RESEARCH ON THE APPLICATION OF NANOCELLULOSE IN SKIN CARE CREAM FORMULATION

NGHIÊN CỨU ỨNG DỤNG NANOCELLULOSE TRONG CÔNG THỨC KEM CHĂM SÓC DA

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ABSTRACT

Nanocellulose has been gaining the attention of researchers for last 10 years, especially in the application in the cosmetics industry. Nanocellulose is a highly biocompatible and environmentally friendly material with a market value of 416 million USD in 2022 and is expected to reach 1.3 billion USD in 2030. Vietnam has a diverse and abundant source of lignocellulose that can be used to produce nanocellulose on a large scale. Nanocellulose was created by Research group from the School of Chemistry and Life Sciences, Hanoi University of Science and Technology from sulfate pulp has fibril form (Cellulose Nanofibril - CNF) with the fiber diameter less than 100nm and the fiber length of microns. This study is the first time that evaluated the applicability of the above CNF in supporting the texture of the cream formulation such as emulsification, thickening and dispersion. In addition, some activities such as moisturizing, moisture lock, skin adhesion and sensory evaluation were also evaluated. The results showed that nanocellulose at a concentration of 5 - 15% had a good combination with the emulsifier ceteareth-25 at a concentration of 5 - 10% and carbomer at a concentration of 1.0 - 1.5%. The cream formulation had good oil-in-water homogenization, stable texture and are promising to be applied in cosmetic and pharmaceutical cream.

Keywords: *Nanocellulose, CNF, emulsifier, Pickering, droplet, cosmetic, cream.*

TÓM TẮT

Trong 10 năm trở lại đây, nanocellulose đang ngày càng thu được sự quan tâm của các nhà nghiên cứu, đặc biệt là ứng dụng trong ngành mỹ phẩm. Nanocellulose là một vật liệu có tính tương thích sinh học cao và thân thiện môi trường với thị trường năm 2022 đạt 416 triệu USD và dự đoán đạt 1.3 tỷ USD vào năm 2030. Việt Nam có nguồn lignocellulose đa dạng, phong phú có thể sử dụng để sản xuất nanocellulose ở quy mô lớn. Nanocellulose do nhóm nghiên cứu của Trường Hóa và Khoa học Sự sống, Đại học Bách khoa Hà Nội tạo ra từ bột giấy sulfate có dạng sợi (Cellulose Nanofibril - CNF) với đường kính xơ sợi < 100nm và chiều dài sợi cỡ micron. Nghiên cứu này đánh giá khả năng ứng dụng của CNF nêu trên trong hỗ trợ kết cấu của dạng bào chế kem như khả năng nhũ hóa, làm dày và phân tán. Ngoài ra cũng đánh giá thêm một số hoạt tính như khả năng giữ ẩm, khóa ẩm, độ bám dính và các đánh giá cảm quan. Kết quả thu được cho thấy nanocellulose ở hàm lượng 5 - 15% có khả năng kết hợp tốt với chất nhũ hóa ceteareth-25 ở hàm lượng 5 - 10% và carbomer-980 với hàm lượng 1 – 1,5%. Công thức dạng kem có độ đồng hóa dầu trong nước tốt, kết cấu ổn định, kéo dài thời gian bảo quản, hứa hẹn có thể ứng dụng trong các loại mỹ phẩm và thuốc dạng kem.

Keywords: *Nanocellulose, chất nhũ hóa, Pickering, nhũ tương, mỹ phẩm, kem.*

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1. INTRODUCTION

Nanocellulose, a class of nanomaterials derived from cellulose, has garnered significant attention due to its unique properties and diverse applications across various industries. When broken down into nanostructures, cellulose exhibits remarkable mechanical strength, lightweight characteristics, high surface area, and biocompatibility. These qualities make nanocellulose an ideal material for applications in different industry [1].

Based on factors like length, diameter, aspect ratio, and composition, nanocellulose can be categorized into four types: spherical nanocellulose particles (NCSP), cellulose nanocrystals (CNC), cellulose nanofibrils (CNF), and bacterial nanocellulose (BNC) [2].

CNFs are a natural material originating from wood or plants consisting of a soft and long structure. The dimensions are typically 3 - 100nm in cross section and typically up to 100 µm in length (ISO/TS 20477, 2017) [3]. CNFs are generated by mechanically laminating cellulose fiber, and the nanometer-scale fibrillation gives beneficial characteristics such as extensive aspect ratio, biodegradable, robust, transparent, hydrophilic, large surface area, low thermal expansion, high stiffness, and decreased density [4].

Because of these unique features, CNFs is applied in many different fields such as electronic devices, packaging, biomedical materials, protective coating, drug delivery, especially cosmetic.

