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The Formulation of Nanogel Allantoin uses Nano Allantoin made by the Ionic Gelation Method between Chitosan and Tripolyphosphate

Formulasi Nanogel Allantoin Menggunakan Nano Allantoin yang Dibuat Melalui Metode Ionic Gelation antara Kitosan dan Tripolifosfa

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ABSTRACT

Allantoin is a kind of chemical compound as an anti-irritant and wound healer. Allantoin has a safe component characteristic and has not toxic but is easily degraded when used orally. On the other hand, modified allantoin through nano gels can increase stability and provide a maximum therapeutic effect. This study aims to determine the effects of the tripolyphosphate in different concentrations as a cross-linking way on the characteristics of nano allantoin and determine the physical characteristics and stability of the selected formula allantoin nano gels.

Allantoin nanoparticles were prepared using the ionic gelation method between chitosan polymer and tripolyphosphate crosslinker. The concentration of allantoin used was 0.5%, chitosan was 0.2% in 1% glacial acetic acid, and tripolyphosphate was made in 3 variations, consisting of 0.3%; 0.2%; and 0.1%. The characteristics of nanoparticles include particle size, zeta potential, and sorption efficiency. Hydroxypropyl methylcellulose (HPMC) was used as a gelling material. The characteristics of nano gels include organoleptic, homogeneity, spreadability, adhesion, and viscosity.

The results of the nano allantoin characteristic test showed the lower the tripolyphosphate concentration, the smaller the particle size, the stable zeta potential, and the higher the adsorption efficiency. Nano allantoin formula number 3 was chosen as the formula for the manufacture of nano gels, which had the smallest particle size (160.73±2.4 nm), stable zeta potential (31.27±2.76 mV), and the highest adsorption efficiency (60.01±0,5%. Formula 3 was proven to be able to be formulated in the form of nano gels and had good gel physical properties standards. The cycling test stability test showed that the preparation was stable during storage.

Keywords: allantoin; ionic gelation; tripolyphosphate; nano gels.

ABSTRAK

Allantoin adalah senyawa kimia yang berfungsi sebagai antiperadangan dan penyembuh luka. Allantoin memiliki sifat komponen aman dan tidak beracun, tetapi mudah terdegradasi jika digunakan secara oral. Di sisi lain, allantoin yang dimodifikasi melalui nano gel dapat meningkatkan stabilitas dan memberikan efek terapeutik maksimal. Penelitian ini bertujuan untuk menentukan pengaruh tripolifosfat dalam konsentrasi yang berbeda sebagai cara pengikatan silang pada karakteristik nano allantoin dan menentukan karakteristik fisik serta stabilitas dari formula pilihan nano gel allantoin.

Nanopartikel allantoin disiapkan menggunakan metode ionic gelation antara polimer kitosan dan silang tripolifosfat. Konsentrasi allantoin yang digunakan adalah 0,5%, kitosan sebesar 0,2% dalam asam asetat glasial 1%, dan tripolifosfat dibuat dalam 3 variasi, yaitu 0,3%; 0,2%; dan 0,1%. Karakteristik nanopartikel termasuk ukuran partikel, potensial zeta, dan efisiensi penyerapan. Metil selulosa hidroksipropil (HPMC) digunakan sebagai bahan pembentuk gel. Karakteristik nano gel meliputi penilaian organoleptik, homogenitas, kelarutan, daya lekat, dan viskositas.

Hasil dari uji karakteristik nano allantoin menunjukkan semakin rendah konsentrasi tripolifosfat, semakin kecil ukuran partikel, semakin stabil potensial zeta, dan semakin tinggi efisiensi penyerapan. Formula nano allantoin nomor 3 dipilih sebagai formula untuk pembuatan nano gel, yang memiliki ukuran partikel terkecil (160,73±2,4 nm), potensial zeta stabil (31,27±2,76 mV), dan efisiensi penyerapan tertinggi (60,01±0,5%). Formula 3 terbukti dapat diformulasikan dalam bentuk nano gel dan memiliki standar sifat fisik gel yang baik. Uji stabilitas selama penyimpanan menunjukkan bahwa persiapan tersebut stabil.

