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Research Article

FORMULATION AND CHARACTERIZATION OF BUCCAL FILM NANOEMULSION APIGENIN AS ANTIDIABETIC

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Abstract
Apigenin (4`,5,7-trihydroxyflavone) is a flavonoid of subclass flavones that have antidiabetic therapeutic activity but limitations BCS Class II low solubility of 2.16 µg/L. To overcome these limitations, the development of nanoemulsion formulation technology, increasing solubility increases dissolution, absorption and bioavailability. It is incorporated into the buccal film for easy application and direct access to the systemic circulation. This study aims to obtain apigenin nanoemulsion with the best characterization and buccal film that meets the characterization. The method was carried out experimentally in the manufacture of nanoemulsions by spontaneous emulsification, a buccal film by solvent casting, and the characterization. 10 nanoemulsion formulas met the characterization with globule size <20.34nm, polydispersity index <0.131, zeta potential close to 0mV, pH 6.23-6.59, %transmittance close to 100% and best F10 incorporated into buccal film has 29x the solubility compared to apigenin with p≤0.05. All buccal films met the characterization with F3 having a 2x faster onset of release than F1&F2 with 86.07% diffusion and 97.9333 mg/sheet. Thus, it was concluded that the formulation and characterization of buccal film fulfilled the characterization and F10 apigenin nanoemulsion increased the solubility 29-fold with the buccal film F3 having a faster onset of release.
Keywords: Apigenin; BCS; Solubility; Nanoemulsion; Buccal Film.

FORMULASI DAN KARAKTERISASI *BUCCAL FILM* NANOEMULSI APIGENIN SEBAGAI ANTI DIABETES

Abstrak
Apigenin (4`,5,7-trihydroxyflavone) merupakan flavonoid subkelas flavon memiliki aktivitas terapeutik antidiabetes, namun memiliki keterbatasan termasuk BCS Kelas II kelarutan rendah 2,16 µg/L. Untuk mengatasi keterbatasan kelarutan, dilakukan pengembangan teknologi formulasi nanoemulsi, meningkatnya kelarutan dapat meningkatkan disolusi, penyerapan dan bioavailabilitas. Diinkoporasikan kedalam buccal film mempermudah pengaplikasian serta akses langsung ke sirkulasi sistemik. Penelitian ini bertujuan mendapatkan nanoemulsi apigenin dengan karakterisasi terbaik serta diperoleh buccal film yang memenuhi karakterisasi. Metode dilakukan secara eksperimental pembuatan nanoemulsi dengan metode emulsifikasi spontan, buccal film dengan metode solvent casting, dan karakterisasinya. Diperoleh 10 formula nanoemulsi memenuhi karakterisasi dengan ukuran globul <20,34nm, indeks polidispersitas<0,131, potensial zeta mendekati 0mV, pH 6,23-6,59, %transmitan mendekati 100% dan terbaik F10 diinkoporasikan kedalam buccal film memiliki kelarutan 29x lipat dibanding apigenin dengan p≤0,05. Semua buccal film memenuhi karakterisasi dengan F3 memiliki onset waktu pelepasan lebih cepat 2x lipat dibandingkan F1 dan F2 dengan terdifusi 86,07% dan kadar 97,9333 mg/lembar. Sehingga, disimpulkan formulasi dan karakterisasi buccal film nanoemulsi apigenin memenuhi karakterisasi serta F10 nanoemulsi apigenin meningkatkan kelarutan 29x lipat dengan buccal film F3 memiliki onset pelepasan lebih cepat.
Kata Kunci: Apigenin; BCS; Kelarutan; Nanoemulsi; Buccal Film.

1. Introduction

Apigenin (4`,5,7-trihydroxyflavone) is one of the most widely distributed flavonoids in plants and belongs to the flavone subclass[1]. Sources of apigenin are found in many fruits and vegetables[2]. Precisely, the main source of apigenin is dominantly found in celery (Apium graveolens) with an apigenin content value of 338.5 mg/kg dry weight[3].

