial products derived from *Spirulina platensis* with the main representative which is natural cosmetics products. Also, the process of developing the best formula to enhance the performance of vitamin, protein and pigment in spirulina products is also in need, especially when we are considering preserving in the most natural way.

Table A: Composition in Nutritional value of S. platensis dried per 100 g (3.5 oz)*
(%DV: Percentages are roughly approximated using US recommendations for adults.
1 Oz = 28.35g

Energy	1,213 kJ (290 kcal)
Carbohydrates	23.9 g
Sugars	3.1 g
Dietary fiber	3.6 g
Fat	7.72 g
Saturated	2.65 g
Monounsaturated	0.675 g
Polyunsaturated	2.08 g
Protein	57.47 g
Tryptophan	0.929 g
Threonine	2.97 g
Isoleucine	3.209 g
Leucine	4.947 g
Lysine	3.025 g
Methionine	1.149 g
Cystine	0.662 g
Phenylalanine	2.777 g
Tyrosine	2.584 g
Valine	3.512 g
Arginine	4.147 g
Histidine	1.085 g
Alanine	4.515 g
Aspartic acid	5.793 g
Glutamic acid	8.386 g
Glycine	3.099 g
Proline	2.382 g
Serine	2.998 g

Vitamins	**Quantity**	**%DV**
Vitamin A equiv. beta-Carotene lutein zeaxanthin	29 µg 342 µg 0 µg	4% 3%
Thiamine (B1)	2.38 mg	207%
Riboflavin (B2)	3.67 mg	306%
Niacin (B3)	12.82 mg	85%
Pantothenic acid (B5)	3.48 mg	70%
Vitamin B6	0.364 mg	28%
Folate (B9)	94 µg	24%
Vitamin B12	0 µg	0%
Choline	66 mg	13%
Vitamin C	10.1 mg	12%
Vitamin D	0 IU	0%
Vitamin E	5 mg	33%
Vitamin K	25.5 µg	24%
Minerals	**Quantity**	**%DV**
Calcium	120 mg	12%
Iron	28.5 mg	19%
Magnesium	195 mg	55%
Manganese	1.9 mg	90%
Phosphorus	118 mg	17%
Potassium	1363 mg	29%
Sodium	1048 mg	70%
Zinc	2 mg	21%
Others	**Quantity**	
Water	4.68 g	

The materials of interest in this brief review are primarily hydrogels, which are polymer networks extensively swollen with water. Hydrophilic gels that are usually referred to as hydrogels are networks of polymer chains that are sometimes found as colloidal gels in which water is the dispersion medium. (Enas M. Ahmed, 2013)

Researchers, over the years, have defined hydrogels in many different ways. The most common of these is that hydrogel is a water-swollen, and cross-linked polymeric network produced by the simple reaction of one or more monomers. Another definition is that it is a polymeric material that exhibits the ability to swell and retain a significant fraction of water within its structure, but will not dissolve in water. (F.L. Buchholz, 1998) (L. Brannon-Peppas, 1991) (Yuhui Li, 2013). They possess also a degree of flexibility very similar to natural tissue due to their large water content.

Alginate is a hydrophilic and linear polysaccharide composed of (1–4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers, which are derived primarily from brown seaweed and bacteria. Simple gelation can be formed when divalent cations such as Ca^{2+}, Mg^{2+}, Ba^{2+}, or Sr^{2+} cooperatively interact with blocks of G monomers to form ionic bridges.

Alginate has been used in a variety of medical applications including cell encapsulation, tissue engineering and drug delivery, because it gels under gentle conditions, has low toxicity, and is readily available. Despite its advantageous features, alginate may not be an ideal candidate for tissue engineering because it does not specifically degrade. Ionically cross-linked alginate hydrogel degrades *via* an ion exchange process involving loss of divalent ions into the surrounding medium, and undergoes an uncontrolled dissolution. Alginate has been covalently coupled with lectin and RGD to enhance cell ligand-specific binding properties due to lack of cellular interaction in its molecular structure for tissue engineering applications. (Tan, 2010)