CNFs are generally used as a stabilizer and thickener for liquid systems. It is particularly suited for controlling the viscosity of dispersions and/or emulsions, and thus the applicability and feeling of use [5]. CNFs produce cosmetic formulations with improved compatibility and tolerance to salt as a stabilizing aid or a gel-forming component. In addition, the CNFs filled with sensitive sensory gel is improved by any reduction in adhesion, rigidity, and agglomeration, as well as a decreased viscosity of the cosmetic composition after application [6].

The excellent moisture retention capabilities of CNFs (up to 75 - 100%) surpasses that of other nanocellulose varieties, owing to their hydrophilic nature and unique structure with a compact nanofibrillar network. Additionally, the water affinity of CNFs can be enhanced even further through surface carboxylation following TEMPO oxidation. As a result, CNFs are commonly used as moisturizing agents, offering superior performance compared to traditional polymers like collagen or hyaluronic acid [5].

Pickering emulsions are surfactant-free emulsions stabilized by colloidal solid particles that are partially wetting to both the oil and water phases. CNFs with a higher aspect ratio can facilitate the formation of an interconnected network around the oil droplets, contribute to the viscosity of the aqueous phase, and improve the storage stability of the CNF-stabilized emulsion [7].

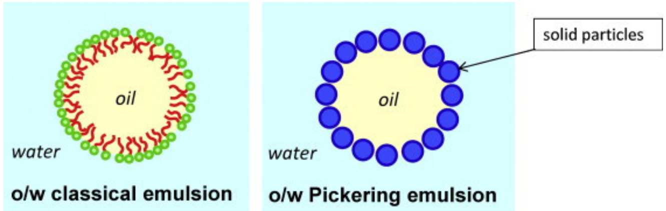


Figure 1. Demonstrations of classical emulsion and Pickering emulsion [Physicochemical and Engineering Aspects 439: 23-34]

Pure CNFs have minimal inherent UV-blocking properties, they can be modified to enhance UV protection in cosmetic by incorporating other materials like lignin or UV blockers. Huang *et al.* added nanoscale lignin from pre-hydrolysate into CNF suspension to prepare the lignin-reinforced CNF composite film. The residual lignin retained in lignocellulosic nanofibril was found to act as natural macro-molecular UV absorber. Moreover, lignocellulosic nanofibril film exhibited stable UV shielding performance under high temperature, UV irradiation, acidic or alkaline conditions, providing lignocellulosic nanofibril film with a long-term use capacity [8]. Recently, CNFs decorated with TiO_2 and ZnO nanoparticles having high refractive index and UV absorbance are used to produce transparent nanocellulose films. The deposition of TiO_2 nanoparticles through physical interaction adds good UV resistance to nanocellulose fibers [2].

In Vietnam, Le Quang Dien *et al.*, have published many works on nanocellulose produced from sugarcane bagasse, cassava bagasse, rice husks, wood, etc [9-11]. In which, CNFs are produced from acacia wood, by limited hydrolysis method, using a dilute sulfuric acid agent system supplemented with hydrogen peroxide to obtain CNFs with fiber diameter < 100nm, with efficiency reaching 57 - 62% compared to pulp. With the features of CNFs and the resulting white, odorless powder form, it is very suitable for inclusion in cosmetic formulations.

Cream formulation is a semisolid emulsion containing one or more active substances, dissolved or dispersed, and may be defined as a biphasic system in which the dispersed or internal phase is finely and uniformly dispersed in the continuous or external phase. According to the dispersed phase nature, it is possible to acquire an oil-in-water cream (o/w) or a water-in-oil cream (w/o) [12]. A cream formulation include an oil phase (wax, oil or fatty acid), a water phase such as water, preservative, emulsifiers, thickeners and other additive such as antioxidant, moisturizer, fragrance and will be evaluated the homogeneity, viscosity, pH, accelerated stability [13].

2. MATERIALS AND METHODS

2.1. Materials and equipment

All chemicals used in the study were of cosmetic grade.

- Medium chain triglycerides (MCT oil), Shea butter, Crosslinked polyacrylic acid (carbomer 980), C16-18 alcohols ethoxylated with 25mol EO average molar ratio (ceteareath-25), triethanolamine (TEA) were purchased in cosmetic grade.

- Nanocellulose in CNF forms were prepared from sulphate pulp by Le Quang Dien et. al.

- Deionizing H_2O was prepared in-house using Hamilton water distiller WSB/4 Budget Water Still.

- Overhead stirrer IKA RW20 and homogenizer SH-HZD from SH Scientific was used for mixing and homogenizing.

2.2. Research methods

2.2.1. Cream formulation preparation

- In cream formulation, the semisolid emulsion consists of oil phase and water phase dispersed into each other in the appearance of emulsifiers, thickeners to improve active ingredients penetration through the skin.

- For oily excipients, Shea butter was used as an active ingredient. It consisted of several fatty acids (both saturated and essential unsaturated ones like omega-6 and omega-9) and various vitamins such as A and especially E which promote healthy skin cell growth with antioxidants property. MCT oil was used as hydrophobic solvent to help dissolve Shea butter and other active ingredients if necessary. In the water phase, carbomer-980 was used as thickener to stabilize the texture.

