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In Vitro Release Ability of Nanoparticles Poly-Lactic-Co-Glycolic-Acid (PLGA) Gel Containing Pegagan Leaves Ethanolic Extract (*Centella asiatica L.*)

Mardiyanto, Elsa Fitria Apriani*, M. Pandu Kalingga Jati
Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Sriwijaya University, Ogan Ilir, Indonesia

*Corresponding author: elsafitria@mipa.unsri.ac.id

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Abstract

Background*: Pegagan (Centella asiatica L.) leaves are proven to contain high concentrations of flavonoid compounds as antioxidants. Flavonoids are unstable compounds due to environmental influences such as light, humidity, pH, and oxygen. The stability of pegagan extract was proven to be improved by making the extract into nanoparticle preparations.* ***Objective****: This study aims to formulate nanoparticles of pegagan into gel preparations and determine their release ability with the Franz diffusion test using a cellophane membrane compared to pegagan gel not formulated into nanoparticles.* ***Methods****: Nanoparticles were made using poly-lactic-co-glycolic acid polymers and then formulated into gels with various concentrations of Carbopol 934, namely 1, 1.5 and 2%. The gel nanoparticles were then subjected to the characterization of the preparation, stability test and release test of the preparation.* ***Results****: A concentration of 1% Carbopol 934 provides the best evaluation of gel preparations where the gel produced was homogeneous, pH was around 6.2, viscosity was 3417.12 cPs, spreadability was 5.1 cm, and adhesion was 209.33 seconds. The stability test showed no significant organoleptic and pH changes ($p>0.05$). The release kinetics model occurs at zero order. F1 has a higher reaction kinetics constant (k) than the other formulations, so drug release occurs faster.* ***Conclusion****: The best formula of pegagan (F1) nanoparticle gel was proven to have good physical stability and release ability.*

Keywords: *Centella asiatica L; Nanoparticles; Gel; Franz Diffusion*

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INTRODUCTION

The development of herbal medicine in Indonesia is growing rapidly. One of the plants that are widely used in health is *Centella asiatica* L. leaves. *Centella asiatica* L. leaves are known to have an anti-inflammatory (Park *et al*., 2017), neuroinflammatory (Gelders *et al*., 2018), antioxidant (Jiang *et al*., 2016), anti-obesity (Rameshreddy *et al*., 2018), antidiabetic (Zheng *et al*., 2018), wounds healing (Sawatdee *et al*., 2016), anti-hypertension (Liu *et al*., 2017), etc. The ethanolic extract of pegagan leaves was proven to contain alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins, and reducing sugars (Arumugam *et al*., 2011). Flavonoids are the main compounds found in pegagan leaves. Based on research conducted by Quyen *et al*. (2020), the total flavonoid content of *Centella asiatica* L. leaves was higher than the total phenolic content, namely 23.03±2.89 mgQE/g and 2.14±0.29 mgGAE/g, respectively.

Flavonoids are phenolic compounds with a C6-C3-C6 chemical structure (Panche et al., 2016). Flavonoids are beneficial for health because they can protect the body from reactive oxygen species. Flavonoids will oxidize free radicals to become more stable and less reactive (Panche et al., 2016). However, the effectiveness of flavonoids can be reduced because flavonoids are less-stable compounds. Flavonoids are compounds sensitive to light, oxygen, pH, and temperature (Ioannou et al., 2012). Based on the research of Ioannou et al. (2020), the antioxidant activity of flavonoids was significantly reduced when flavonoids were exposed to excess heat for 2 hours. In addition, research by Ramesova et al. (2011) also proved that flavonoids degrade when exposed to atmospheric oxygen. To increase the stability of the flavonoids, the ethanolic extract of *Centella asiatica* L. leaves was formulated into nanoparticles.

Nanoparticles is a technology that aims to develop the dosage form size in the nano range, namely 200-500 nm (Mardiyanto et al., 2018). The polymer used is Poly-lactic-co-glycolic Acid (PLGA). While the stabilizer used is Polyvinyl Alcohol (PVA), which protects the nanoparticle preparation from the influence of temperature (Mardiyanto et al., 2018). The nanoparticles of *Centella asiatica* L. were then formed into a gel preparation.