Hyaluronic acid (HA) is a naturally occurring non-sulfated glycosaminoglycan that is widely distributed throughout the ECM of all connective tissues in human and other animals. Hyaluronic acid plays an essential role in many biological processes such as tissue hydration, nutrient diffusion, proteoglycan organization, and cell differentiation. HA is especially prevalent during wound healing and in the synovial fluid of joints. Hyaluronic acid is a GAG consisting of multiple repeating disaccharide units of N-acetyl-D-glucosamine and D-glucuronic acid. Hyaluronic acid is naturally degraded by hyaluronidase, which is ubiquitous in cells and in serum. Due to its good biocompatibility, biodegradability, as well as excellent gel-forming properties, HA and its derivatives have been widely explored as hydrogels for tissue engineering. Hyaluronic acid hydrogels can be formed by covalent crosslinking with hydrazide derivatives, esterification, and annealing. Additionally, hyaluronic acid has been combined with both collagen and alginate to form composite hydrogels. (Tan, 2010)

Peel-off mask is one of the most popular forms of cosmetic skincare product nowadays thanks to its various benefits. Peel off masks adhere to the top layer of dead skin and the dirt in clogged pores. As it is peeled off after it dries, it lifts off all the micro particles of dust and dirt, which effectively cleans the skin. Moreover, with visible reduction of pore size and evidently firmer skin, a post peel off will leave the skin looking younger as it appears brighter and more taut. Peel off masks also absorb excess oil from the skin while they unclog and purify the pores, giving a natural matte and clarified complexion.

Put in consideration such benefits, we firmly believe that peel off mask, alongside with hydrogel mask, would be suited for the application of *Spirulina platensis* into cosmetic product to develop a mask formula which focuses on moisturization for the skin. In this research, we will assess different characteristics of peel off mask to determine its appropriation for our objectives.

Objectives: This study investigates in introducing bioactive spirulina platensis to cosmetic products in an organic way with some main research objectives:

1. Optimization of preparation method for crude S. platensis for nutritional preserving and phycocyanin standardization in raw material.
2. Formulation of Alginate hydrogel skincare mask.
3. Evaluation of permeability of phycocyanin as well as other vitamins in hydrogel mask.

Main work tasks and protocols:

1. Phycocyanin extraction and quantification.

In phycocyanin quantification experiment, Spirulina powder 40 mg was added into a 10-ml. centrifuge tube with 10 ml. of the 100 mM phosphate buffer (100-mM Phosphate buffer contains 10.64 g. K_2HPO_4 and 5.29g. KH_2PO_4 per liter, pH 7.), vortex to mix well. Store the tube in refrigerator overnight. After 24 hour, vortexing the tub to mix well and then centrifuge 5 minutes at 10°C at 3500 RPM. Read the absorbency of each replicate at 620 nm, using phosphate buffer as blank.

2. Mask formula building:

The formula building process was divided into 2 main phase based on the development of experiments throughout the whole project. Phase 1 Formula is derived from article research and suggestions from our supervisors with the existing statistics to build up caffein gel (a gel with the same desired texture). The phase's purpose was to test the appropriate concentration of spirulina platensis to be added in the final formula, also test the combination of difference mask carrier component: PVA; HPMC; CMC; Gelatin. Phase 2 formula is hydrogel mask building base on researched paper

Phase 1: August-October/2019

A. Spirulina concentration test: The following formula is built up to test the suitable concentration of spirulina platensis in mask formula. Mask ingredients includes PVA, CMC, Fragrance, Alcohol, Leucidal, Hyarulonic acid as listed below, with the same concentration amongst 4 samples and their controls. Spirulina platensis concentration are varied throughout 4 samples with concentration: 0,83%;3,33%;5,33% and 8,3%.

Table A: Formula for varied spirulina concentrations

Ingredients	**Sample**							
	F1	F2	F3	F4	F1(-)	F2(-)	F3(-)	F4(-)
PVA	3	3	3	3	3	3	3	3
CMC	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fragrance	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Alcohol	2	2	2	2	2	2	2	2
Leucidal	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Hyarulonicacid	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Spirulina	0.2	1	1.5	2	-	-	-	-
Aquadest ad	20	20	20	20	20	20	20	20

B. Peel-off mask formulation test: After testing the concentration of spirulina, we test different types of peel off mask carriers: PVA, CMC, HPMC, Gelatin and their combinations to compare the mask textures. Three samples respectively: F1: CMC+HPMC; F2: CMC+Gelatin; F3: PVA+CMC. The other ingredients are remained the same within 3 samples.

F1(-);F2(-); F3(-) are the control without spirulina.