- In this mixture of immiscible liquid, nanocellulose (CNF) was evaluated the emulsifying activity with or without appearance of thickener or other emulsifier.

- The procedure of making cream formulation is briefly demonstrated in scheme 1.

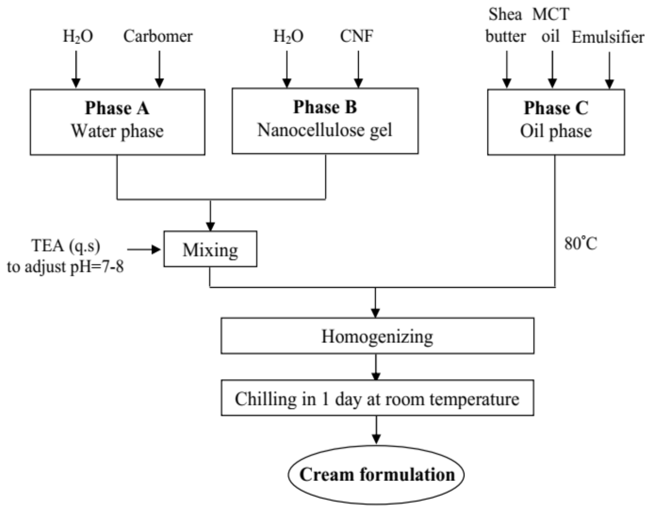
1. Carbomer was soaked for 4 hours in deionizing water in phase A until carbomer is completely dissolved in water. This step will be applied in formulations using thickener.

2. CNF was soaked and mixed well in phase B to form the nanocellulose gel. TEA was added in the mixture of phase A and phase B to adjust pH of the mixture when stirring at 100 rpm in 5 mins. This mixture was considered as water-phase.

3. Phase C was prepared by mixing all the oil excipients at 90 - 100°C then was cooled to 50 - 60°C. Ceteareth-25 emulsifier was just used in a few formulations. This solution was considered as oil phase.

4. Phase C and the mixture of phase A and phase B were homogenized at 4200rpm, at 50°C, in 20 mins.

5. The products after homogenization were chilled at room temperature 1 day before evaluations.



Scheme 1. General procedure of emulsifying process to make cream formulation

2.2.2. Evaluation method

- **Spreading ability:** The sample is taken at about 0.1g, spread evenly on a glass slide. Cover the second glass slide and press firmly until a circular mark with a diameter of about 2cm appears. Observe the resulting mark with the naked eye. If the preparation does not have tiny particles, the sample is considered to meet the requirements. The final product should be uniform, not lumpy, and free of from visible extraneous matter.

- **Homogeneity:** The sample is taken at about 0.1g, spread evenly on a glass slide. Cover it with a second slide and press firmly until a circular mark with a diameter of about 2cm appears. Observe the obtained mark with the naked eye. If the preparation does not have tiny particles, the sample is considered to meet the requirements. The final product must be homogeneous, free of lumps, and free of from visible extraneous matter. The slide was observed with an optical microscope at the magnification of 100 times the actual size to figure out the morphology of the oil droplet in water environment. Pictures were taken with a magnification of 3.

- **Stability:** The sample is placed in a jar with a lid then left at 50°C for 7 days for accelerated aging test. The texture was observed via microscope image and evaluated after periods. The texture is considered to be broken when the droplets was aggregated or disappeared along with 2 distinct phases was observed separated via eye-test. For the viscosity, the sample is taken at about 0.15g on a glass slide then place the glass slide 90° on the table. Movement of the sample was observed for 1 hour at room temperature. The sample was considered as acceptable when there is no movement on the glass slide.

3. RESULTS AND DISCUSSION

3.1. Preliminary assessment on emulsifying property of CNF

Table 1. Cream formulations using CNF as emulsifier (F0.1-2)

	Water phase		Oil phase	
F0.1 formulation	H_2O	10.8g (77.1%)	Shea butter	0.6g (4.3%)
	CNF	**1.0g (7.1%)**	MCT oil	1.6g (11.4%)
F0.2 formulation	H_2O	10.8g (67.5%)	Shea butter	0.6g (3.7%)
	CNF	**3.0g (18.7%)**	MCT oil	1.6g (10.0%)