Gel preparations have several advantages because they have a higher water component, making drug dissolution easier. Gels consisting of one phase have a faster drug release than creams consisting of two phases. This is evidenced by the research of Patel et al. (2009), where the release profile of psoralen gel preparations was greater than psoralen cream, namely 48.11 ± 2.1% and 25.96 ± 1.2. The release of active substances in gel preparations is also strongly influenced by gelling agents. The greater the concentration of the gelling agent used will produce a thick gel preparation that makes it difficult to release the active substance (Forestryana et al., 2020). The gelling agent used in this study was Carbopol 934. Carbopol 934 is a gelling agent that is commonly used because it has high compatibility and stability. In addition, the use of Carbopol 934 also provides a good release of active substances (Madan and Singh, 2010).

Based on the description above, the researchers formulated a nanoparticle gel of *Centella asiatica* L. with variations in carbopol 934 concentration. The gel was characterized and tested for release ability using the Franz Diffusion Cell method and measurement of flavonoid content using UV-Vis Spectrophotometry.

MATERIALS AND METHODS

Materials

The materials used were *Centella asiatica* L. Leaves (Palembang, Indonesia), Poly-Lactic-Co-Glycolic Acid (Sigma Aldrich, Singapore), Polyvinyl alcohol (Sigma Aldrich, Singapore), cellophane membrane (Merck, Indonesia), KH2PO4 (Merck, Indonesia), NaOH (Merck, Indonesia), glycerin (Bratachem, Indonesia), methylparaben (Bratachem, Indonesia), propylparaben (Bratachem, Indonesia), aquadest (Bratachem, Indonesia), quercetin (Sigma Aldrich, Singapore), carbopol 934 (Multisera Indos, Indonesia), and 96% ethanol (Bratachem, Indonesia)

Tools

The tools used in this study were analytical balances with readability of 0.001 g and 0.0001 g (Nesco®), pH meter (Thermo Scientific ®), rotary evaporator (IKA), franz diffusion cell (Pyrex®), Cup and Bob viscometer (Grace M3400®), and UV-Vis spectrophotometry (Biobase®).

Preparation of Centella asiatica L. leaves ethanolic extract

Centella asiatica L. leaves are obtained from Palembang, Indonesia. Leaves were made into a herbarium and identified at the Plant Conservation Center at the Purwodadi LIPI Botanical Garden. *Centella asiatica* L. leaves are dried to obtain dry simplicia. The simplicia was then macerated using 96% ethanol in a ratio of 1:5 for five days while stirring occasionally (Apriani et al., 2021). The filtrate obtained

was then concentrated using a rotary evaporator at a temperature of 60ºC to get a thick extract.

Preparation of nanoparticles of Centella asiatica L.

The manufacture of nanoparticles following the research of Apriani et al. (2022). The nanoparticles were made using the Emulsion Solvent Evaporation Method. A total of 158 grams of extract and 50 grams of PLGA were dissolved in ethyl acetate solvent and acted as oil phase. In a different container, 40 mg of PVA was dissolved in aquadest and served as the aqueous phase. The oil phase was added drop by drop into the water phase above the homogenizer at a speed of 750 rpm for 1 hour. Next, aquadest was added up to 25 ml. The resulting solution was then evaporated for 24 hours.

Preparation of nanoparticles gel

The gel preparation in this study refers to the research of Mardiyanto et al. (2022) with modifications. The concentration of the materials used can be seen in Table 1. Carbopol 934 was dispersed in distilled water and added TEA to form a gel mass. Methylparaben and propylparaben were dissolved in glycerin and put into a gel mass. Nanoparticles of pegagan were added little by little and stirred until homogeneous. After homogeneous, add the remaining distilled water up to 100 mL.

Table 1. Formula for nanoparticles gel

Materials	Concentration Formula (%)		
	F1	F2	F3
Nanoparticles of *Centella asiatica* L.	25	25	25
Carbopol 934	1	1.5	2
Glycerin	2	2	2
Methylparaben	0.18	0.18	0.18
Propylparaben	0.02	0.02	0.02
TEA	qs	qs	qs
Aquadest (ad)	100	100	100

Evaluation of nanoparticles gel

The evaluation of the nanoparticle gel included organoleptic tests, homogeneity tests, pH tests, viscosity tests, spreadability tests, and adhesion tests.