Table B: Formula for peel-off mask test: F1: F1: CMC+HPMC; F2: CMC+Gelatin; F3: PVA+CMC

Ingredients	**Sample**					
	F1	**F2**	**F3**	**F1(-)**	**F2(-)**	**F3(-)**
PVA (g)	-	-	2.5	-	-	2.5
CMC	0.5	0.5	0.5	0.5	0.5	0.5
HPMC (g)	0.4	-	-	0.4	-	-
Gelatin (g)	-	6	-	-	6	-
Glycerin	1	1	1	1	1	1
Leucidal(ml)	0.2	0.2	0.2	0.2	0.2	0.2
Alcohol (g)	2.5	2.5	2.5	2.5	2.5	2.5
Fragrance(ml)	0.1	0.1	0.1	0.1	0.1	0.1
Hyarulonic acid (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Aquadest ad	20	20	20	20	20	20
Spirulina (g)	1.5	1.5	1.5	-	-	-

CMC solution was prepared by carboxymethyl cellulose to water and stirred until complete solubilization.

A homogeneous solution of PVA was obtained by dissolving distilled water at 95 C in 30 minutes and after that use homogenizer to stir vigorously.

Next, CMC and PVA solutions were mixed. Spirulina and preservatives were dissolved in glycerol before being added to the PVA and HPMC solution. Leucidal were poured into ethylene alcohol then added to the gel base and homogenized.

Phase 2: October- November/2019

C. Optimize Formula:

Table C: Optimized formula with other components: F1: PVA+CMC+PEG; F2: PVA+Gelatin; F2: PVA+Gelatin (with increased amount of PVA in compare to F2); F3: Sodium alginate + Xanthan Gum and Guar Gum.*

Ingredients	F1	F2	F2*	F3	F1 (-)	F2 (-)	F2*(-)	F3 (-)
Hydrolyzed PVA	1g	8 g	10g	-	1g	8 g	10g	-
CMC	0.1g	-	-	-	0.1g	-	-	-
PEG	0.67 ml	-	-	-	0.67 ml	-	-	-
Powder Gelatin		2 g	2g			2 g	2g	
Sodium alginate	-	-	-	1.2g	-	-	-	1.2g
Xanthan Gum	-	-	-	0.15 g	-	-	-	0.15 g
Guar Gum	-	-	-	0.15 g	-	-	-	0.15 g
Glycerin	1.5g	2g	2g	1.5g	1.5g	2g	2g	1.5g
Ethanol	3g	-	-	0.75g	3g	-	-	0.75g
Fragrance	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Leucidal	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml	0.2ml
Al_2O_3	0.05g	0.05g	0.05g	0.05	0.05g	0.05g	0.05g	0.05
Sodium hyaluronic	0.1 ml	0.1ml	0.1ml	0.1ml	0.1 ml	0.1ml	0.1ml	0.1ml
Spirulina	1g	1g	1g	1g	-	-	-	-
H2O	Ad 20g	Ad 20g	Ad 20g	Ad 20g	Ad 20g	Ad 20g	Ad 20g	Ad 20g

FORMULA 1 (PVA+CMC+PEG)

CMC solution was prepared by through adding **100 mg** sodium carboxymethyl cellulose to **5 mL DI water** and stirred until complete solubilization.

A homogeneous solution of PVA was obtained by dissolving **1 g of PVA in 5 mL** distilled water at 95 C in 30 minutes under vigorous stirring.

Next, CMC and PVA solutions were mixed at a volume ratio of **1:1**. Then, the **0.75 g of PEG powder** was added into the mixed solution with stirring until the solution being transparent.

(we have to change 0.75 gram powder into equal liquid PEG we have)

After **placed at room temperature (RT) for 20 min**, the solution that became turbid was poured into the mask templates. Finally, the templates were placed in the 20 C refrigerator for 20 h.

Spirulina and preservatives were dissolved in glycerol before being added to the PVA and HPMC solution. Leucidal were poured into ethylene alcohol then added to the hydrogel base and homogenized.

FORMULA 2:

Hydrolyzed PVA mixed with gelatin power in a water bath at 60C and mix until the mixture is homogenous.

Spirulina and preservatives were dissolved in glycerol before being poured into ethylene alcohol then added to the PVA gelatin solution and homogenized. The mixture was then stirred until homogenous.

FORMULA 3

Sodium alginate was dispersed into distilled water and homogenized for 10 minutes.

