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Original Research Article

Design, Evaluation, and Activity Testing of Buccal Film Apigenin Nanoemulsion Drug Delivery System as Antidiabetic Agent

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ABSTRACT

Apigenin (4',5,7-trihydroxyflavone) is a flavonoid with antidiabetic potential, but has low water solubility (2.16 µg/L). To overcome this challenge, nanoemulsion formulations were developed to improve apigenin solubility. This research aimed to produce an apigenin nanoemulsion (APG-Ne) that meets quality requirements and can be incorporated into buccal film to facilitate application. Apigenin nanoemulsions (APG-Ne) (F1 – F3) were formed by the self-nanoemulsion method, while buccal film apigenin nanoemulsions (Buccal film APG-Ne) (F1 – F5) were made using the solvent-casting method. All formulations were evaluated for their organoleptic and physicochemical properties. The antidiabetic activity of the formulations was evaluated *in vitro* by alpha-glucosidase inhibitory assay, and *in vivo* using alloxan-induced diabetic rabbits. APG-Ne met the requirements for self-nanoemulsion with globule size of < 29.74 nm, polydispersity index (PDI) < 0.9766, pH 6.21 - 6.20, and zeta potential close to 0 mV. The best formula was F3 APG-Ne with significantly improved solubility 34 times higher than that of pure apigenin. All buccal film APG-Ne formulations met the quality criteria, with F5 showing the highest percentage drug release (71.06 ± 0.08% and 0.1879 ± 0.07 g/film sheet). APG-Ne (F3) and buccal film APG-Ne (F5) exhibited potent antidiabetic activity by significantly inhibiting α-glucosidase enzyme (IC_{50} = 55.23 ppm, and 48.03 ppm, respectively), and lowering blood glucose in alloxan-induced diabetic rabbits, producing a 66 ± 11.14 mg/dL decrease in blood glucose after seven days of treatment. Therefore, buccal film APG-NE is an innovative drug delivery system with improved solubility and bioavailability of apigenin as potential antidiabetic agent.

Keywords: Apigenin, Solubility, Biopharmaceutical Classification System, Nanoemulsions, Buccal Film, Alpha-glucosidase enzyme.

Introduction

According to the International Diabetes Federation (IDF), Indonesia is among the top 10 countries or regions with the highest prevalence of diabetes among the adult population of 20 - 79 years, with about 19.5 million people living with diabetes in 2021, and predicted to increase to 28.6 million by 2045.[1] Diabetes is categorized into four categories, namely; type 1 diabetes, type 2 diabetes, gestational diabetes, and specific diabetes caused by other factors. Type 2 diabetes mellitus occurs due to increased blood glucose levels caused by reduced insulin secretion from β-pancreatic cells and insulin resistance.[2]

Apigenin is a flavonoid with three hydroxyl substituents that belongs to the flavone subclass, an aglycone from several natural glycosides with the molecular formula $C_{15}H_{10}O_5$ and a molecular weight of 270.24 g/mol.[3] The primary sources of flavones are parsley *(Petroselinum crispum*) and peppers *(Piper nigrum)*, with total flavone contents of 13.526 mg/100 g and 4.98 mg/100 g, respectively.[4]

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Additionally, celery *(Apium graveolens)* is the dominant source of apigenin, containing 338.5 mg/kg dry weight.[5] Apigenin has been shown to posses antidiabetic properties by lowering blood glucose levels in the blood and increasing insulin levels in streptozotocin-induced rats.[6] Although apigenin has therapeutic potential as an antidiabetic agent, it is constrained by its poor bioavailability, which can be attributed to its low rate of dissolution, solubility, and gastrointestinal absorption, which reduces its therapeutic effectiveness.[7] Apigenin has low solubility in water with a value of 2.16 µg/L at pH 7.5, and as a result, it is included in Class II of the Biopharmaceutical Classification System (BCS).[8] Therefore, enhancing the solubility is essential to improve the dissolution rate and bioavailability of apigenin.

Formulation technology has been developed to improve solubility by making nanoemulsions using the spontaneous emulsification method (Self Nanoemulsion) to overcome the limitations of solubility and bioavailability of apigenin. Nanoemulsions are chosen as a formulation technology to improve solubility because they can increase dissolution rate and bioavailability, avoid enzyme degradation, and improve the physical and chemical stability of the preparation on long-term storage.[9] The nanoemulsion is inserted into buccal film to facilitate the use of apigenin nanoemulsion (APG-Ne) as an antidiabetic agent. Buccal film is a formulation designed to deliver medication through the oral cavity system. The buccal mucosa has good accessibility, allowing drugs to be absorbed directly through the jugular vein into the systemic circulation. The jugular vein is the pathway by which drugs bypass the liver from the digestive system.[10] In addition, this pathway can avoid acid hydrolysis in the digestive tract and bypass the first-pass metabolism in the liver, thereby increasing the bioavailability of the drug.[11]

Therefore, this study aims to acquire the APG-Ne formula based on the best evaluation results and the buccal film APG-Ne that meets the evaluation criteria and provides the best dose as an antidiabetic drug formulation. The development of the buccal film APG-Ne is expected to overcome the obstacle of poor solubility of apigenin. In addition, the formula makes it easy for patients with difficulty swallowing, such as pediatric and geriatric patients, and can be administered to less

cooperative patients during therapy. Thus, it is an innovation to improve solubility and bioavailability and maintain steady blood levels for more extended release of apigenin for a more effective therapeutic effect as antidiabetic.