Organoleptic and homogeneity test

Organoleptic and homogeneity tests were performed visually. The parameters that were observed included odour, colour, and dosage form of the gel.

pH test

The pH of the nanoparticles gel was measured using a pH meter.

Viscosity test

The viscosity test was carried out using a cup and bob viscometer with a speed of 60 rpm.

Spreadability test

The spreadability test was carried out using a scaled round glass. Measurement of the diameter of the spread is carried out longitudinally and transversely. The load used is from 50 to 150 grams.

Adhesion test

The gel preparation is placed on a slide and covered with a glass of another object, then given a weight of 1 kg for 3 minutes. Determination of adhesion is done by measuring the time it takes for the two slides to come off.

Stability test of nanoparticles gel

The stability test was carried out using the Cycling Test method at a temperature of 4±2ºC and 40±2ºC. The test was carried out for up to 6 cycles, and observations were made at the end of the cycle. The parameters that were observed included organoleptic and pH.

Measurement of the total flavonoid content of nanoparticles gel

A total of 0.1 sample solution was added with 0.1 mL of 1 M potassium acetate, 0.1 mL of $FeCl_3$, and up to 5 mL of distilled water. The absorbance of the solution was measured at a maximum wavelength for quercetin, which is 425 nm to obtain the total flavonoid content in the sample solution. Quercetin was used as a standard (Indarti et al., 2019).

In vitro release evaluation

In vitro, release evaluation was carried out on the three nanoparticle gel formulas (F1, F2, and F3) and the gel formula which was not formulated into nanoparticles as F4. The membrane used is the cellophane membrane. The receptor compartment solution was phosphate buffer pH 7.4 (Nurleni et al., 2019). The test was carried out at 37ºC at a speed of 750 rpm. The number of samples used is 1 gram. Sampling was conducted at 0, 15, 30, 45, 60, 90, and 120 minutes and then the total flavonoid content in the sample was measured. After obtaining the total flavonoid content, the cumulative % drug release was calculated according to equation 1.

$$Cumulative\ \%\ Drug\ Release = \frac{Flavonoid\ content\ in\ receptor\ compartment\ at\ minute\ n}{Flavonoid\ content\ in\ nanoparticles\ gel} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ [1]$$

Furthermore, the cumulative % drug release data is plotted into several models of release kinetics, namely zero order (cumulative % drug release vs time), first order (log cumulative % drug release vs time), Higuchi's model (cumulative % drug release vs squre root of time), and Korsmeyyer Peppas (log cumulative % drug release vs log time) to see the release model that occurs.

Data analysis

Data analysis was performed using SPSS for the evaluation of nanoparticles gel. A normality test was performed using Shapiro Wilk. If the data is normally distributed ($p>0.05$), then it is continued with the one-way ANOVA test, but if the data is not normally distributed ($p<0.05$), then it is continued with the Kruskal-Wallis test.

RESULTS AND DISCUSSION

The ethanolic extract of pegagan leaves has a thick texture, blackish green colour, and a distinctive smell. The yield percentage produced is 9.156%. The yield percentage produced is smaller than the previous research conducted by Djoko et al. (2020), namely 16.31%. This difference is due to different extraction processes. The study of Djoko et al. (2020) was sifted at a size of 40 mesh so that the smaller the particle size, the larger the surface area of the simplicia in contact with the solvent. However, this result still meets the requirements of the Indonesian Herbal Pharmacopoeia, which is not less than 7.2% (Kemenkes RI, 2017).

The ethanol extract of pegagan leaves was then formulated into nanoparticles to increase the stability of the flavonoids. Flavonoids are compounds that are easily degraded in the presence of oxygen, high temperature, and light (Ioannou et al., 2020; Kemit et al., 2019; Ramesova et al., 2011; Sharma et al., 2015). The nanoparticle formula refers to the research of Apriani et al. (2022), where the resulting particle size was 288.1667±3.4195 nm, the polydispersity index was 0.371±0.0045, and the zeta potential was -10.6333±0.1154.

Nanoparticles of pegagan were formulated into gel preparations with varying concentrations of gelling agents. The gelling agent used in this study was Carbopol 934 with a successive concentrations of F1, F2, and F3 were 1; 1.5; and 2%. This concentration refers to the research of Asmi (2013) and Zheng et al. (2016). In Asmi's research (2013), the gel viscosity results obtained at carbopol 934 concentrations below 1% did not meet the requirements for suitable gel viscosity (< 2000 cPs). Meanwhile, in the research conducted by Zheng et al. (2016), the viscosity of the gel at a concentration of 3% carbopol 934 was very high, resulting in a minor release ability of the gel. The results of the evaluation of the nanoparticle gel can be seen in Table 2.