Xanthan gum, Guar gum were then added to glycerin, and a mixture of xanthan gum-guar gum-glycerin Spirulina and preservatives were dissolved in glycerol mixture above then added ethylene alcohol

The mixture was added to the hydrogel base and homogenized until a semisolid gel formed. The semisolid gel was then poured into a glass mold and dipped into a 2% calcium chloride mixture for 60 minutes.

3. Functional testing and optimization.

In this experiments, we use the techniques for testing the products, such as dissolution testing and physical testing. Dissolution testing, which is applied for the ready-to -be mask, is about to test the realease. In physical testing, we use the observation of the appearance of gel by eyes and the homogenization under microscope, oraganolepic (check visually the color, the consistency and the smell), homogenecity (visually), pH (1% solution was prepared and check pH), physical stability at room temperature, irritation test (test on skin to check for allergy) and mask shape formation ability.

4. Evaluation and report writing

2.2 A recall of the project implementation timeline

(Please copy in this section the proposed implementation timeline as it is in the accepted proposal and check which tasks have been completed/ on-going/ will be implemented (if suitable)).

No.	Implementation contents	Expected time
1	Research	07/18 – 09/18
2	Experimenting	09/19 – 10/19
3	Testing	10/20 – 11/20
4	Product improving and finishing (Report writing)	11/21 – 12/15

2.3 Technical results

(Please describe in this sections the details of technical results that have been achieved during the first year of project implementation with highlight in the main/ significant/ or promising results that make important contribution (or potentially have important contribution) to the community and that are deserve for further investigation or communication (or publication). Illustration of achieved results by figures/ draws/ tables is appreciated. Comparison between the achieved results with those in the current state of the art is important and appreciated.).

Apparatus dissolution result:

In order to evaluate the permeability of Phycocyanin through dialysis membrane which can provide preliminary information to predict the efficiency of mask, we realize that the dissolution test demonstrate the best about the release of Phycocyanin throught the dialysis membrane.

Firstly, in organisation of apparatus experiment, prepare Phosphate Buffer monobasic 0.1M, pH 5.6 - 5.8 (effect : stimulation skin's environment). Preparing vessels which contain distill water about 500 ml per 1 vessel, label and increase the temperature up to 32oC . Add 3 gram sample in dialysis membrane, put into the vessels and stir. Secondly, collect the dissolution solution in each vessel after 10, 20, 30, 40, 50 and 60 minutes and measure the absorbance of each solution by UV- Vis measurement (wavelength of Phycocyanin = 620 nm). Note: The negative control was replace by the sample with all the substance set in the table.

1.1 Phase 1 :

Table SA: Spirulina concentration test: using dissolution apparatus in 60 min in OD

Time (min)	10	20	30	40	50	60
Control	0,014	0,002	0,002	0,003	0,003	0,004
Sample 1 (0,83%)	0,012	0,015	0,02	0,02	0,025	0,028
Sample 2 (3.33%)	0,01	0,012	0,015	0,02	0,025	0,026
sample 3 (5.33%)	0,013	0,016	0,018	0,02	0,03	0,032
sample 4 (8,3%)	0,014	0,023	0,032	0,04	0,05	0,052