Kata Kunci: allantoin; ionic gelation; tripolyphosphate; nano gel

1. INTRODUCTION

Allantoin is a natural anti-irritant and skin protector, and biochemically it can accelerate wound healing by stimulating the growth of new skin cells [1]. The development of allantoin in nanotechnology is still rarely studied. Nanodrug systems have advanced toxicity reduction, release, and increased treatment efficiency[2]. On the other hand, allantoin has a safe characteristic component and have not toxic but is easily degraded when used orally. Besides, modified allantoin through nano gels can increase stability and provide the desired therapeutic effect[3].

The development of a study related to ionic gelation allantoin encapsulation shows that chitosan and tripolyphosphate can be used as allantoin carriers[4]. The principle is to create an ionic interaction between the charged amino group of chitosan positively and the polyanion crosslinker negatively. Furthermore, the polyanion crosslinker used consisted of sodium tripolyphosphate because it has a penetrating character good, stable, and nontoxic membrane. Besides, allantoin combined with chitosan produces a good speed of wound healing and anti-inflammatory effects[5]. On the other hand, chitosan acts as a carrier for the nanoparticle system, which can increase biomolecules and bioavailability due to has good diffusion ability[6]. Increasing the concentration of crosslinkers can affect the particle size to become significant. Nano allantoin is made into nano gels for topical application. Nanogels can protect charged drugs, have high stability, and maximize drug release on the skin[8].

This study aims to determine the effect of tripolyphosphate in different concentrations as a cross-linking material on the characteristics of nano allantoin, understand the characteristics of the gel physical properties, and the selected formula allantoin nano gel stability.

2. METHODS

2. 1. TOOLS AND MATERIALS

The instrument used in this study w**as a** UV-Vis spectrophotometer, particle size analyzer, zeta sizer, magnetic stirrer, analytical balance, centrifuge, pH meter, cup and bob viscometer, oven, refrigerator, mortar, stamper, glassware, and non-glass tools.

The materials used in this study were allantoin, chitosan, sodium tripolyphosphate, aquadest, glacial acetic acid, NaOH, technical hydroxymethyl cellulose, glycerin, propylene glycol, propylparaben, and methylparaben.

2. 2. PROCEDURE

Preparation Nano Allantoin

Table 1. Allantoin nanoparticle formulas

Material	Concentration (%)		
	F1	F2	F3
Allantoin	0.5	0.5	0.5
Chitosan	0.2	0.2	0.2
Tripolyphosphate	0.3	0.2	0.1

Chitosan Solution Preparation. Chitosan was dissolved in 1% glacial acetic acid solution and homogenized by a magnetic stirrer.

Tripolyphosphate Solution Preparation. Tripolyphosphate in each concentration was dissolved in 40 mL of distilled water using a magnetic stirrer [9].

Allantoin Nanoparticle Preparation. Allantoin was dissolved in a chitosan solution using a magnetic stirrer. It was dripped constantly into the chitosan mixture and allantoin solution at room temperature (25^{o}C) using a 1500 rpm homogenizer within 30 minutes

Nanoparticle Characterization

Particle Size and Zeta Potential Determination. Measurement of particles and zeta potential using allantoin nanoparticle suspension dispersion in distilled water at 25˚C tested using PSA and zeta sizer[9].

Entrapment Efficiency Determination. Efficiency determination by allantoin nanoparticles centrifugation at 6000 rpm for 15 minutes. Absorption is measured at the maximum wavelength, calculated through the calibration curve equation[9].

Selected Formula of Nano Allantoin Determine

The selected formula for allantoin nanoparticles was determined by comparing three allantoin nanoparticle formulas based on the d on the nanoparticle's characters. The characteristics of the selected formula have the smallest particle size, stable zeta potential (> 30 mV), and significant percent entrapment efficiency.

Preparation Allantoin Nanogels

Table 2. Allantoin Nanogel formula

Material	Concentration (%)
Allantoin nanoparticles	47
HPMC	3
Glycerin	5
Propylene glycol	10
Propyl parabens	0.01
Methyl parabens	0.03
Aquadest	ad 100

While stirring, the hydroxymethyl cellulose gelling agent was developed with an aquadest 70oC in a hot mortar. Other additives are included at the base. The selected nano allantoin formula was added to the base; meanwhile, stirring was carried out until it was mixed, homogeneous, and became a good gel mass.

Characteristics of Allantoin Nanogel Physical Properties

Allantoin nano gel tested the characteristics of the physical properties, including organoleptic, pH, homogeneity, adhesion, and viscosity.