Materials and Methods

Chemicals and reagents

Apigenin (Hefei Dielegance Biotechnology Co., Ltd), Sunflower oil (Jan Dekker International), Kolliphor®RH40 (BASF), Polyethylene glycol 400 (PEG 400), Sodium alginate (Na Alginate), Sucralose, and Aquadeion (Merck, Tbk), Sodium carboxymethyl cellulose (Na CMC) (Dai-Ichi Kogyo Seiyaku Co., Ltd, Tbk), acarbose (Dexa Medica), p-nitrophenyl-α-D-glucopyranoside (PNPG) substrate (Sigma-Aldrich N1377-1G), and α-glucosidase enzyme (Sigma-Aldrich G6003-100UN).

Pre-formulation of apigenin

The tests carried out in the pre-formulation of apigenin include organoleptic, pH (Ionix, Singapore), functional group analysis using Fourier Transform Infrared - Attenuated Total Reflectance (FTIR-ATR, Malaysia), and solubility (Agilent Cary 60, Malaysia).

Design of APG-Ne formula by self-nanoemulsion (SNE) method

Three formulas were made with concentrations of 11 mg, 13 mg, and 15 mg apigenin/1 gram SNE using the self-nanoemulsion method using the best nanoemulsion base combination components with a ratio of 1:8:1 (Sunflower oil: Kolliphor®RH 40: PEG 400).[12] The design of the APG-Ne formula is listed in Table 1.

APG-Ne was made using the SNE method, which involves mixing the oil, surfactant, and co-surfactant phases and stirring using a magnetic stirrer (IKA® C-MAG HS7, Germany) for 20 minutes. Then, apigenin was added, and stirred again with a magnetic stirrer for 10 minutes and sonicated for 1 hour. Thereafter, aquadeion was added, then stir again with a magnetic stirrer. Nanoemulsions was formed spontaneously with the addition of aquadeion and light stirring.[13]

APG-Ne evaluation

APG-Ne was evaluated based on the following parameters; organoleptic, pH (Ionix, Singapore), solubility (Agilent Cary 60, Malaysia), functional groups (FTIR-ATR, Malaysia), TEM (TEM JEOL JEM 1400, Japan), PDI, globule size and zeta potential (Malvern Instruments Ltd., Zetasizer, Canada).

Design of buccal film APG-Ne formula

Buccal film was produced using solvent casting method, the most common technique in producing buccal film.[14] The formula design of buccal film APG-NE consists of 5 formulas with variations of active substances, as listed in Table 1. Na CMC and Na Alginate were dissolved in aquadeion and stirred with a magnetic stirrer (IKA® C-MAG HS7, Germany). Thereafter, APG-Ne, PEG 400, and sucralose were added to the solution and stirred until homogeneous, then the remaining aquadeion was added. The solution was poured into a mold and subjected to drying at 50°C in an oven (Memmert®, Germany) for a duration of 24 hours until a film was formed. Subsequently, the film was cut into 3 x 3 cm² sizes using a sterile scalpel.[15]

Evaluation of the buccal film APG-Ne

The buccal film APG-NE was evaluated by performing the following tests; organoleptic, viscosity (Brookfield viscometer, RVDV 10, United Kingdom), and pH (Ionix, Singapore) of Wet Mixture Film Apigenin Nanoemulsion (WMF APG-Ne), uniformity of film thickness (Mitutoyo® Micrometer, Japan) and weight (Mettler Toledo, Switzerland), folding fastness, surface pH (Ionix, Singapore), tensile strength, %break elongation, functional group (FTIR-ATR, Malaysia), release test (Franz diffusion cell, Ward`s Science, United Kingdom), and determination of buccal film APG-NE level (Agilent Cary 60, Malaysia).

Table 1: Formula Design for APG-Ne and Buccal Film APG-Ne

APG-Ne Formula Design with SNE Method				
Composition		**Amount (% w/v)**		
		F1	**F2**	**F3**
Apigenin (mg)	Active Pharmaceutical	11	13	15
Sunflower Oil:	Oil	1:8:1 (1 gram)		
Kolliphor®RH40:	Surfactant			
PEG 400 (g)	Co-Surfactant			
Aquadeion (g)	Solvent	ad 20 gram		

Buccal Film APG-NE Formula Design with Solvent Casting Method							
Component		**Amount (% w/v)**					
		F1	**F2**	**F3**	**F4**	**F5**	**Blank**
APG-Ne (Equivalent to 15 mg/g SNE)	Active Pharmaceutical	1	2	3	4	5	-
Na CMC	Polymer	2.5	2.5	2.5	2.5	2.5	2.5
Na Alginat	Polymer	0.1	0.1	0.1	0.1	0.1	0.1
PEG 400	Plasticizer	0.975	0.975	0.975	0.975	0.975	0.975
Sukralose	Sweetener	0.01	0.01	0.01	0.01	0.01	0.01
Aquadeion	Solvent	ad 100					

In vitro antidiabetic assay of APG-Ne and buccal film APG-Ne
Sodium phosphate buffer (360 µL of 0.2 M pH 6.8) was placed into a vial, followed by the addition of acarbose (5, 7, 9, 11, 13 ppm), APG-NE (20, 40, 60, 80, 100 ppm), buccal film APG-Ne (20; 40; 60; 80, 100 ppm) and 170 µL of PNPG substrate. The mixture was incubated for 5 minutes in a water bath (37℃). The enzyme α-glucosidase (170 µL) was added and the mixture was re-incubated for 25 minutes at the same temperature. The reaction was stopped by the addition of 1000 µL of Na_2CO_3 (200 mM), thereafter, the absorbance was measured at 405 nm using a UV-visible spectrophotometer (Agilent Cary 60, Malaysia).[16] The percentage inhibition of α-glucosidase was calculated using the formula below:

$$\%\ Inhibition = \frac{(Ac - Acb) - (As - Asb)}{(Ac - Acb)}\ x\ 100$$

Where;
Ac: Absorbance of the negative control (phosphate buffer and enzyme)
Acb: Absorbance of the control blank (phosphate buffer without enzyme)
As: Absorbance of the sample (inhibitor and enzyme)
Asb: Absorbance of the sample blank (inhibitor without enzyme)

In vivo antidiabetic assay of APG-Ne and Buccal Film APG-Ne
Animals
Twenty-four (24) 2.5 – 3 months old male albino rabbits of the New Zealand strain weighing between 0.67-1.9 kg were obtained from the Asta Rabbit Tasikmalaya, Tasikmalaya Regency, West Java, Indonesia. The rabbits were housed in well-ventilated cages and acclimated to laboratory conditions for five days, with a room temperature of 22 ± 2℃ and a 30% - 70% relative humidity.