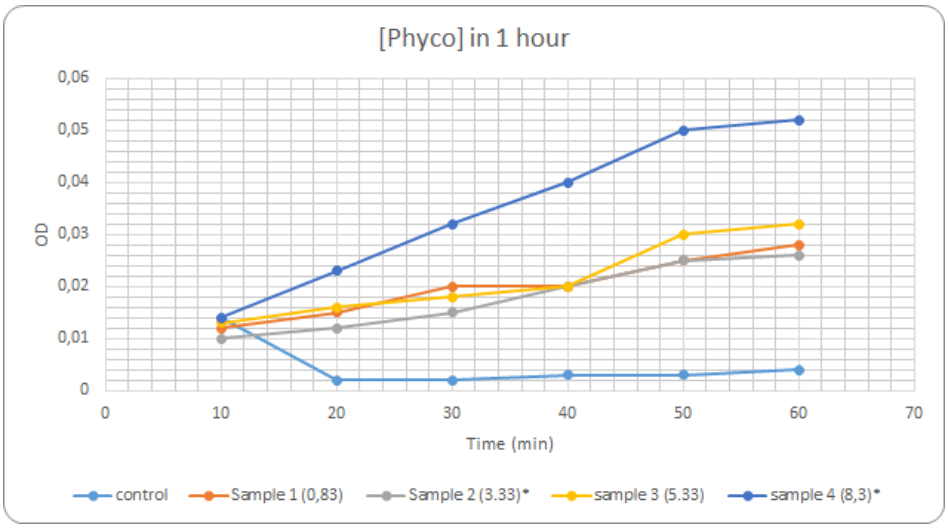


Figure 1 (Table A): Spirulina concentration test: using dissolution apparatus in 60 min, measured at 6 time points (10 min; 20 min; 30 min ; 40 min ; 50 min and 60 min), at wavelength: 620 nm. Control: sample without spirulina; sample 1: 0,83% spirulina; sample 2: 3,33% spirulina; sample 3: 5,33% spirulina; sample 4: 8,3% spirulina (Number of trials: 2).

Table SB: Optimization mask formulation test by dissolution apparatus in OD

Time (min)	10	20	30	40	50	60
Sample 3 (PVA)	0,014	0,025	0,03	0,035	0,046	0,051
Sample 1 (HPMC)	0,007	0,009	0,013	0,025	0,037	0,04
Sample 2 (Gelatin)	0,001	0,016	0,019	0,026	0,03	0,037

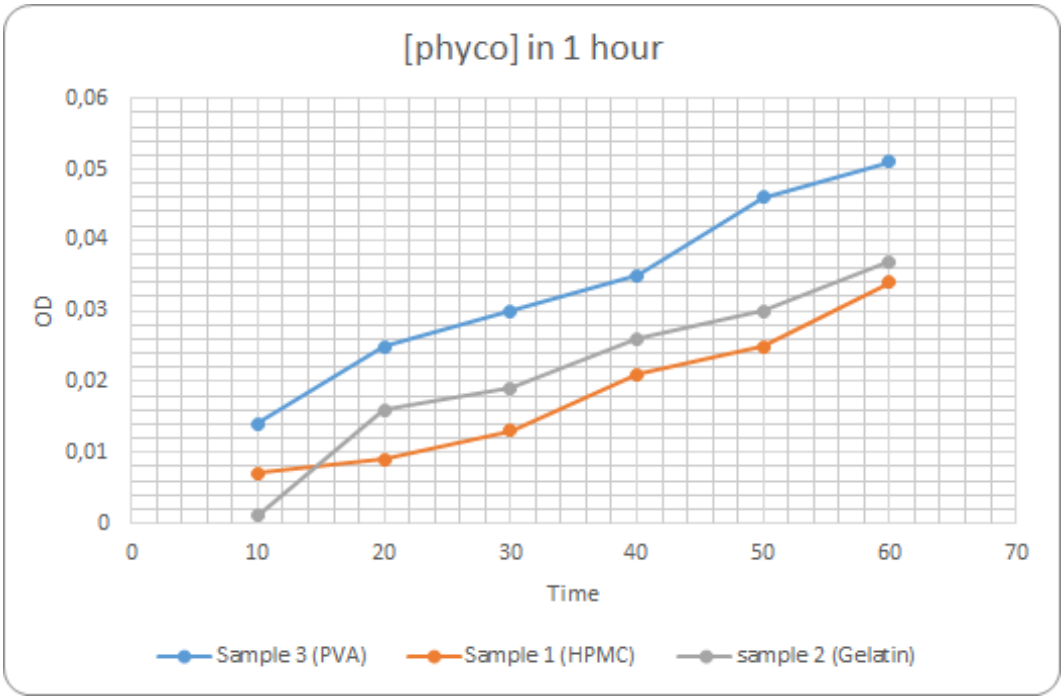
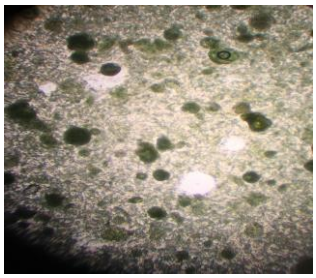
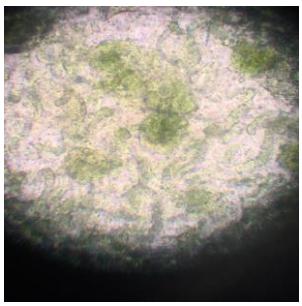
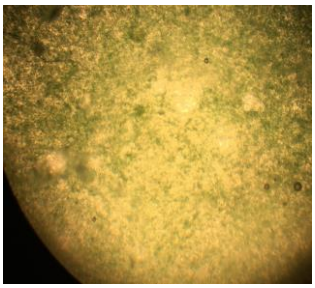
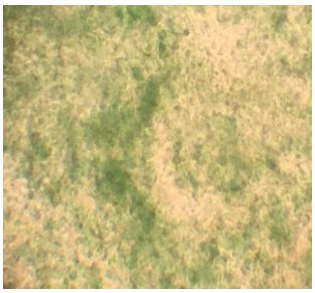
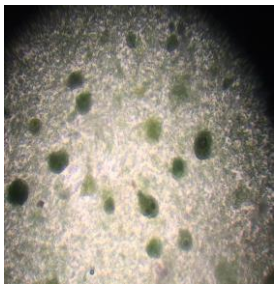
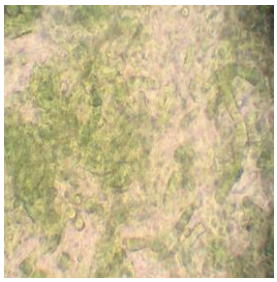