Apigenin has various therapeutic activities such as anti-inflammatory, antioxidant, and antiproliferative activities[4]. In addition, apigenin has antidiabetic activity in animal models of diabetes testing[5]. The mechanism of apigenin as an antidiabetic with the ability to renew pancreatic cells produced by the excretion of insulin, so that apigenin can activate glycogen formation through an increase in glycogen content in the liver[2].

Apigenin has good potential as an antidiabetic but has limitations regarding its bioavailability value, where apigenin is included in the BCS (Biopharmaceutical Classification System) Class II category with low solubility in water of around 2.16 g/L at pH 7.5 so it affects the dissolution rate and its absorption becomes low thereby reducing its therapeutic activity[6]. the solution that can be done is to make efforts to increase the solubility of apigenin to increase its dissolution rate and bioavailability[7].

The development of increasing the solubility of apigenin that has been carried out including solid dispersion using the hot melt extrusion can increase the solubility of apigenin in water by 18.25 times[8], Microemulsions with various oil phases, surfactants, and co-surfactants can increase the solubility of apigenin in water by 15.26 times[9], and Bio-SNEDDS with various bioactive oils, mono and Di-glyceride oil, surfactants and co-solvents can increase the solubility of apigenin in water by 12.36 times[10].

The selection of excipients in pharmaceutical preparations, especially in the manufacture of SNE is a critical factor because they must meet the requirements for toxicity and compatibility in the body. It is necessary to pay attention to the type of oil, surfactant, and cosurfactant used, in that the excipients must not be irritating and free from toxicity problems, both

chronic and acute. The spontaneous nano emulsification process is influenced by the specific properties of the oil, surfactant, and cosurfactant, the concentration and ratio of oil-surfactant-cosurfactant, and the temperature at which the spontaneous nanoemulsion is formed. Therefore, only certain combinations of excipients can form spontaneous nanoemulsion systems[11].

Some of the advantages obtained from using nanoemulsions compared to other dosage forms are increasing the speed of absorption, reducing inter-subject and intra-subject variability and the effect of food on drug absorption, not affecting the fat digestion process, increasing drug entrapment capacity, increasing lipophilic drug solubility and increasing bioavailability. From the manufacturing technique also nanoemulsion is a method that is easy to make and can be mass produced with a high level of reproducibility, capable of delivering hydrophobic and hydrophilic drugs, increasing drug efficacy so that drug doses can be lowered and side effects become lower[11,12].

To facilitate the application of nanoemulsions apigenin as an antidiabetic, then it was incorporated into a buccal film dosage form. preparations buccal films are preparations that use the oral cavity as a delivery system (buccal) have excellent accessibility and do not undergo first-pass metabolism in the liver due to direct access to the systemic circulation through the jugular vein[13]. Another advantage of using buccal film preparations is that they have a relatively larger surface area than other oral preparations such as tablets so the disintegration and dissolution processes occur quickly. In addition, the use of hydrophilic polymers with small contact angles and large scatter coefficients results in better film wetting and faster disintegration time[13].

This study aims to obtain an apigenin nanoemulsion formula with the best characterization evaluation and to obtain a buccal film nanoemulsion with good apigenin

and meet the evaluation of the characterization of buccal film.

2. Materials and Methods

2.1. Tools

Oven (Memmert), analytical balance (MettlOven (Memmert), analytical balance (Mettler Toledo), measuring cup (Iwaki pyrex), beaker (Iwaki pyrex), dropper pipette, magnetic stirrer (IKA Germany), orbital shaker (Oregon KJ-201BD), Brookfield viscometer (RVDV 10), pH meter (Ionix), volumetric flask (Iwaki pyrex), petri dish (RRC), screw micrometer (RRC), Petri dish (NORMAX), micropipette (Socorex®), UV-visible spectrophotometer (Agilent Cary 60), Fourier Transform Infrared Spectroscopy (FTIR) (FTIR Attenuated Total Reflectance), particle size & zeta potential Analyzer (PSA) (Malvern Zetaasir), sonicator (Krisbow® & Powersonic 420), Franz diffusion cell (Ward`s science), 1 mL syringe (Onemed®) and glass beakers commonly used in the laboratory.