Ethical approval
The protocol for the use of rabbits as experimental animals in this study received approval from the Health Research Ethics Committee of Bakti Tunas Husada University, Tasikmalaya, Indonesia, under the ethical approval certificate number 047/E.02/KEPK-BTH/VII/2024.

Induction of diabetes
Before induction, the rabbits were fasted for 8 hours and their blood glucose levels (Day 0) were measured. Then, the rabbits were induced by an intravenous dose of 150 mg/kg BW of alloxan. Diabetes was confirmed when a 2-hour postprandial blood glucose value ≥ 200 mg/dL was achieved.[17]

Evaluation of antidiabetic activity
After the diabetic state has been achieved (Day 3), all test animals were treated according to their group (Table 2) for seven days (Days 4 to 10). At the end of the treatment period, blood samples were drawn through the rabbit ear veins, and blood glucose levels were determined 2 hours after treatment using the Accu-Check® active blood glucose meter kit (Roche, Germany). The buccal film APG-Ne was administered by placing it in the rabbit buccal area.[18]

Statistical analysis
Data were presented as mean ± standard deviation (SD). The data were analyzed statistically using the IBM SPSS Statistics version 29 software, and normality was tested using the Shapiro-Wilk test. Differences between means were analyzed using One-Way analysis of variance (ANOVA), followed by LSD post hoc test. P-value < 0.05 was regarded as significant at 95% confidence level.

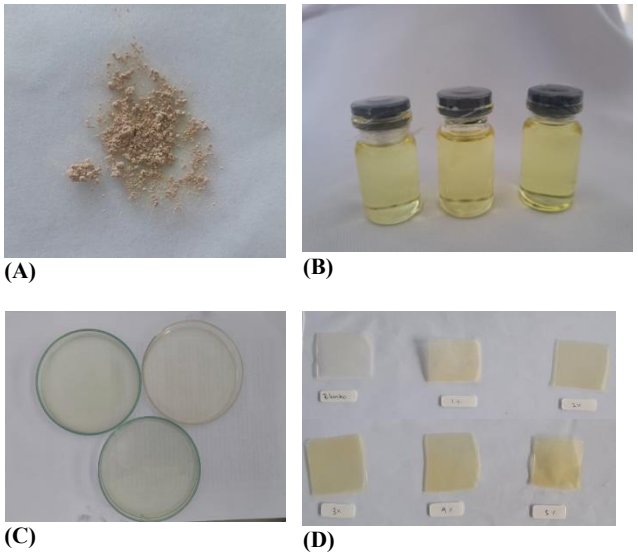
Results and Discussion

Organoleptic and physicochemical properties of pre-formulated apigenin
The pre-formulated apigenin appeared as a yellow, odorless powder (Figure 1), with a pH of 6.56 ± 0.007.

Table 2: Antidiabetic activity Study Design

Group	Test Design
I	Rabbits were induced with Alloxan 150 mg/kg BW, then given buccal film APG-Ne at a dose of 19.5 mg/1.5 kg BW Rabbits were placed in the buccal area 1 x 1 day for 7 days.
II	Rabbits were induced with Alloxan 150 mg/kg BW, then given APG-Ne at a dose of 19.5 mg/1.5 kg BW Rabbits were placed in the buccal area 1 x 1 day for 7 days.
III	Rabbits were induced with Alloxan 150 mg/kg BW, then given pure apigenin at a dose of 19.5 mg/1.5 kg BW, Rabbits were placed in the buccal area 1 x 1 day for 7 days.
IV	Positive Control (Rabbits were induced with Aloxan 150 mg/kg BW, then given Glimepiride 1 mg at a dose of 19.5 mg/1.5 kg BW Rabbits were placed in the buccal area 1 x 1 day for 7 days.
V	Normal Control.
VI	Negative Control (Alloxan only).

The UV spectrum showed a λ max of 274 nm. A standard curve of apigenin at the λmax produced a linear regression equation y = 0.0025x – 0.0113 (R^2 = 0.9975) (Figure 2). From the standard curve, the solubility of apigenin was determined as 0.9 ± 0.001 g/L (Figure 3). In addition, functional group analysis of apigenin, APG-Ne, and buccal film APG showed absorption peaks at 3499 - 3201 cm^{-1} which corresponds to the presence of hydroxyl group (wave number = 3500 - 3200 cm^{-1}).[19]



(A) **(B)** **(C)** **(D)**

Figure 1: Organoleptics of Apigenin **(A)** APG-Ne **(B)** WMF APG-Ne **(C),** and Buccal Film APG-Ne **(D)**