Table 2. Evaluation results of nanoparticles gel

Parameter	Formula			Sig
	F1	F2	F3	
Organoleptic	Yellow, distinctive smell, soft	Yellow, distinctive smell, slightly stiff	Yellow, distinctive smell, stiff	-
Homogeneity	Homogeneous	Homogeneous	Homogeneous	-
pH	6.20 ± 0.08	6.17 ± 0.17	6.00 ± 0.08	0.216
Viscosity (cPs)	3417.42 ± 55.59	3937.46 ± 127.82	4754.67 ± 277.97	0.001*
Spreadability (cm)	5.1 ± 0.08	3.7± 0.08	3.1 ± 0.08	0.000*
Adhesion (sec)	209.33 ± 8.99	249.00 ± 8.29	301.67 ±10.27	0.000*
Stability	No physical changes, pH 6.5	No physical changes, pH 6.4	No physical changes, pH 6.4	0.192

Note: * indicates that there is a significant difference in each test group ($p<0.05$)

The gel preparation has good characteristic if they have a pH in the skin range of 4.5-6.5 (Okuma et al., 2015; Nikam, 2017), viscosity in the range of 2000-4000 cPs (Ardana et al., 2015), spreadability of 5-7 cm (Apriani et al., 2018), and adhesion of 2-300 seconds (Yusuf et al., 2017). The resulting gel preparation is yellow, has a characteristic odour, homogeneous, and has a soft to stiff texture due to the effect of differences in the concentration of Carbopol 934 used. The pH of the gel preparation was around 6 due to the addition of TEA to the formula. Carbopol 934 has an acidic pH range of 2.75 – 3.5 (Rowe et al., 2009). When added TEA which has a pH in the range of 7.76 (Fiume et al., 2013), Carbopol 934 will expand and form a gel mass. TEA also makes the pH of the preparation higher so that it meets the pH requirements of the skin range. The gel viscosity shows that F1 and F2 meet the requirements. F1 has the lowest viscosity compared to F2 and F3. The

viscosity can affect the results of spreadability and adhesion. The higher the viscosity, the smaller the spreadability of the gel, but the adhesion will be longer. The viscosity, spreadability, and adhesion statistical results showed a significant difference between groups ($p<0.05$). This is due to differences in the concentration of Carbopol 934 used. The higher the concentration of Carbopol 934 used, the higher the viscosity of the gel preparation. Carbopol 934 is a polymer that has an -OH group; when in contact with water in an alkaline environment, hydrogen bonds will occur, which cause swelling (Kaur et al., 2018). Spreadability and adhesion will affect the release ability of the preparation. Gels that have high spreadability and adhesion allow the drug to be maximally absorbed into the skin (Febrianto & Mia, 2020). The stability test results also showed that the three formulas did not significantly change in physical and pH ($p>0.05$).

The nanoparticle gel was followed by a release test using the Franz Diffusion Cell method using a cellophane membrane. In this test, pegagan gel which was not formulated into nanoparticles, was used as a comparison (F4). This test was carried out to determine the percentage of flavonoid content that was released at time intervals. The results can be seen in Table 3.

The release kinetics constant from zero order, first order, Higuchi's model, and Korsmeyer Peppas were calculated from the slope of the appropriate plots, and the regression coefficient (r^2) was determined in Table 4.

Table 3. Cumulative % Drug Release

Time (min)	Cumulative % Drug Release			
	F1	F2	F3	F4
0	7.93	5.49	0.67	2.31
15	15.19	12.35	3.07	5.26
30	20.65	17.47	5.78	8.12
45	25.62	26.51	10.78	9.37
60	32.87	35.14	16.71	12.45
90	42.19	40.76	21.01	14.57
120	58.17	47.74	23.76	20.62