2.2. Materials

The ingredients used include Apigenin (Hefei Dielegance Biotechnology Co., Ltd), Sunflower Oil (Jan Dekker International), Kolliphor®RH40 (BASF), PEG 400 (Merck, Tbk), HPMC K15M (Dipa Prasada Husada), Na. CMC (Dai-Ichi Kogyo Seiyaku Co., LTD), Tween 80 (BASF), Aquadeion (Merck, Tbk).

2.3. Methods

2.3.1. Pre-Formulation and Characterization of Apigenin

Pre-Formulation and Characterization Tests were carried out on apigenin including organoleptic, pH, functional groups, %transmittance, and solubility.

2.3.2. Apigenin Nanoemulsion

Preparation Apigenin Nanoemulsion Preparation 10 formulas were made using the spontaneous emulsification method listed in table 1 with varying concentrations of the active substance, namely 1 - 10 mg apigenin/1 g SNE using the optimum combination base of nanoemulsion. best with a 1:8:1 ratio (Sunflower oil: KolliphorâRH40: PEG 400) based on the best optimization results[14].

2.3.3. Nanoemulsion Characterization

Organoleptic testing of samples was observed through the senses in terms of color, clarity, and smell[15].

Testing pH by dissolving 1 gram of sample in 5 mL of the aquadeion and measuring using a pH meter[15]. The pH value of saliva in the mouth ranges from 5.6 to 7.4[16].

Solubility test using UV-Vis Spectrophotometer using 125 mg of sample in a 50 mL aquadeion volumetric flask and then shaking for 24 hours. Next, the sample was filtered and the absorbance of the filtrate was measured at a wavelength of 200-400 nm[8].

Samples were analyzed through Fourier Transform Infrared Spectroscopy (FT-IR) to identify compounds at frequencies and wave numbers that indicate functional groups and to determine whether or not there is an interaction between active pharmaceutical ingredients and excipients[17]. Scanning was carried out with a wave number of 4000-600 cm^{-1} using FTIR ATR (Attenuated Total Reflectance)[18].

Test of %transmittance with 100 µL of sample added with 5 mL of aquadeion, then the %transmittance was measured through a UV-Vis spectrophotometer at a wavelength of 200-400 nm[19].

Size globule and polydispersity index were analyzed by photon correlation spectroscopy which monitors the scattering of light due to Brownian of the globules and zeta[20].

Components in the buccal film were made of 3 formulas using the solvent casting with variations in the active substance and 1 blank formula listed in table 2 using the best buccal film[21].

2.3.4. Evaluation Characterization of Buccal Film Nanoemulsion Apigenin (Buccal Film Ne-APG)

Organoleptic tests were carried out including visual observations such as color, clarity, and odor of the wet mixture and buccal film the resulting[22].

Table 1. Apigenin Nanoemulsion Formulation

Composition		Amount									
		F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Apigenin	Active Pharmaceutical	1	2	3	4	5	6	7	8	9	10
Sunflower oil: Kolliphor RH40: PEG 400 (g)	Oil: Surfactant : Co-Surfactant	1:8:1 (1 gram)									
Aquadeion (g)		ad 20 gram									

Viscosity was measured to see the rheological properties or the ability of pourability using a Brookfield spindle type 4 viscometer with a speed of 50 rpm[20].

Testing the pH of the wet mixture by dissolving 1 gram of the wet mixture in 5 mL of aquadeion and measuring using a pH meter [15]. The pH value of saliva in the mouth ranges from 5.6 to 7.4[16].

Thickness film was measured using a micrometer against three film points and the results obtained were calculated on average[23].

The folding endurance test is carried out manually by folding the film repeatedly in the same place until it breaks or up to 300 times[13].

Weight film is cut to a size of 3x3 cm2 and then weighed using an analytical balance[24].

pH Testing the film by immersing the film using 20 mL of phosphate buffer pH 6.8 in a petri dish for 1 hour at room temperature[25]. The surface pH value was then measured using a pH meter[26]. The pH value of a buccal is in the range of 5.6-7[27].