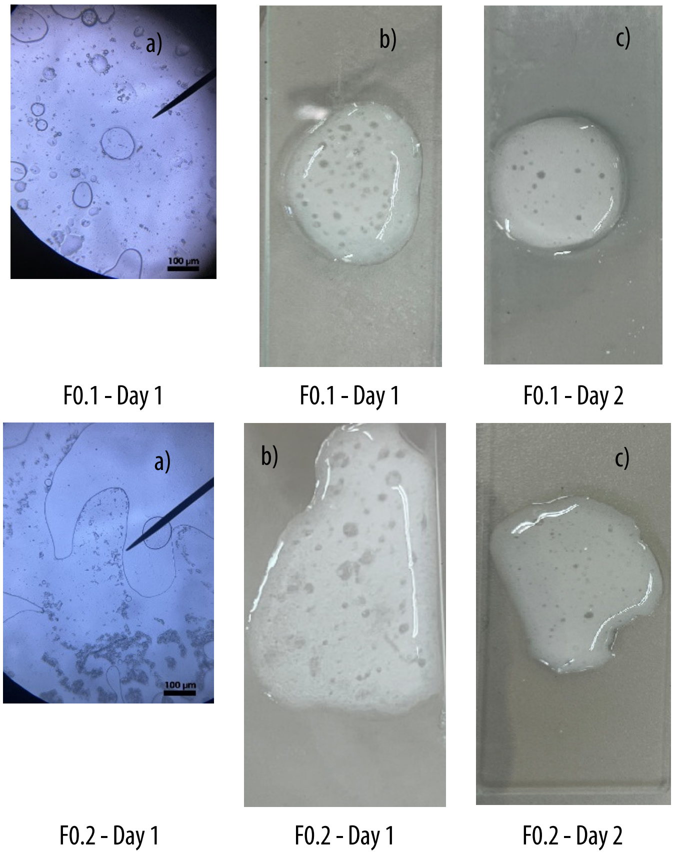


Figure 2. Observation of F0.1-2 on a) microscope and b) image after 1 day and c) image after 2 days

Emulsifying property of CNF alone was evaluated preliminarily in the simplest formulation (F0.1-2) in Table 1. The results were demonstrated in Figure 2.

When the sample was placed on the slide, a big oil droplet was easily observed. When the second slide was pressed before microscope observations, the big oil droplet was broken and aggregated into oil phase, separated from the water-phase. On the microscope image, the unbroken smaller oil droplets were observed in the size of up to 100µm. The formulation was not stable and broken after just 2 days. In general, CNF tended to be able to emulsify o/w formulation but still required enhancer to stabilize the formulation.

3.2. Evaluation on the ratio of thickener on the cream formulation using CNF as emulsifier

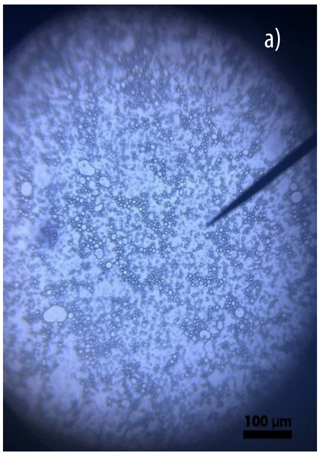
The recipe was added 0.2g of carbomer as thickener for stabilization. 3 formulations (F1.1-3) were prepared using 0.6g, 1.0g and 2.0g of CNF respectively (Table 2). Textures of formulations after 1, 4 and 7 days were evaluated on microscope images in Figures 3 - 5 respectively.

Table 2. Cream formulations using CNF as emulsifier combined with carbomer-980 as thickener (F1.1-3)

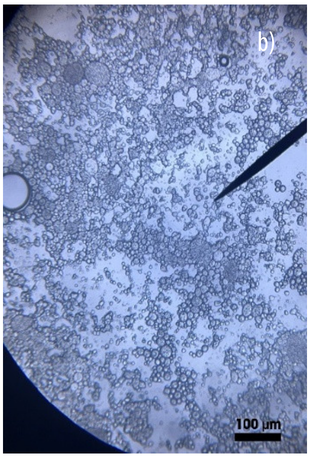
	Water phase		Oil phase	
F1.1 formulation	H_2O	10.8g (78.75%)	Shea butter	0.6g (4.3%)
	CNF	**0.6g (4.3%)**	MCT oil	1.6g (11.2%)
	Cacbomer-980	0.2g (1.45%)		
F1.2 formulation	H_2O	10.8g (76.1%)	Shea butter	0.6g (4.2%)
	CNF	**1.0g (7.1%)**	MCT oil	1.6g (11.2%)
	Cacbomer-980	0.2g (1.4%)		
F1.3 fomulation	H_2O	10.8g (71.5%)	Shea butter	0.6g (3.5%)
	CNF	**2.0g (13.16%)**	MCT oil	1.6g (10.52%)
	Cacbomer-980	0.2g (1.31%)		

In general, all the formulations using thickeners observed smaller oil droplets compared to ones without thickener (F0s). On the other hand, the aggregation of oil droplets still occurred in accelerated aging tests. The increase in the amount of CNF leads to a decrease in the size of oil droplets. The sample using 0.6g CNF obtained the droplet size of 5 - 30µm (Figure 3) while ones using