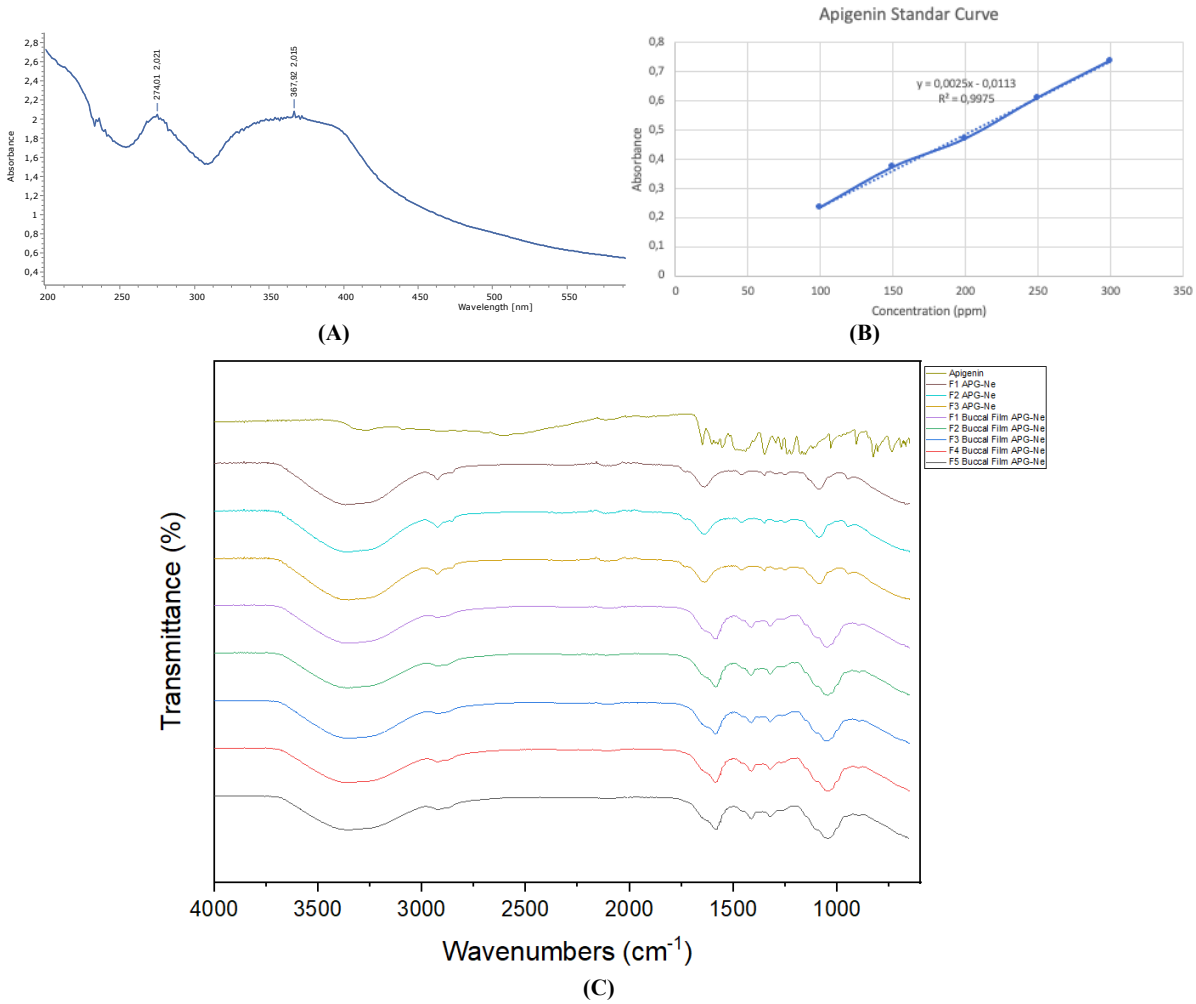


Figure 2: UV spectrum showing the λ max of Apigenin **(A)** Apigenin Standard Curve **(B),** and FTIR spectrum of Apigenin **(C)**

Organoleptic and physicochemical properties of apigenin nanoemulsion (APG-Ne)

APG-Ne was observed as a yellow, odorless, clear emulsion (Figure 1) with a pH ranging from 6.21 – 6.29 (Table 3), consistent with the normal salivary pH range of 5.6 – 7.4.[20] The Zetasizer Malvern® instrument was employed to assess globule size, polydispersity index, and zeta potential through light scattering techniques, thereby facilitating the prediction of emulsion stability.[21] The normal globule size distribution is shown in Figure 3 with APG-Ne globule size of 22.21-29.74 nm (Table 3). These results show that the size of the APG-Ne globules met the criteria for nanoemulsions (with a size requirement of less than 100 nm).[22] The shape of the APG-Ne globule was determined using Transmission Electron Microscopy *(*TEM) and it was found that APG-Ne has a spherical shape (Figure 3), which is consistent with the shape of apigenin-loaded SNEDDS.[23] The APG-NE had a polydispersity index of 0.9399 – 0.9766 (Table 3), which met the requirement for nanoemulsion. A PDI < 0.5 indicate a homogeneous and uniformly distributed globule size, and also suggest that the formulation is stable over a long period of time.[24]

Table 3: APG-Ne evaluation results

Formula	Parameter			
	pH	Globule size (nm)	Polydispersity index	Zeta potential (mV)
F1	6.21 ± 0.03	27.89 ± 0.39	0.9502 ± 0.0013	-7.963 ± 0.41
F2	6.27 ± 0.02	29.74 ± 5.03	0.9399 ± 0.0097	-8.046 ± 0.36
F3	6.29 ± 0.02	22.21 ± 0.03	0.9766 ± 0.0021	-7.233 ± 1.12

Data represent mean ± standard deviation (SD), n=3.