Gel Cycling Test Physical Stability Test

The gel was stored at 4 ± 2^{o}C for 24 hours and transferred to an oven at 40 ± 2^{o}C for 24 hours (one cycle). The treatment was carried out for six cycles. Physical testing is conducted by observing the changes that occur and compared to the manufacturer's initial conditions.

Calibration Curve Creation

The calibration curve is determined by making 100 ppm allantoin mother liquor, determining the maximum wavelength and operating time, and making the allantoin standard curve concentration of 6, 8, 10, 12, 14, 16, 18, and 20 ppm.

Validation of the Analytical Methods

The parameters of the analytical methods include linearity, precision, accuracy, LOD, and LOQ[10].

3. RESULTS AND DISCUSSION

Characterization of Allantoin Nanoparticles

The results of this study showed that all nanoparticle formulas were successfully made into nanoparticle suspensions.

Table 3. Results of characterization of allantoin nanoparticles

Nanoparticle characteristics	Formula		
	1	2	3
Particle size	166.9 ± 2.62 nm	192.43 ± 5.19 nm	160.73 ± 2.4 nm
Zeta potential	19.23 ± 0.59 mV	28.47 ± 2.19 mV	31.27 ± 2.76 mV
Entrapment efficiency	54.82 ± 0.33%	56.47 ± 0.1%	60.01 ± 0.5%

* Description: F1 tripolyphosphate concentration 0.3%; F2 tripolyphosphate concentration 0.2%; and F3 tripolyphosphate concentration 0.1%.

Figure 1. Allantoin nanoparticles

The results of this study related to particle measurement obtained that particle sizes in all formulas had a nanoparticle size range of 10-1000 nm. Whereas formula 1 resulted in particle size (166.9±2.62 nm), zeta potential (19.23±0.59 mV), and sorption efficiency (54.82±0.33%). On the other hand, formula 2 resulted in particle size (192.43±5.19 nm), zeta potential (28.47±2.19 mV), and sorption efficiency (56.47±0.1%). Meanwhile, formula 3 resulted in particle size (160.73±2.4 nm), zeta potential (31.27±2.76 mV), and sorption efficiency (60.01±0.5%).

Ionic gelation interactions occurred due to the positive charge of chitosan binds to the negative one of tripolyphosphat[11]. Tripolyphosphate cross-linking materials have functioned as a polymer nanoparticle stabilizer[12]. This study showed that the higher the concentration of tripolyphosphate, the larger the particle size, the smaller the zeta potential, and the lower the adsorption efficiency. Besides, formula 2 obtained the largest particle size, which was probably caused by a technical error when the dripping tripolyphosphate solution was not constant and caused the particle solids formation to run quickly, making the particle size large.

Many cross-links could increase the chitosan matrix's mechanical strength, making it complex and challenging to break down into smaller sizes. Zeta potential is a kind of measurement to measure the force of repulsion between particles. The zeta potential of nanoparticles plays an important role in physical stability and affects their effectiveness as a drug delivery system[13]. It is used to determine the surface charge characterization on nanoparticles.

Furthermore, nanoparticles with a zeta potential value of more than 30 mV will have higher stability[14]. The positive zeta possible charge is related to the nanoparticles formed through the ionic gelation method, which is the positive charge of the amine group belonging to chitosan that is neutralized by the tripolyphosphate interaction, which has negative control. However, the residual amine group belonging to the chitosan causes a positively charged zeta potential[15]. The zeta potential determination results showed that the greater the tripolyphosphate concentration, the smaller the zeta potential produced.

The entrapment efficiency describes the amount of active substance that is trapped in the nanoparticle system. The large entrapment efficiency indicates that the nanoparticle system is good[16]. The entrapment efficiency was carried out by comparing the total allantoin used during preparation with the amount of the active ingredient allantoin free in the solution. The entrapment efficiency is also used to assess the ability of chitosan and tripolyphosphate nanoparticles as carriers of the active substance allantoin toward the target application. The difference in entrapment efficiency in each formula can be affected by the difference in the concentration of the cross-linking. The greater the tripolyphosphate concentration, the smaller the absorption efficiency. These results indicate that the smaller tripolyphosphate concentration will significantly increase entrapment efficiency[17]. The low entrapment efficiency is probably due to the chitosan polymer's low molecular weight, so the chitosan's free amino groups are easily protonated and lead to higher allantoin encapsulation through ionic interactions[18].