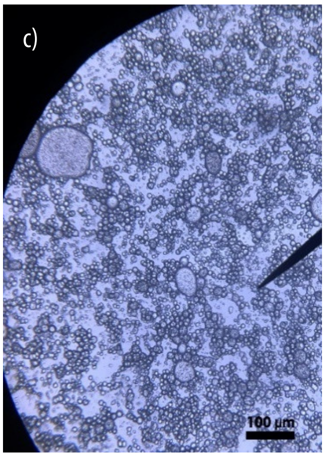
1.0 and 2.0g of CNF gave us much smaller droplet size of 1 - 10µm (Figure 4 and Figure 5). It turns out that carbomer significantly stabilize the formulation using CNF as emulsifier. With 0.2g of carbomer, using of 1.0 - 2.0g of CNF obtained good oil droplet size of 1 - 10µm.



F1.1 - Day 1

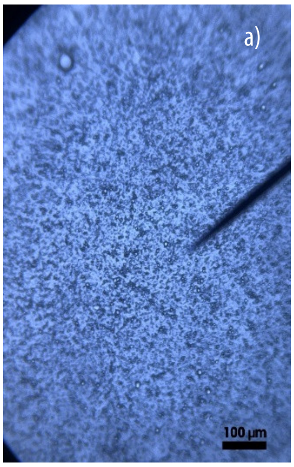


F1.1 - Day 4

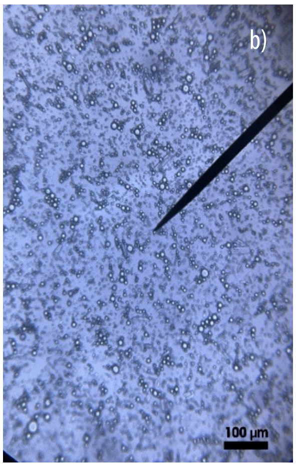


F1.1 - Day 7

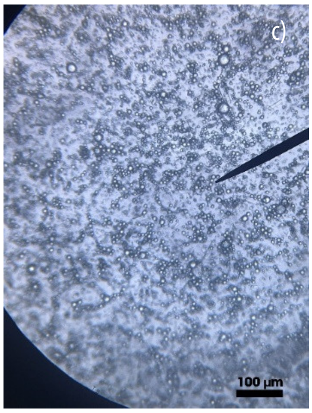
Figure 3. Observation of F1.1 on microscope after a)1 day, b)4 days c)7 days



F1.2 - Day 1

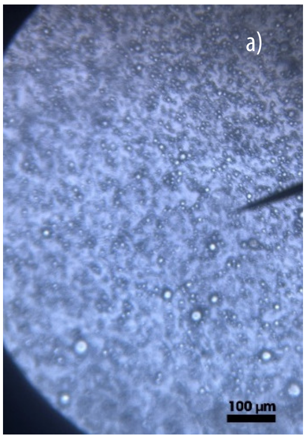


F1.2 - Day 4

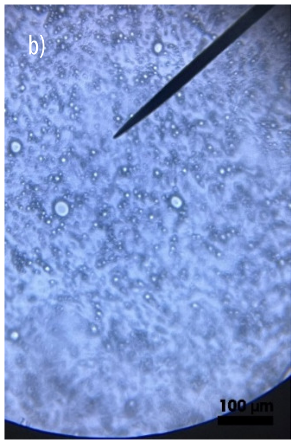


F1.2 - Day 7

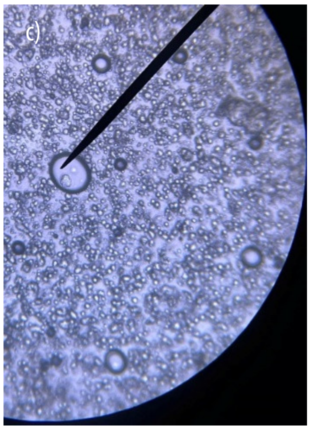
Figure 4. Observation of F1.2 on microscope after a)1 day, b)4 days and c)7 days



F1.3 - Day 1



F1.3 - Day 4



F1.3 - Day 7

Figure 5. Observation of F1.3 on microscope after a)1 day, b)4 days and c)7 days

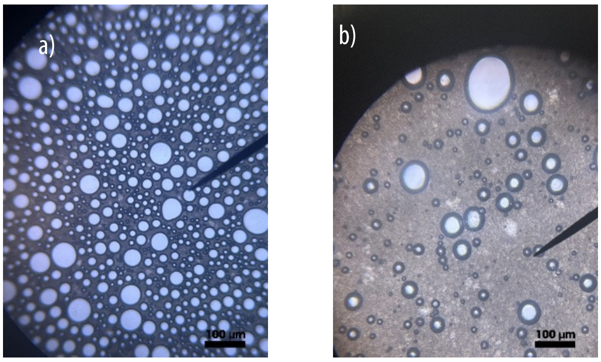
3.3. Evaluation of the ratio of surfactants combined with CNF as an emulsifier system

Zero reference formulations (F0.3-4) were prepared using carbomer-980 (0.2g) as thickener and ceteareth-25 (0.6 and 1.0g) as emulsifier without CNF (Table 3).