In addition, the physical stability of the nanoemulsion is determined by the zeta potential of APG-Ne (-7.233 mV) – (- 8.046 mV) (Table 3), and the presence of a non-ionic surfactant polymer chain within the micelle. The nanoemulsion is sterically stable if the zeta potential value is > +20 mV or < -20 mV.[12]

Based on the solubility test results presented in Figure 3, the formulation demonstrating the highest solubility was F3 (15 mg/1 g SNE), attaining 31.47 ± 0.145 g/L. This constitutes a 34-fold increase in comparison to apigenin, which exhibited a solubility of 0.9 ± 0.001 g/L. The data indicate that increasing the concentration of apigenin within nanoemulsions directly correlates with an enhancement in the drug's solubility. In the conducted study, Kolliphor®RH40 was employed as the surfactant for dissolving apigenin, achieving a solubility of 19.66 ± 0.15 mg/mL.

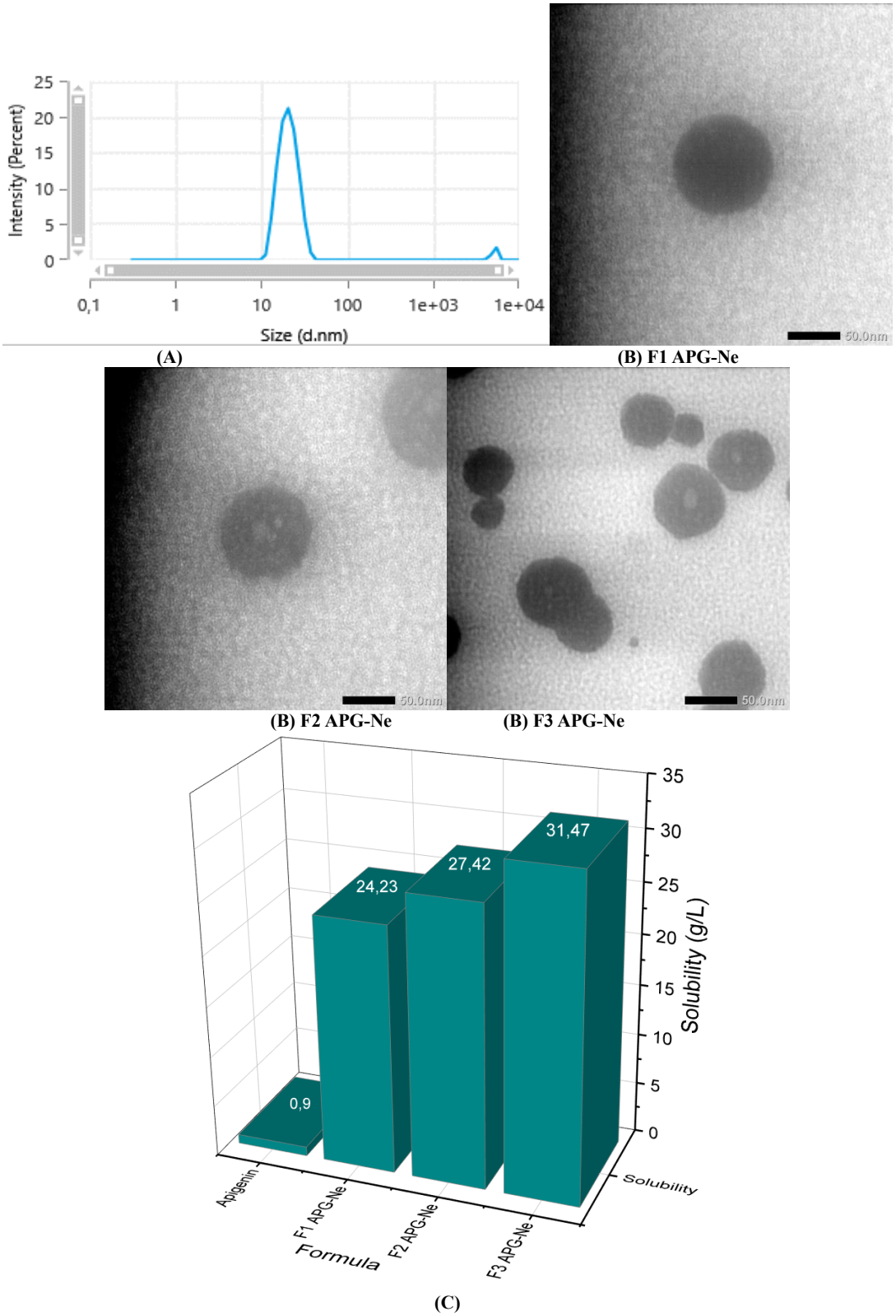


Figure 3: Globule Size Distribution Curve **(A)** Spherical APG-Ne TEM Results **(B)** and Graph of Solubility Test Results **(C)**

Conversely, a lower solubility of 14.03 ± 1.73 mg/mL was reported when PEG 400 was used as a co-surfactant for the dissolution of apigenin.[25]

Surfactants and co-surfactants diminish the surface tension of emulsions by reducing Gibbs free energy and particle size. According to the Noyes-Whitney equation, the rate of dissolution depends on the surface area of the active substance that is in contact the solvent. An increase in this surface area accelerates the absorption.[26] Through the application of nanoemulsion technology to decrease particle size, the active substance's surface area in contact with the solvent is augmented owing to reduced surface tension. This improvement enhances solubility and accelerates drug dissolution.