The results of the characteristics data analysis of the nanoparticles showed that the normality value of the significance p-value on all formulas was > 0.05, indicating the data were normally distributed so that the homogeneity test and the one-way ANOVA statistical test were continued. The results of the homogeneity test have a significance value of p-value> 0.05, indicating that the data is homogeneous. The results of the significant value of p-value were < 0.05. Moreover, one-way ANOVA proved that the results of all the nanoparticle characteristics in formulas 1, 2, and 3 were significantly different on average.

The selected Formula ofAllantoin Nanoparticles Determination

The selected formula for allantoin nanoparticles manufacture of gel preparations is used formula 3 with a concentration of 0.2% chitosan and 0.1% tripolyphosphate, which is the best formula from the allantoin nanoparticles characterization using the ionic gelation method, producing the smallest particle size, stable zeta potential, and biggest entrapment efficiency. Formula, 3 of allantoin nanoparticles was proven to be encapsulated using the chitosan-tripolyphosphate ionic gelation method with the smallest particle size results compared to other formulas (160.73 ± 2.4). Formula 3, to prevent aggregation, had the largest and most stable zeta potential (+31.27 ± 2.76 mV > 30 mV).

The results of the entrapment efficiency in formula 3 obtained the largest % EE value (60.01% ± 0.5), which indicates that the carboxyl group in chitosan can bind strongly to cross-linking and active substances**,** which made the increased entrapment efficiency.

Nanogel Characteristics Test

The organoleptic test was carried out by visual observation, including the preparation form or consistency, gel color, and smell odor[19]. The results of the organoleptic test showed the gel preparation was in the form of a soft semi-solid, which had a slightly transparent white color and had no odor. The consistency of the gel preparation is related to the comfortable user. The soft texture causes the gel to be easily absorbed and spread on the skin. Allantoin nano gel preparations showed air bubbles during manufacturing, which were probably caused by stirring during manufacture; however, they disappeared after storage.

The homogeneity test is important in determining whether the gel is homogeneous or uniform. The homogeneity test results showed good homogeneity, including the colors being distributed and no coarse particles.

Besides, the pH test was carried out to determine the level of gel preparation acidity and safety. The allantoin nano gel showed a safe pH range (4.5-6.5) and did not cause skin irritation, which was on the value of 5.64.

Spreadability describes the ease of gel when it is applied to the skin. However, the preparation is good if it is easy to spread and comfortable to use. Besides, the aqueous gel preparation causes low adhesion and reduced duration of active substance contact. Moreover, the average dispersion test result showed 4.07 cm, which indicates good dispersion of semi-solid preparations (3-5 cm)[20].

Gel adhesion is the ability of preparation to the application way, which means the longer the gel to the absorbed active, the maximum effect it will be given. On the other hand, the test showed good adhesion and**,** based on the requirements test**,** with an average of 75.33 seconds > 1 second[21]. Thus, the HPMC gelling material can swell, form colloids, and bind water well.

The viscosity test determines the preparation consistency that can affect topical use. Viscosity in topical preparations must follow the target of therapy and use, which means the greater the viscosity, the more difficult it is to apply at the application site, while the smaller its viscosity, the easier it is to apply. Viscosity results on allantoin nano gels showed good consistency in the 50-1000 dPa.s[22]. The viscosity results showed that the preparation is easy to apply**,** and the active substance could be absorbed more optimally at the used target.

Physical Stability Test

Physical stability testing, which uses the cycling test method, was tested for six cycles. Furthermore, physical changes are seen and compared to the preparation initial condition of the preparation.

The stability test results of the organoleptic parameter cycling test method did not show any changes in shape, color, or odor. The gel showed no phase separation, and no air bubbles were formed.

The homogeneity stability test showed no change in inhomogeneity, such as color changes or coarse particles, to fulfill the requirements for a homogeneous gel. Hence, nano allantoin components and gelling additives combined to form a homogeneous gel preparation.