Table 4. Release Kinetics Model

Release Kinetics Model	F1		F2		F3		F4	
	r2	k	r2	k	r2	k	r2	k
Zero Order	0.9945	0.4033	0.9606	0.3605	0.9582	0.2089	0.9829	0.1433
First Order	0.9137	0.0065	0.8240	0.0072	0.7712	0.0116	0.8483	0.0069
Higuchi	0.8944	4.4214	0.9388	4.2103	0.8991	2.3397	0.9202	1.6024
Korsmeyer-Peppas	0.9101	0.3917	0.9427	0.4602	0.9646	0.7759	0.9531	0.4370

Based on Table 3, F1 has the highest cumulative percentage of drug release. F4 is a gel preparation of pegagan which is not formulated into nanoparticles and has the smallest cumulative percentage of drug release compared to other formulas. The cumulative amount is strongly influenced by the characteristics of the gel preparation made and the nanoparticle carrier used. F1 has the lowest viscosity compared to F2 and F3, so the resistance to release is lower. When the gel is in contact with the phosphate buffer pH 7.4 (receptor compartment solution), the gel will swell so it will break. At medium pH 7.4, the COO- group in Carbopol 934 will experience electrostatic repulsion among –COO groups and a decrease in hydrogen bonding interactions which causes hydrogel swelling (Gaikwad et al., 2012; Suhail et al., 2020).

PLGA will contact with phosphate buffer solution pH 7.4. The solution will enter the nanoparticle system and cause the nanoparticles to expand to form pores that allow the active substances in them to be constantly released until the degradation process of the PLGA polymer is complete (Fredenberg et al., 2011; Vimal et al., 2016). Furthermore, the drug in the form of nanoparticles will release. Based on Table 4, F1, F2, and F4 have a zero-order release kinetics model because they have the highest linearity with r2 values of 0.9945, 0.9606, and 0.9829, respectively. At the same time, F3 has a release kinetics model of Korsmeyer Peppas with an r2 value of 0.9646. The zero-order rate describes the systems where the drug release rate is independent of its concentration. F1 has a higher reaction kinetics constant (k) than the other formulas, so drug release occurs faster (Figure 1). Based on the results of the evaluation, physical stability, and release ability of the preparation, F1 was determined as the best formula in this study.

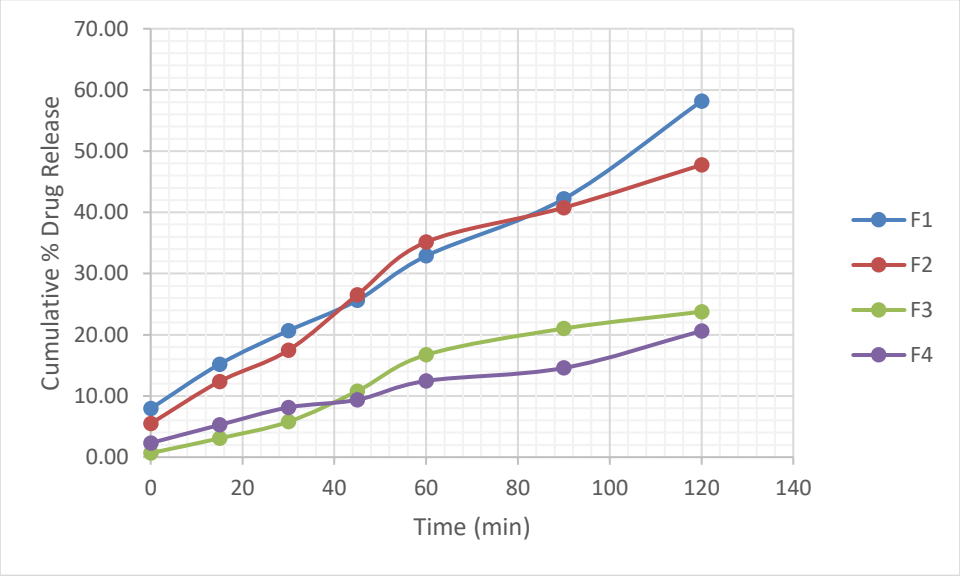

Figure 1. In vitro release curve

CONCLUSION

The concentration of the gelling agent Carbopol 934 influences the evaluation of the gel preparation and the release ability of the gel. The lower the concentration of Carbopol 934 used, the lower the gel's viscosity so that the gel's spreadability will be greater. The low viscosity of the gel will make it easier to release. F1 is the best formula because it has good physical properties and the greatest release ability compared to other formulas.

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