The release test was carried out in vitro using a Franz diffusion cell consisting of 2 parts, namely the donor compartment and the receptor compartment separated by a millipore membrane. The donor compartment is filled with the active substance, while the receptor compartment is filled with phosphate buffer pH 6.8 (salivary fluid pH) which is stirred with a magnetic stirrer at 37°C. Thus, the active substance diffuses through the membrane from the donor compartment to the receptor[28]. Then, the receptor fluid samples were taken periodically and their absorbance was measured through a UV-Vis spectrophotometer at a wavelength of 200-400 nm[8].

Samples were analyzed through Fourier Transform Infrared Spectroscopy (FT-IR) to identify compounds at frequencies and wave numbers that indicate functional groups and to determine whether or not there is an interaction between active pharmaceutical ingredients and excipients[17]. Scanning was carried out with a wave number of 4000-600 cm^{-1} using FTIR ATR (Attenuated Total Reflectance)[18].

An assay using UV-Vis Spectrophotometer by dissolving buccal film nanoemulsion apigenin (3x3 cm2) in 10 mL of aquadeion. Then, the absorbance was measured at a wavelength of 200-400 nm[8].

3. Result

3.1. Pre-Formulation and Characterization

Pre-Formulation and Characterization Tests were carried out on apigenin including organoleptic, pH, functional groups, % transmittance, and solubility.

Table 2. Formula for Buccal Film Nanoemulsion Apigenin

Component Material		Amount (% w/v)			
		F1	F2	F3	Blanko
Nanoemulsion Apigenin (Equivalent to 10 mg/g SNE)	Active Pharmaceutical	0.25	0.5	0.75	-
HPMC K15M	Polymer	0.33	0.33	0.33	0.33
Na. CMC	Polymer	0.67	0.67	0.67	0.67
Tween 80	Chemical Enhancer	0.05	0.05	0.05	0.05
PEG 400	Plasticizer	0.5	0.5	0.5	0.5
Aquadeion	Solvent	ad 100			

Table 3. Results of Evaluation of Ne-APG Characterization

Sample	Result Evaluation				
	Globule Size (nm)	Polydispersity Indeks	Zeta Potential (mV)	pH	%Transmittance
F1	18,69±0,0642	0,066±0,011	-6,49±0,523	6,59±0,0115	95,453±0,4998
F2	18,26±0,1106	0,131±0,013	-17,7±0,566	6,55±0,0057	95,695±0,0724
F3	20,34±0,1136	0,088±0,011	-9,02±0,247	6,51±0,0057	97,854±0,0458
F4	20,08±0,0378	0,080±0,013	-11,0±0,424	6,44±0,0057	98,837±0,013
F5	17,48±0,2639	0,090±0,025	-14,6±0,0707	6,57±0,0115	99,1±0,0584
F6	20,06±0,0550	0,101±0,013	-9,15±0,672	6,32±0,0404	95,85±0,2642
F7	17,85±0,0378	0,113±0,004	-15,4±0,354	6,28±0,0115	96,163±0,1034
F8	19,48±0,1856	0,071±0,009	-8,62±0,424	6,23±0,0057	96,5±0,0504
F9	19,46±0,3842	0,080±0,006	-6,97±0,912	6,44±0,0173	96,705±0,3219
F10	19,21±0,1473	0,056±0,013	-5,53±1,73	6,45±0,0057	98,631±0,3547

***) Each evaluation result is the average standard deviation (n=3).**

3.2. Evaluation of Apigenin Nanoemulsion Characterization

Characterization tests were carried out including Organoleptic, pH, Solubility, Functional Groups, %Transmittance, Globule Size, Polydispersity Index, and Zeta Potential. The test results for the evaluation of the apigenin nanoemulsion characterization are listed in Table 3.

3.3. Evaluation of Characterization of Buccal Film Nanoemulsion Apigenin (Buccal Film Ne-APG)

Characterization tests carried out included wet mixed organoleptic and buccal film, wet mixture viscosity, wet mix pH, thickness uniformity, folding resistance, weight uniformity, surface pH, release test, cluster function, and an assay of the buccal film Ne-APG. The test results for the evaluation of the buccal Ne-APG films are listed in table 4.