Self-nano-emulsifying technology increases bioavailability by lowering the surface tension and enlarging the oil-water interface area. This allows the active substance that is difficult to dissolve in water to

immediately form an oil-in-water emulsion (o/w) when in contact with gastric fluids. The drug dissolves *in situ* and is absorbed through the lymphatic tract without experiencing first-pass metabolism.[27]
Based on the statistical analysis, the formula F3 APG-Ne was selected for buccal film due to its highest solubility, which was 34 times greater than that of apigenin ($p<0.05$). A high solubility increases loading dose, and improves the overall quality of the formulation.[28]

Organoleptic and physicochemical properties of the buccal film APG-Ne
The buccal film APG-Ne appeared as a yellow, odorless, and clear formulation (Figure 1). The formulation was easy to pour into the mold due to its favourable viscosity which is in the range of 140.4 – 312.26 cP (Table 4). In addition, the buccal film APG-Ne had pH in the range of 5.75 – 5.93 (Table 4), and this met the pH requirements of the saliva (pH 5.6 – 7.4).[20]
The evaluation of buccal film content showed that formula F5 contained the highest apigenin content, which was 96.57 ± 0.07 mg/film sheet (Table 4). The increase in weight and thickness also signifies uniformity, which is directly correlated with dose accuracy. Formula F5 weighed 0.1879 ± 0.07 g/sheet of film, and had a thickness of 0.2355 ± 0.0134 mm (Table 4), which met the ideal buccal film thickness requirement (0.05 - 1 mm).[29]

Table 4: Evaluation Results of the Buccal Film APG-Ne

Test Parameter	Formula					
	F1	F2	F3	F4	F5	Blank
Viscosity WMF APG-Ne (cP)	155.06 ± 0.46	156.26 ± 0.23	190.4 ± 5.38	162.53 ± 1.97	312.26 ± 1.4	140.4 ± 0.4
pH WMF APG-Ne	5.91 ± 0.01	5.87 ± 0.0057	5.93 ± 0.04	5.81 ± 0.0057	5.75 ± 0.05	5.83 ± 0.02
Thickness Film (mm)	0.1266 ± 0.0033	0.1267 ± 0.015	0.1366 ± 0.0088	0.1633 ± 0.0057	0.2355 ± 0.0134	0.0838 ± 0.006
Weight Film/Sheet 3x3 cm^2 (g)	0.1316 ± 0.02	0.1433 ± 0.03	0.1493 ± 0.04	0.1732 ± 0.06	0.1879 ± 0.07	0.0791 ± 0.02
Folding Resistance	>300 times	>300 times	>300 times	>300 times	>300 times	>300 times
Surface pH	6.74 ± 0.01	6.77 ± 0.01	6.81 ± 0.02	6.84 ± 0.03	6.91 ± 0.01	6.97 ± 0.01
Tensile Strength (MPa)	18.91 ± 0.23	19.71 ± 0.53	20.22 ± 0.28	20.37 ± 0.35	20.48 ± 0.48	22.39 ± 0.25
Break Elongation (%)	4.36 ± 0.28	4.61 ± 0.32	4.84 ± 0.28	4.85 ± 0.25	5.30 ± 0.31	4.63 ± 0.27
Assay (mg/sheet film)	11.32 ± 0.07	13.22 ± 0.0092	22.49 ± 0.03	74.82 ± 0.12	98.57 ± 0.07	-

Data represent mean ± standard deviation (SD), n=3.

Folding durability test on all the formulas resulted in >300 times the folding resistance (Table 4). This indicates that the resulting buccal film has the flexibility of a film, not easy to tear, and met the ideal buccal film requirement (>300 times).[10] Furthermore, a surface pH test was carried out to ensure safety during use (buccal route) because an inappropriate pH will cause discomfort to the patient due to damage to the mucosal membrane.[29] The surface pH of the buccal film APG-Ne was found to be 6.74 – 6.97 (Table 4), which again met the pH requirement of the saliva and buccal mucosal (pH 5.6 – 7.4).[30]
In general, an ideal buccal film should have sufficient flexibility and elasticity to be able to adjust to the movement of the mouth comfortably when used. In addition, a buccal film must also have sufficient mechanical strength to resist abrasion arising from the movement of the tongue. Therefore, high tensile strength and percent break elongation are ideal mechanical characteristics for buccal film.[31] The tensile strength and %break elongation obtained in this study were 18.91 – 22.39 MPa, and 4.36 – 5.30%, respectively (Table 4), showing that the buccal film met the mechanical characteristics based on the JIS 1975 (Japanese Industrial Standard), which propose a minimum tensile strength of 0.39226 MPa. From the results of the study, the buccal film had sufficient flexibility and elasticity coupled with a high tensile strength and %break elongation. The flexibility, and elasticity of buccal film is usually influenced by polymers and plasticizers.[32,33] Without these materials, the resulting film is hard and easily tears when pressure is applied. Meanwhile, the presence of plasticizers increases the film's elasticity and improves its physical and mechanical properties.[34]
The buccal film APG-Ne drug release was evaluated using Franz diffusion cell membrane, with phosphate (pH 6.8) as the receptor compartment and the buccal film as the donor compartment. Liquid samples were taken from the receptor compartment at specific time interval to measure their absorbance.[35] The results showed that the buccal film APG-Ne exhibited a constant increase in the percentage of drug released, with formula F5 achieving the highest percentage of drug released of 71.06 ± 0.08% at 240 minutes compared to other formulas (Figure 4). Formula F5 showed percentage drug release twice that of F1 (38.4 ± 0.34%), indicating that the onset of drug release was faster in F5.

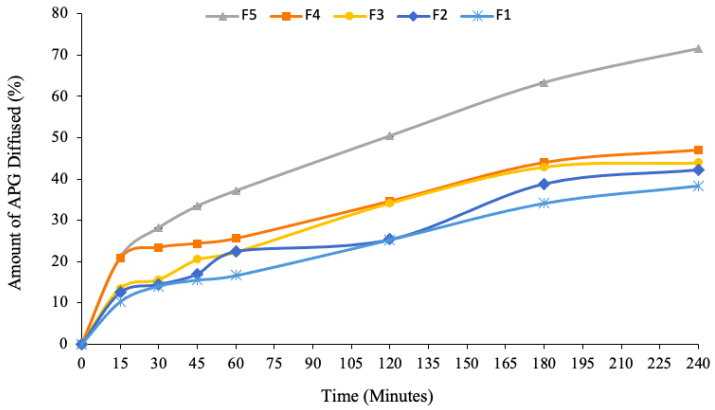


Figure 4: Amount of released Apigenin (APG) versus time (minutes).
Each curve of F1 Buccal Film APG-Ne (-), F2 (-), F3 (-), F4 (-) and F5 (-) The result of the average standard deviation is n=3.