Figure 2 (Table B): Peel-off mask formulation test by dissolution apparatus: 3 samples, which used 3 different polymers (such as Poly-vinyl Alcohol, Hydroxy Propyl Methyl Cellulose, and Gelatin), were measured in 620 nm at 1 hour (10 min, 20 min, 30 min, 40 min, 50 min and 60 min) (Number of trials: 2).

Mask Testing and Optimization:

Table Mask testing and Optimization 1 : Observation of 3 samples (Poly-vinyl Alcohol, Hydroxy Propyl Methyl Cellulose, and Gelatin) in 7 parameters, such as organoleptic, homogeneity, pH, physical stability at room temperature, irritation test, peeling time, and observation the homogenize of the gel under microscope.

No	Parameter	Observation		
		Sample 1	Sample 2	Sample 3
1	Organoleptic	Consistency Semiliquid Coleur: green Odor: citric	Consistency Semiliquid Coleur: green Odor: citric	Consistency Semiliquid Coleur: green Odor: citric
2	Homogeneity	Inhomogeneity	Normal homogeneity	Inhomogeneity
3	pH	7.4	7.5	7.4
4	Physical stability at room temperature	Stable	Stable	Stable
5	Irritation test	No	No	No
6	Peeling time	cannot peel	cannot peel	9 minute 20 second
7	Observation the homogenize of the gel under microscope	Not homogenize totally Scope 10/0.25  Scope 40/0.65 	Homogenize moderatelly Scope: 10/0.25  Scope: 40./0.65 	Not homogenize totally Scope: 10/0.25  Scope 40/0.65 

1.2 Phase 2:

After forming masks following data of Table C and observing the texture of the masks, we selected sample F2* and F3 to further test spirulina release by dissolution apparatus:

Note that F2* is the upgraded version of F2 by level up the concentration of PVA for better consistency.

Table SC: Hydrogel mask dissolution apparatus test in OD

Time (min)	10	20	30	40	50	60
F2*	0,04	0,045	0,07	0,09	0,12	0,19
F2*(-)	0,035	0,039	0,064	0,072	0,088	0,096
F3	0,005	0,006	0,008	0,008	0,017	0,023
F3(-)	0	0,005	0,005	0,006	0,007	0,007

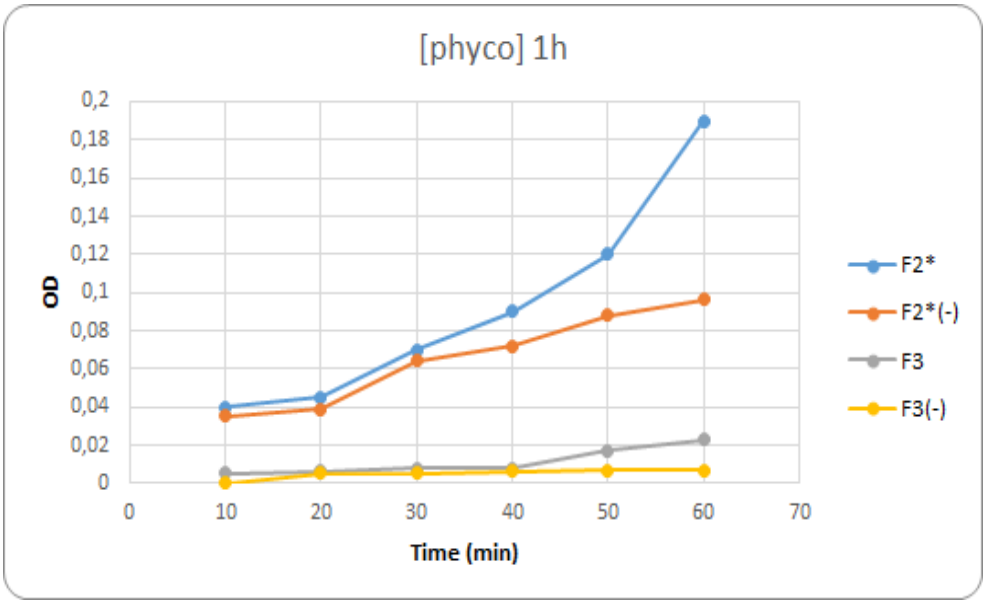
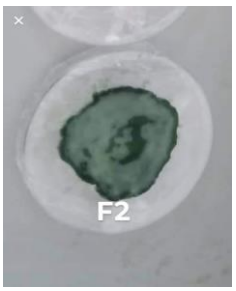