4. Discussion

4.1. Pre-Formulation and Characterization

Apigenin was obtained from Hefei Dielegance Biotechnology Co., Ltd. The results of the organoleptic characterization test for apigenin are odorless and yellow and have a pH of 6,8±0,0461.

The apigenin solubility test was carried out first by determining the maximum wavelength (max) using a UV-Vis spectrophotometer with a concentration of 2000 ppm, apigenin max was obtained at 334 nm and absorbance of 0.683 as shown in Figure 1.

Then, a standard curve with 5 concentration series was made with the obtained linear regression equation y = 0.0003x + 0.0133 a correlation of R2 = 0,9998. The correlation coefficient value of 0.9998 is close to 1 which means there is a linear relationship between concentration and absorption and fulfills Lambertbeer's law. The solubility test of apigenin with a concentration

Table 4. Results of Evaluation of the Characterization of Buccal Film Ne-APG

Test Parameters	Formula			
	F1	F2	F3	Blanko
Viscosity CBF-Ne APG (cP)	125,33±2,3094	117,33±2,3094	82,67±2,3094	54,67±2,3094
pH CBF-Ne APG	6,60±0,0057	6,61±0,0057	6,62±0,0057	6,55±0,0057
Thickness Film (mm)	0,0788±0,0050	0,0866±0,0057	0,1333±0,01	0,0667±0,0087
Folding Resistance	>300 times	>300 times	> 300 times	>300 times
Weight Film/Sheet 3 cm2 (mg)	57,67±0,1527	82,3±0,2	100,8±0,2516	54,73±0,1527
Surface pH	6,89±0,0057	6,88±0,0057	6,87±0,0057	6,87±0,01524
Assay (mg/sheet film)	24,3110±0,0193	54,8533±0,2029	97,9333±0,6333	-