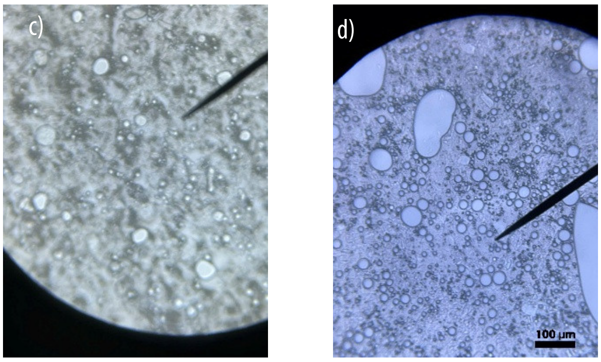
Table 3. Cream formulations using carbomer-980 combined ceteareth-25 and without CNF (F0.3-4)

	Water phase		Oil phase	
F0.3 formulation	H_2O	10.8g (78.3%)	Shea butter	0.6g (4.3%)
	Cacbomer-980	0.2g (1.4%)	MCT oil	1.6g (11.6%)
	Ceteareath-25	**0.6g (4.3%)**		
F0.4 formulation	H_2O	10.8g (76.1%)	Shea butter	0.6g (4.2%)
	Cacbomer-980	0.2g (1.4%)	MCT oil	1.6g (11.2%)
	Ceteareath-25	**1.0g (7.1%)**		

F0.3 formulation **F0.4 formulation**



F0.3 - Day 1 F0.3 - Day 7



F0.4 - Day 1 F0.4 - Day 7

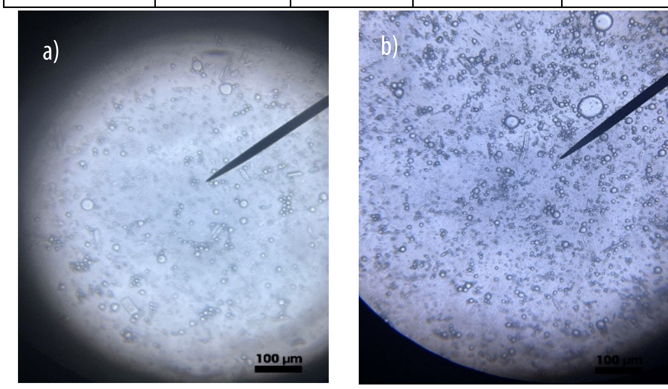
Figure 6. Observation of F0.3 and F0.4 on microscope after a) 1 day, and b) 7 days

F0.3 obtained uniform droplets with size of 10-40 µm but droplets of F0.4 are much smaller. It was obvious that 1.0g of ceteareth-25 could emulsify the formulation much better than using just 0.6g. However, both samples after 7 days showed clearly the aggregation of the oil droplets. F0.3 could be improved by using more emulsifiers or thickeners therefore the work below will figure out the role of CNF in supporting the emulsion.

To evaluate and compare the role of CNF versus ceteareth-25 as emulsifier. Four formulations were prepared using combos of emulsifiers (Table 4). Textures of formulations after 1 and 7 days were evaluated on microscope images in Figures 7 - 10, respectively.

Table 4. Cream formulations using carbomer-980 combined ceteareth-25 and CNF (F2.1-4)

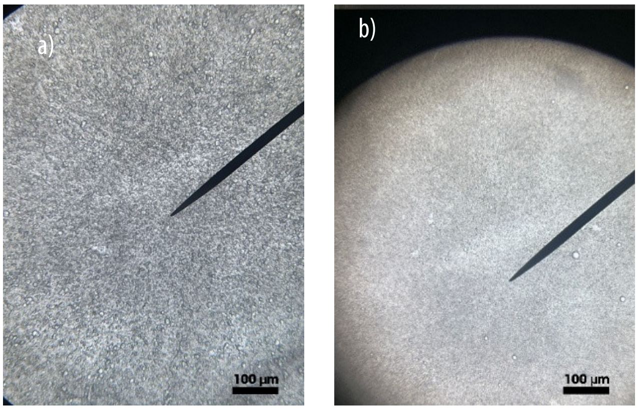
	Water phase		Oil phase	
F2.1 formulation	H_2O	10.8g (75%)	Shea butter	0.6g (4.2%)
	Cacbomer-980	0.2g (1.4%)	MCT oil	1.6g (11%)
	CNF	**0.6g (4.2%)**	**Ceteareath-25**	**0.6g (4.2%)**
F2.2 formulation	H_2O	10.8g (73.0%)	Shea butter	0.6g (4.05%)
	Cacbomer-980	0.2g (1.3%)	MCT oil	1.6g (10.8%)
	CNF	**1.0g (6.8%)**	**Ceteareath-25**	**0.6g (4.0%)**
F2.3 formulation	H_2O	10.8g (73.0%)	Shea butter	0.6g (4.0%)
	Cacbomer-980	0.2g (1.3%)	MCT oil	1.6g (10.8%)
	CNF	**0.6g (4.0%)**	**Ceteareath-25**	**1.0g (6.8%)**
F2.4 formulation	H_2O	10.8g (68.4%)	Shea butter	0.6g (3.8%)
	Cacbomer-980	0.2g (1.3%)	MCT oil	1.6g (10.1%)
	CNF	**2.0 g (12.6%)**	**Ceteareath-25**	**0.6g (3.8%)**