Figure 3 (Table 3): Hydrogel masks contain 4 samples (F2*, F3 and the negative control themselves) in 620 nm at the time point (10 min, 20 min, 30 min, 40 min, 50 min and 60 min). (Number of trials: 2).

Mask testing and optimization:

Table Mask testing and Optimization 2 : Observation of 3 samples F1, F2 and F3 in 6 parameters, such as organoleptic, homogeneity, pH, physical stability at room temperature, irritation test, and mask formation

No	Parameter	Observation		
		F1	F2 (PVA+gelatin)	F3 (add CaCl2) (Sodium alginate + xanthan gum + guar gum)
1	Organoleptic	Inconsistency semiliquid color: green (a little yellow) Odor: citric (far) Closer (algea unique smell)	Consistency gel color: green odor: citric	Consistency gel color: green odor: citric
2	Homogeneity	Inhomogeneity	Normal homogeneity	Homogenized
3	pH	7.4	6.5	6.31
4	Physical stability at room temperature	Stable	Stable	Stable
5	Irritation test	No	No	No
6	Mask formation	Fail to form shape	Form mask shape 	Form mask shape 

Hydrogel mask samples were stored at room and cold temperature for 7 days for physical stable and pH stability test

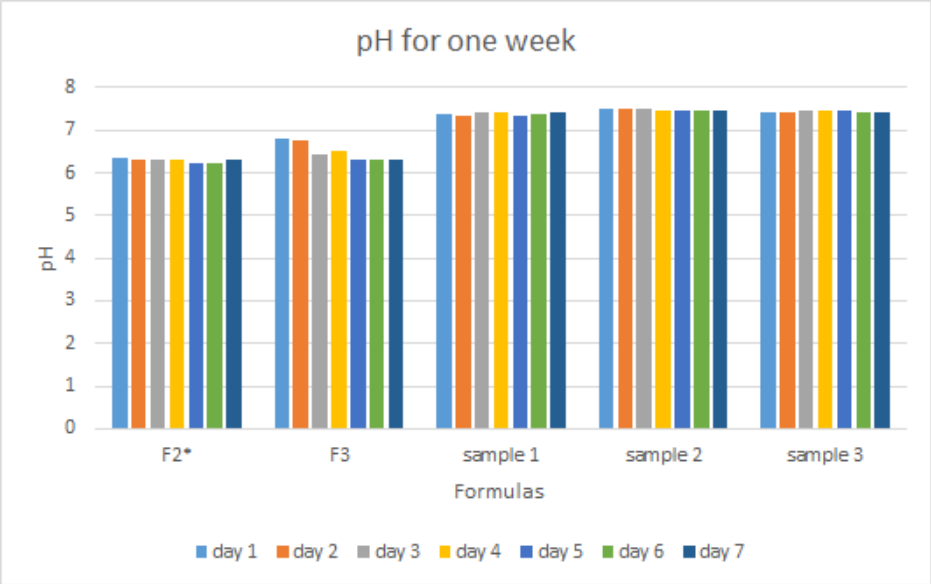


Figure 4: pH test for 1-week storage of varies sample of the hydrogel mask.