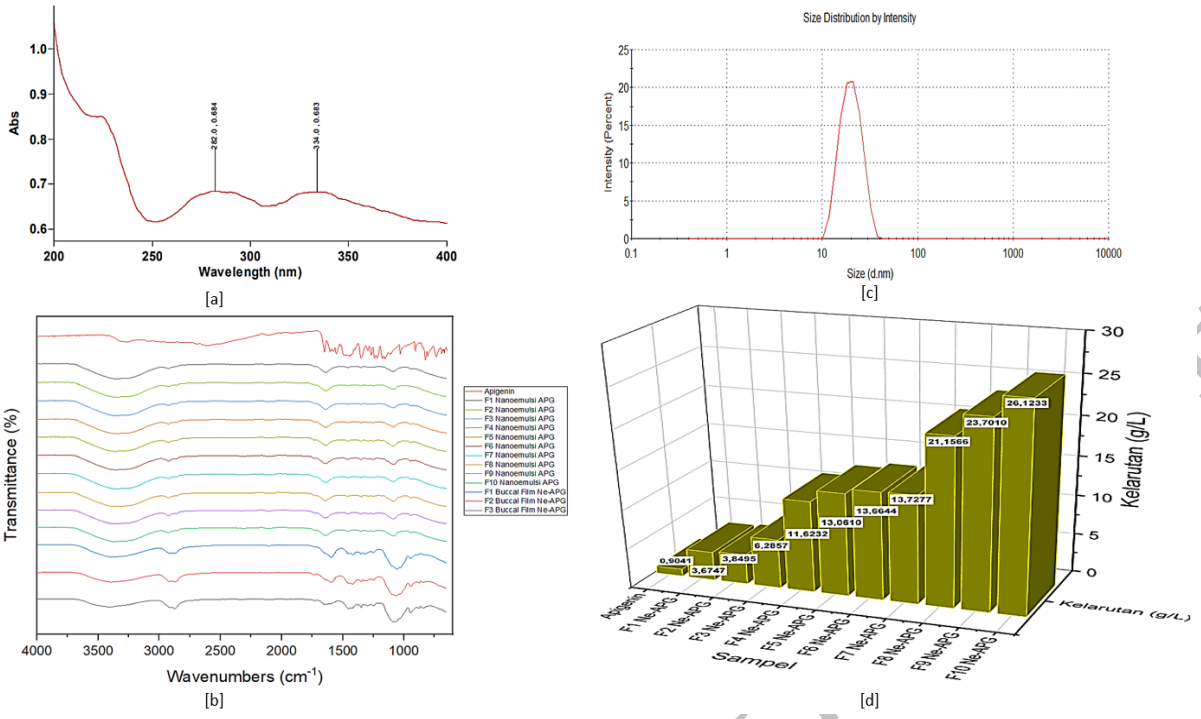

Fig 1. (a) The result of determining the maximum wavelength (λmax) of Apigenin. (b) Results of FT-IR Apigenin, Nanoemulsion, and Buccal Film Ne-APG. (c) Globule Size Distribution Curve. (d) Graph of Apigenin Nanoemulsion Solubility Test Results. Each graph of Apigenin and F1-F10 Apigenin Nanoemulsion is the result of the average standard deviation, n=3.

of 2500 ppm resulted in apigenin solubility of 0.9041 g/L±0.0082 and %transmittance of 24.961%±0.1123.

Testing of functional groups on apigenin, apigenin nanoemulsion, and buccal films of apigenin nanoemulsion was carried out using FT-IR (Fourier Transform Infrared) which is listed in Figure 1 which has a hydroxyl group in the wave number range of 3412 - 3287 cm-1, present in the number wave 3500 - 3200 cm^{-1} is where the hydroxyl group[29].

4.2. Evaluation of Apigenin Nanoemulsion Characterization

The organoleptic test of apigenin nanoemulsion on 10 formulas produced a transparent yellow color, odorless and clear with a pH value in the range of 6.23 - 6.59 listed in Table 3, this met the requirements for the pH value of saliva in the mouth, namely 5.6 – 7.4[16].

Based on the results of the apigenin nanoemulsion globule size test on 10 formulas, it was found that the globule size ranged from 17.48 to 20.34 nm as listed in table 3 (with the normal globule size distribution curve) listed in Figure 1, the nanoemulsion globule size requirements were <100 nm[30]. Oil and surfactants produce a stable system that supports a decrease in globule size when in the form of nanoemulsions[31]. The use of surfactants and co-surfactants in nanoemulsion formulation technology can reduce surface tension causing a decrease in Gibbs free energy, thereby causing a decrease in particle size[32].

The %transmittance test showed a clear and transparent visual with the results of apigenin nanoemulsion listed in table 3 obtained in the %transmittance range of 95.453% - 99.1% compared to apigenin which is 24.961%, this has met the requirements for a good nanoemulsion %transmittance ranging from 90-100% where the greater the transmittance value, it can be estimated that the nanoemulsion droplets have reached nanometer size with clear and transparent visuals[33].

Testing the polydispersity index is the uniformity of the globule size and the physical stability of a dispersion system where a value of<0.2 indicates a uniform and monodispersed globule size distribution and if it is close to 1, it indicates a polydispersity system[34]. The

requirement for the polydispersity index value of nanoparticle preparations is <0.5[35]. Based on the test results of apigenin nanoemulsion, 10 formulas met the requirements which were in the range of 0.056 – 0.131 listed in Table 3, indicating that the resulting globule size distribution was uniform and stable in the long term (uniform size distribution).

The zeta potential test showed the physical stability of the nanoemulsion containing dispersed globules through repulsion between particles of the same charge when they are close together[36]. Based on the measurement results, the zeta potential value of apigenin nanoemulsion for the 10 formulas listed in table 3 is in the range (-17.7) mV - (-5.53) mV (close to 0), where the zeta potential value is greater (+20 mV) or less than (-20 mV) will be sterically stable by the presence of non-ionic surfactant polymer chains in micelles[14].