These findings suggest that formula F5 possesses a superior potential for enhanced therapeutic efficacy due to its maintenance of a higher concentration of active substances within the body.[36] In addition, the kinetics of drug release in the buccal film APG-Ne followed the first order, i.e., the rate of drug release in the system depends on concentration, which was proven by the highest concentration of apigenin in formula F5 (5%) resulting in faster drug release.[37]

In vitro antidiabetic activity of APG-Ne and buccal film APG-Ne

The anti-diabetic activity of APG-Ne and buccal film APG-Ne was evaluated *in vitro* by measuring the inhibitory activity against the enzyme α-glucosidase. Acarbose was used as the standard antidiabetic agent. PNPG (p-nitrophenyl-α-D-glucopyranoside) was used as the source of carbohydrates that the enzyme will breakdown into glucose and p-nitrophenol.[16] The result of the α-glucosidase inhibitory assay is presented in Figure 5. The IC_{50} values were 55.23 ppm, 48.03 ppm, and 8.78 ppm for APG-Ne, buccal film APG-NE, and acarbose, respectively. The smaller the IC_{50} value, the higher the ability to inhibit the enzyme α-glucosidase. Based on these results, acarbose falls within the very active category as an antidiabetic agent (IC_{50} <11 ppm), while APG-Ne and buccal film APG-Ne are included in the active category as antidiabetics (IC_{50} = 11 – 100 ppm).[38] Apigenin inhibits the enzyme α-glucosidase by slowing down the formation and delivery of glucose, thereby reducing blood glucose after meals. By lowering blood glucose, apigenin reduces pressure on the pancreas caused by high blood glucose. It can also increase insulin sensitivity and potentially protect pancreatic function to prevent diabetes complications. One of the treatment strategies for diabetes involved the use of α-glucosidase inhibitors such as acarbose and miglitol. These agents slows down the digestion and absorption of carbohydrates in food, thereby helping to regulate blood glucose levels after meals. However, these drugs are often associated with gastrointestinal side effects such as diarrhea and abdominal pain as well as possible liver and kidney damage, resulting in long-term complications.[39] Therefore, buccal film APG-Ne can be used as an alternative to acarbose in diabetes treatment.

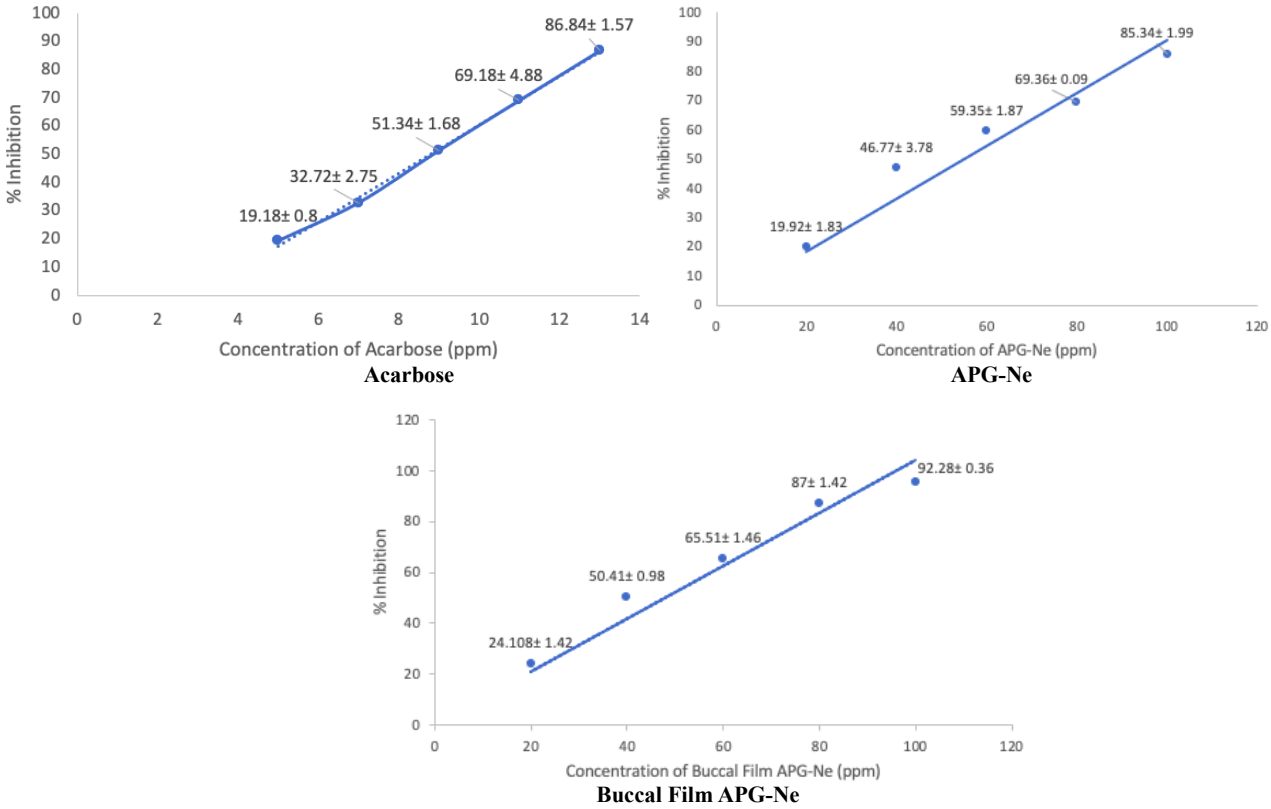


Figure 5: Alpha-glucosidase inhibitory activity of Apigenin, APG-Ne, and Buccal Film APG-Ne (mean ± SD, n=3)