Discussion:

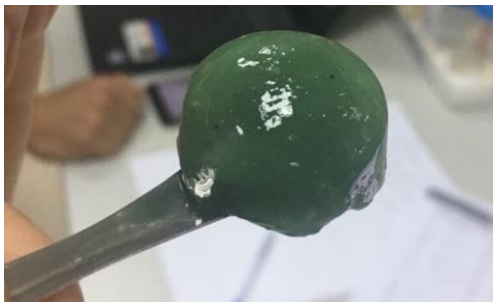
After literally research, the formula building process was divided into two-phase: peel off and hydrogel mask. In the first phase, the mask was based on the formula provided by our supervisor. The concentration of spirulina was varied to figure the optimization of phycocyanin releasing from the mask and checked the homogeneity between four samples.

Dissolution testing is used for the extent and rate of solution formation measurement from the mask sample. The test shows the samples' bioavailability and therapeutic effectiveness. Dissolution tests and drug release are terms used interchangeably. Homogeneity test used the microscope to check whether the formula is homogenous

After the dissolution test and homogeneity test, we concluded that formula with concentration 7.5 % of spirulina was the best amongst four samples. Next, we optimized the ingredients of the gel mask. Formula 1 containing HPMC was not suitable because it cannot peel easily; while formula 2 including gelatin became solid too fast. Formula 3 suits our objectives most visually. Besides, with the dissolution test, formula 3 shows the promising result of phycocyanin release.

In phase two: hydrogel mask formation, three formulas were built after researching articles. Formula 1 was not stable in normal conditions and become liquid after an amount of times. Formula 2 and Formula 3 remain their solidity, potentially suitable for dissolution testing. Moreover, Formula 2 needs to improve its homogeneity by increasing PVA amount to Formula 2*. We bring these two formulas 2* and 3 in to specify the release of phycocyanin by dissolution apparatus. The results indicate that a higher amount of phycocyanin released in formula 2* in the period of 60' test. Thus, the combination of gelatin and PVA in formula 3 shows promising formula for spirulina mask.

After the formula build- up, we did a series of experiments of physical characteristics of mask with Formula 1, 2 and 3. Firstly, the organoleptic test shows the same results for green color and citric odor for all 3 formulas. However, the consistency gel is only a trait for formula 2 and 3 whereas, formula 1 shows inconsistent semi-liquid. Also, this formula shows the lowest homogeneity. The pH remains stable in the range from 6.3 to 7.4 (figure 4), nonirritant for skin applying. All of the 3 formulas maintain their stability within 2 weeks and we observed microbe molds after 2 weeks at room temperature.



A **B**

Image A: Hydrogel mask bead product from Spirulina Platensis.
Image B: Spirulina Platensis form mask is deeply filled in $CaCl_2$.
(Image is cut from our video)

2.4 Self-assessment of the project progress

(Please describe herein what have been done and what have been not done in compared with the approved project implementation timeline. In the latter case, please provide your evidences/ explanations.
Please state how this project has supported the research development/ research capability in your group/ laboratory/ department).

Accomplished:

We have successfully extracted Phycocyanin from *Spirulina platensis and* developed a mask formula which satisfies many of our goals (structure and organic ingredients). We have designed and optimize the protocol to evaluate the permeability of phycocyanin in spirulina through the skin, using the dissolution apparatus. We have conducted several tests to check the quality of the product (dissolution test, physical test and various observations with naked eyes or with microscope).

Unfinished tasks:

Besides the accomplished task, we have not optimize the formula of the product due to lack of time, materials and equipments (the materials and equipments have not been carefully considered while doing the proposal and only realized later during the process). Some tests were not conducted or failed (antioxidant test, microbe test…). Design the formulation and process of producing S. platensis capsules in laboratories; Design the formulation and process of producing cosmetic masks in the form of water beads (because there had been a change in the form of the mask from water gel beads to normal gel mask due to lack of time and necessary equipments). Create a way of preserving the products eco-friendly (due to lack of time and miscalculation of the time of the testing so we could not have time for coming up with a way of preservation for the product).

Improvements in the future:

According to finished and unfinished tasks, we need to complete the unfinished tests like antioxidant test or microbe test, redo the failed tests or the tests with undesired results, optimize the mask formula focusing on the release of phycocyanin, and continue searching for a method to carry out the initial approach of making cosmetic masks under the form of water beads.

Hanoi, December 15th, 2019

Assessment of Department's Director *(Signature & full name)*	**Assessment of Advisor** *(Signature & full name)*	**Group leader** *(Signature & full name)*