F2.1 - Day 1 F2.1 - Day 7

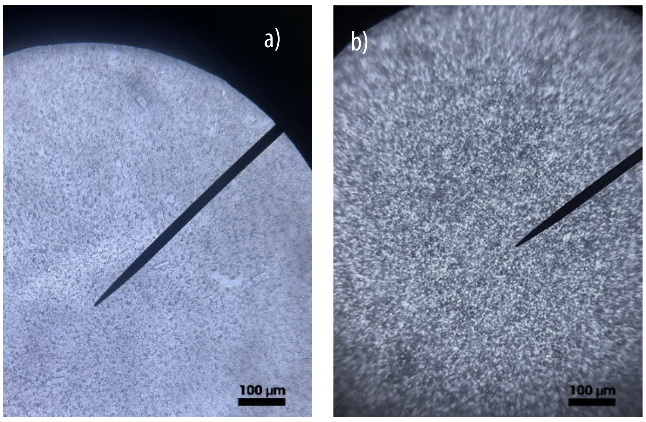
Figure 7. Observation of F2.1 on microscope after a)1 day and b)7 days

F2.1 containing the same amount of carbomer-980 and CNF compared to F1.1 and the same amount of carbomer and ceteareth-25 to F0.3 but the textures was maintained well after 7 days. The combination of CNF and ceteareth-25 indeed improved the stability of the emulsion.



F2.2 - Day 1 F2.2 - Day 7

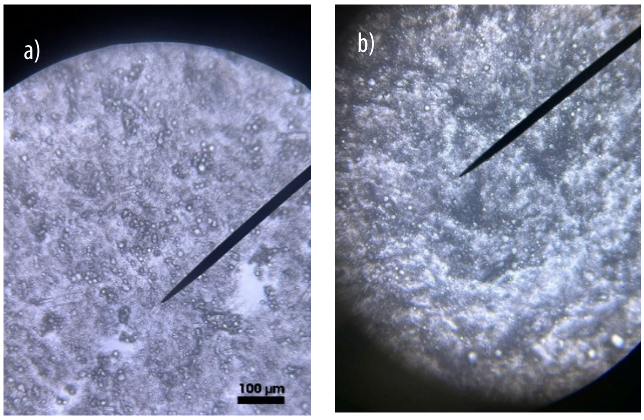
Figure 8. Observation of F2.2 on microscope after a)1 day and b)7 days



F2.3 - Day 1 F2.3 - Day 7

Figure 9. Observation of F2.3 on microscope after a)1 day and b)7 days

Microscope images of F2.2 and F2.3 using the same total amount of the emulsifiers of 1.6g showed very fine oil droplet sizes. Droplet sizes are around 0.5 to 2.0µm and were maintained nearly unchanged after 7 days of accelerated aging tests.



F2.4 - Day 1 F2.4 - Day 7

Figure 10. Observation of F2.4 on microscope after a)1 day and b)7 days

When the amount of CNF increased to 2.0g in F2.4, the droplet size increased compared to F2.2 using the same amount of ceteareth-25. This agreed with the property of Pickering emulsifier like CNF. Depending on the purpose of the cream, F2.4 can be considered to apply when the stability was still good.

The eye-tested viscosity of F2.2, F2.3 and F2.4 was also conducted (Figure 11) and obtain no movement of the cream formulations after 1-hour observation, confirmed the acceptable viscosity of them.

The optimized formulation in F2.2 and F2.3 was compared to some commercialized cream on the market (Figure 12). Droplets from all creams look similar in