Picture 2. Allantoin nanogel before and after stability test

Table 4. Results of the cycling test stability

Stability test	Parameter	Average±SD	Description
Before cycling test	Organoleptic	Semi-soft solid, slightly transparent white color, and odorless	-
	Homogeneity	Homogeneous	-
	*p*H	5.64	-
	Coverage	No-load: 3.67 ± 0.21 50 g: 4.13 ± 0.15 100 g: 3.67 ± 0.21 150 g: 4.13 ± 0.15	-
	Stickiness	75 seconds ± 1	-
	Viscosity	400 ± 0 dPa.s	-
After cycling test	Organoleptic	Semi-soft solid, slightly transparent white color, and odorless	Stable
	Homogeneity	Homogeneous	Stable
	*p*H	5.64	Stable
	Coverage	No-load: 3.1 ± 0.1 50 g: 3.3 ± 0.1 100 g: 3.5 ± 0.1 150 g: 3.93 ± 0.15	*Sig 2 tailed T-test* 0.391> 0.05 0.308> 0.05 0.103> 0.05 0.058> 0.05
	Stickiness	78.33 seconds ± 1.53	*Sig 2 tailed T-test* 0.149> 0.05
	Viscosity	400 ± 0 dPa.s	Stable

The study showed that the allantoin nano gel results after the cycling test did not show pH changes and were still in the safe pH range for the skin. Thus, it showed that the combination of HPMC, glycerin, and propylene glycol could produce a stable pH gel and not cause skin irritation.

The stability test on dispersion decreased compared to the beginning of manufacture. Thus, it can be caused by an unstable temperature so that the HPMC polymer is disturbed and causes the water molecules in the gel to be reduced.

The results of the adhesion stability test showed an increase **in** adhesion value to 78 seconds. Thus, it can be caused the storage temperatures to change so that the water content of the gel decreases and can stick for longer. Viscosity after testing needs to be observed to determine the cycling test effects on changes in gel viscosity.

Calibration Curve Creation

The allantoin mother liquor was made at a concentration of 100 ppm. Furthermore, the allantoin wavelength was determined by observing the mother liquor absorption using UV-Vis spectrophotometry. The maximum wavelength results at 227 nm with an absorbance value of 0.4720.

These results follow the literature[4]. Operating time determines the optimal measurement time when it takes place and is characterized by stable absorbance. The continuously increasing absorbance from minute to minute cannot be used as an operating method, so the measurement is not optimal. The readings at minute 0 to minute 30 had stable absorption, indicating the optimal time for absorbance readings. The calibration curve series solution was prepared by diluting 100 ppm allantoin mother liquor into eight different concentrations. Furthermore, the standard solution at each concentration was determined for absorption using a UV-Vis spectrophotometer at a wavelength of 227 nm. Whereas linear regression value y= 0.014214x + 0.043464 and r= 0.9991 was obtained.

The validation of the analytical method

The validation of analytical methods is the process of assessing analytical methods based on experiments to confirm whether the methods used meet the applicable provisions[10]. The validation parameters of the analytical method include linearity, precision, accuracy, LOD, and LOQ[10].

Linearity indicates the ability of an analytical method to obtain test results following the sample analysis concentration in a specific concentration range. The results of linear regression analysis based on UV-Vis spectrophotometry at a wavelength of 227 nm obtained the linear regression equation y= 0.014214x + 0.043464 and r= 0.9991. However, the results of the linearity test had a correlation coefficient with good criteria where the value of r = 0.9991[10].

The accuracy test showed the closeness degree of the results to the actual analyte content. The recovery percentage value was 101.33%. The test results show that the analytical method used has good accuracy because it followed the requirements range (98% -102%)[10].

Precision measures the analysis closeness results from various repeated measurements at one concentration. The results of the precision test showed a CV value of 0.0125%. These results are included in the excellent category because they follow the requirements (CV < 2%)[10].

The limit of detection and limit of quantification aims to determine the lowest concentration of analyte that can be detected in the sample. The detection limit test showed the detection limit value of 0.7369 g/mL. Meanwhile, the quantity limit testing resulted in a 2.2331 /g/mL quantity limit.

4. CONCLUSION

The difference in the tripolyphosphate concentration affected the nanoparticles' characteristics, including particle size, zeta potential, and adsorption efficiency. The results of the particle size formulas 1, 2, and 3 were 166.9±2.62, respectively; 192.43±5.19; 160.73±2.4. Furthermore, the zeta potential test results on formulas 1, 2, and 3 were +19.23±0.59, respectively; +28.47±2.19; and +31.27±2.76. The adsorption efficiency in formulas 1, 2, and 3 were 54.82%±0.33, respectively; 56.47%±0.1; 60.01%±0.5. Meanwhile, the results of the nanoparticle characterization showed that formula 3 was better than formulas 1 and 2. A tripolyphosphate concentration of 0.1% could be formulated into a gel preparation that met the good gel physical properties standards and physical stability of the preparation.

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