The results of the solubility test can be seen in Figure 1. Based on the results of the solubility test of apigenin nanoemulsion, the formula that produces the highest solubility is F10, which is 10 mg/1 gr SNE with a 29-fold increase in solubility compared to pure Apigenin. Thus, the higher the concentration of the active substance in the nanoemulsion, the higher the increase in drug solubility. In this study, Kolliphor RH40 as a surfactant was able to dissolve apigenin of 19.66 mg/mL±0,15 compared to Pine Oil Emulsifier 8.30 mg/mL±1,73 and Labrasol 7,70±0,88. Then, PEG 400 as a co-surfactant was able to dissolve apigenin at 14.03 mg/mL±1.73 compared to Ethanol 3.53 mg/mL±0.10, 1,2-Propanediol 1.74 mg/mL±0.16 and Plurolâ 0.35 mg/mL± 0.04[9]. The use of surfactants and co-surfactants can reduce the surface tension which results in a decrease in the Gibbs free energy which causes a decrease in the particle size. Equation Noyes & Whitney shows that the dissolution rate is directly proportional to the surface area of the active substance in contact with the solvent. If the surface area of the active substance is increased, the absorption process will increase[37]. Thus, by reducing the particle size using nanoemulsion formulation technology, it can increase the surface area of the active substance in contact with the solvent by lowering the surface tension to increase the solubility and dissolution of the drug.

Based on the results of statistical data analysis with SPSS, F10 was chosen, namely 10 mg/1 g of SNE Apigenin to be incorporated into the buccal film because it had the highest solubility increase of 29 times compared to pure Apigenin with a significance value of ≤0.05 which showed a significant difference and the higher the concentration used will facilitate the loading dose preparation the buccal film and all characterization tests meet the requirements[38].

4.3. Evaluation of Characterization of Buccal Film Nanoemulsion Apigenin (Buccal Film Ne-APG)

The organoleptic test of the wet mixture and buccal film against 3 Formulas produced a transparent yellow color, odorless and clear, while the blank was transparent, odorless, and clear as shown in Figure 2.

Viscosity testing of the film aims to determine the viscosity of the wet mixture because the viscosity produced is related to the rheological properties or the ability of ease of casting. The results obtained are in the range of 54,67 - 125,33 cP listed in table 4, up to the 4th Formula produced produces ease in pouring into the mold. Then, the pH of the wet mixture that was produced was suitable, namely 6.55 - 6.62 listed in table 4 including the pH of oral saliva ranging from 5.6 - 7.4[16].

Testing the thickness and weight of buccal film apigenin nanoemulsion film is directly related to the accuracy of the dose in the film. This is evident in the results of the determination of the buccal film where the higher the concentration of the active substance used, the greater the content contained in the film weight and thickness film which is directly related to the amount of drug in the film, where F3 with a concentration of 0.75 thickness and weight film compared to other formulas, namely 0.1333 mm and 100.8 mg with levels of 97.9333 mg/ film listed in table 4. The results of the film of the 4 formulas are in the range of 54.73 – 100.8 mg which

is listed in table 4. Meanwhile, the thickness of the film obtained listed in table 4 is in the range of 0.0677 – 0.1333 mm which has met the requirements for the ideal thickness of the buccal film, which is 0.05 – 1 mm[39].

The folding endurance test was carried out to determine the flexibility of the film which indicates that film resulting results obtained from the 4 formulas showed folding resistance of more than 300 times as listed in table 4. Thus, the buccal film apigenin nanoemulsion produced met the requirements of film, namely if it had folding resistance >300 times[13].

The surface pH test aims to ensure film that the resulting buccal. The surface pH value of a buccal film ranges from 5.6 to 7[26]. Of the 4 buccal formulas film, apigenin nanoemulsion film has met the requirements. Because films with too large an acid or alkaline pH can affect the application area and cause damage to the oral mucous membranes, causing patient discomfort[31].

Drug release testing was In vitro carried out using a Franz diffusion cell membrane in which there were 2 compartments, namely the receptor and donor compartments. The receptor compartment is filled with a phosphate buffer solution of pH 6.8, while the donor compartment is filled with buccal film apigenin nanoemulsion by stirring using a magnetic stirrer at 37°C so that the active substance in the film can diffuse through the membrane from the donor compartment to the receptor[28]. 5 mL of fluid from the receptor compartment was taken periodically, namely at 15, 30, 45, 60, 120, 180, and 240 minutes to measure its absorbance through a UV-Vis spectrophotometer at a wavelength of 334 nm and each receptor fluid was taken. Solution with 5 mL of phosphate buffer solution. The results of the drug release test for buccal films can be seen in Figure 2.