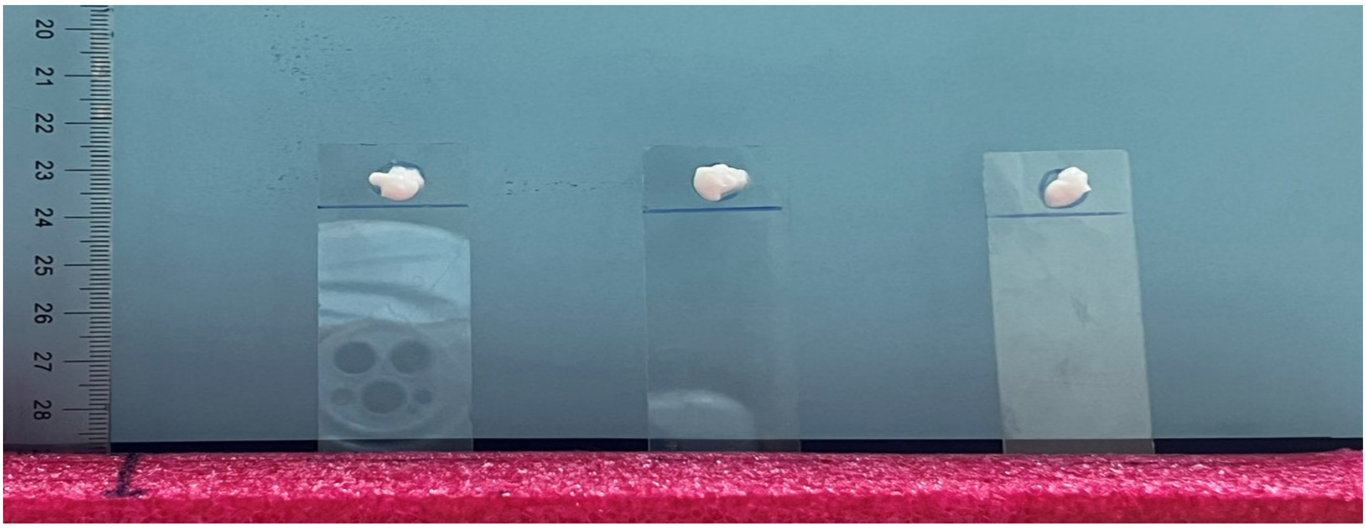


Figure 11. Viscosity observation of formulation a) F2.1; b) F2.2; c) F2.3 at day 7

droplet size and morphology. Moreover, a few more Shea butter solids are obtained in the cream from La Rocheposay because this formulation used more such excipient.

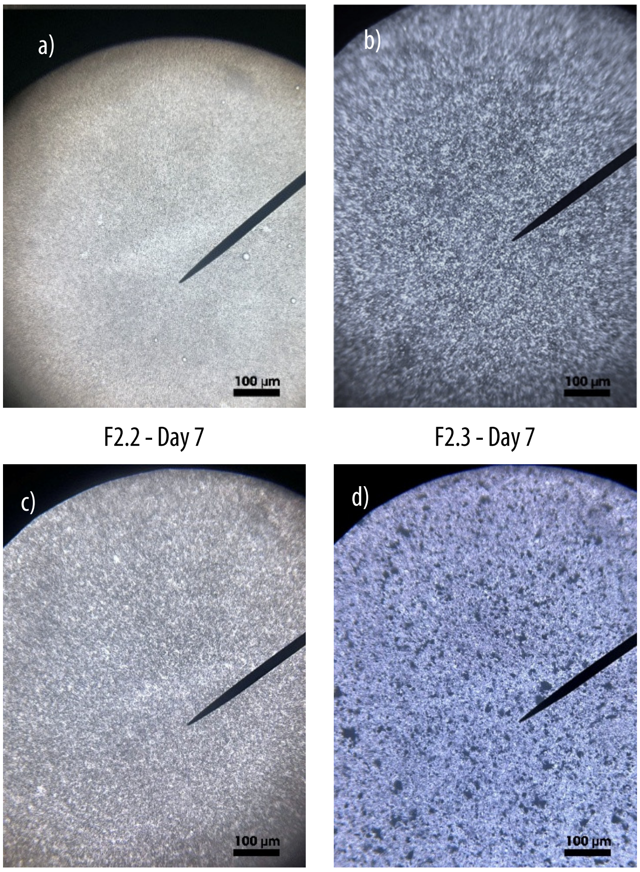


Figure 12. Observation of optimized formulation on microscope with a) F2.2; b) F2.3; c) Hand & Nagelcream from Kamill; d) Cicaplast Baume B5+ cream from La Rocheposay

4. CONCLUSION

Nanocellulose (CNF) showed the ability to emulsify an emulsion. The stability of the emulsion could be significantly improved by using carbomer-980 as thickener or ceteareth-25 as surfactant-based emulsifier. Especially, the combination of CNF and ceteareth-25 (total 1.6g) produce oil droplet sizes of 0.5 - 2.0µm which are noticeable. The optimized formulation was obtained when using CNF at a concentration of 4 - 12% combined with the emulsifier ceteareth-25 at a concentration of 3-7% and carbomer-980 as thickener at a concentration of 1.3 - 1.5%. The increase in the amount of nanocellulose obtained bigger droplet size confirmed the Pickering emulsifying property of our CNF and should be considered to use.

The texture of optimized formulation is comparable to commercial skin-care creams that opened practical applications of the results in developing a nanocellulose skin-care cream in health science and technology.

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THÔNG TIN TÁC GIẢ

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