In vivo antidiabetic activity of APG-Ne and buccal film APG-Ne

The *in vivo* antidiabetic activity of APG-Ne and buccal film APG-Ne was evaluated using alloxan-induced diabetic rabbits (*Oryctolagus cuniculus*). Glimepiride was used as the positive control. Glimepiride is an oral antidiabetic drug of the sulfonylurea class with a mechanism of action similar to flavonoids through increased insulin secretion in the pancreas.[40] From the result as shown in Figure 6, formula F5 of buccal film APG-NE significantly reduced blood glucose in diabetic rabbits, resulting in a 66 ± 11.14 mg/dL decrease in blood glucose level after 7 days of treatment. Similarly, formula F3 of APG-Ne reduced blood glucose level in the diabetic rabbits by 57 ± 8.23 mg/dL after 7 days. For the groups treated with glimepiride and pure apigenin, blood glucose levels decreased by 49 ± 8.24 mg/dL, and 45 ± 10.69 mg/dL, respectively after treatment for 7 days. Based on these results, the buccal film APG-NE produced the most significant ($P < 0.05$) reduction in blood glucose levels compared to APG-Ne, pure apigenin, and glimepiride. This is because the buccal film APG-NE administered by buccal route avoid acid hydrolysis within the gastrointestinal tract, bypasses first-pass metabolism in the liver, and is directly transported to the systemic circulation via the jugular vein. In this way, the bioavailability of the drug is increased, resulting in excellent accessibility to the tissues.[11] Apigenin exerts it antidiabetic action by inhibiting adrenaline-induced stimulation of α_2 receptor in pancreatic beta-cells, resulting in decreased blood glucose. It aids insulin release and increases glycogen levels in alloxan-induced diabetic rabbits by acting directly on the pancreas to stimulate insulin release. The hormone insulin then activates the enzyme glycogen synthase to initiate glycogenesis. When there is an increase in blood glucose, the pancreas secretes insulin to stimulate glucose storage in the form of glycogen in the liver and muscles.[41] In addition, apigenin functions as an inhibitor of sodium-glucose co-transporter 2 (SGLT2), thereby diminishing blood glucose levels by obstructing glucose reabsorption in the kidneys.[42,43] In hyperglycemic conditions, apigenin prevents the excessive production of reactive oxygen species, thereby reducing oxidative stress.[44] Apigenin binds free radicals through the hydroxy (OH) groups in its structure, and thus function as a potent free radical scavenger resulting in strong antioxidant activity, which contributes to its hypoglycemic effect.[45]

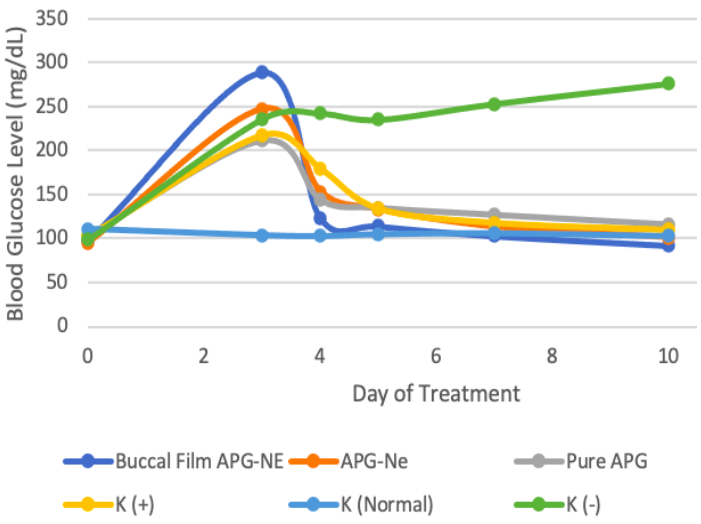


Figure 6: Hypoglycaemic effect of Apigenin, APG-Ne, and Buccal Film APG-Ne in alloxan-induced diabetic rabbits

Conclusion

From the findings of the study, all apigenin nanaemulsion (APG-Ne) formulated met the requirement for nanoemulsion with formula F3 APG-Ne having the highest solubility, 34 times higher than pure apigenin, making it easier for loading onto buccal film. In addition, all buccal film APG-Ne formulations met the evaluation criteria, with the F5 buccal film APG-Ne showing a faster drug release which was twice as fast as that of F1 buccal film APG-Ne in the release of active substances, leading to increased therapeutic effectiveness. *In vitro* and *in vivo* antidiabetic activity evaluation revealed that the three formulations (pure apigenin, APG-Ne, and buccal film APG-NE) possess potent antidiabetic activity, which was comparable to that of acarbose in inhibiting α-glucosidase enzyme, and glimepiride in lpwering blood glucose in alloxan-induced diabetic rabbit *(Oryctolagus cuniculus)*. Among the three formulations, buccal film APG-NE had the highest antidiabetic activity *in vitro* and *in vivo*. Thus, buccal film APG-NE is a novel drug delivery system that improves solubility and bioavailability of apigenin, and maintain steady blood levels for more extended release of apigenin for a more effective therapeutic effect as antidiabetic agent.

Conflict of Interest

The authors declare no conflict of interest.

Authors` Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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