In Figure 2 it can be seen that the F3 buccal apigenin nanoemulsion film constantly diffuses % increases with time with the highest % diffused result at 240 minutes producing 86.07% diffused % compared to F1 and F2 only producing 59.04 % diffused % for F1 and 71.09% for F2. So, F3 is the fastest drug release and the highest amount where the peak drug release is diffused 2 times faster with the amount of % diffused is 86.07% compared to F1 and F2. Thus, F3 has a faster onset time of drug release in releasing the active substance from the film with the highest amount of % diffused. Therefore, the greater the amount of drug released, the greater the level of active substances that survive in the body to increase the effectiveness of the resulting therapy[40].

Based on the test results of drug release, buccal film apigenin nanoemulsion where the release kinetics are first order, the release rate in the system depends on the concentration[41].

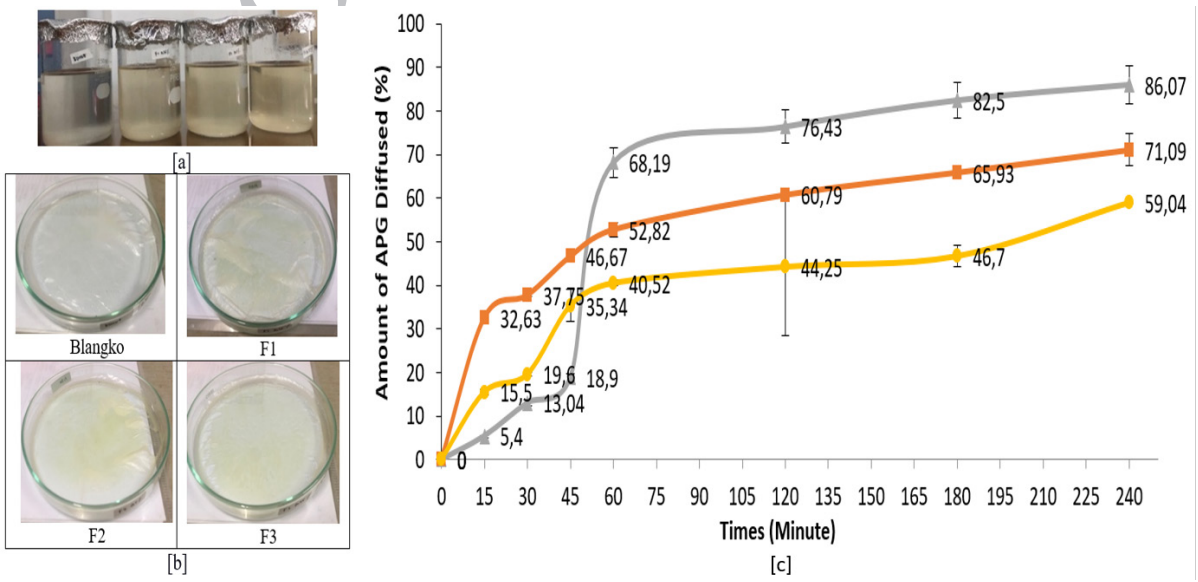

Fig 2. Organoleptic Buccal Film Ne-APG [a], Wet Mixed Organoleptic Buccal Film [b], and Amount of diffused Apigenin (APG) (%) versus time (minutes). Each curve of F1 Buccal Film Ne-APG (—), F2 (—), and F3 (—) are the result of the average standard deviation, n=3 [c].

This was proven when F3 with the highest concentration of 0.75% resulted in faster drug release and the highest number of diffused drugs with 2x faster diffusion time of drug release than F1 and F2.

5. Conclusion

Based on the results of the research, the formulation and characterization of 10 apigenin nanoemulsion formulas fulfilled the evaluation requirements with the best formula F10 to be used as buccal film, which had the highest solubility, namely 29 times compared to pure apigenin with $p \leq 0.05$, which would facilitate loading doses of preparations buccal film. formulas buccal film met the evaluation requirements with F3 having a faster onset of drug release time in releasing the active substance from preparation film 2x as compared to F1 and F2 which affects the effectiveness.

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