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Optimisation and Characterisation of Curcumin Nanoparticles with Chitosan-TPP Combination Using Ionic Gelation Method as a Cut Wound Healer

Ferdinta D setyawan[1] *, Ilham Kuncahyo[2], Wiwin Herdwiani[3]
Department of Pharmacy, Faculty of Pharmacy, Setiabudi University, Surakarta
Corresponding Author: Ferdinta D setyawan ferdintas14@gmail.com

A R T I C L E I N F O

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A B S T R A C T

Curcumin has low oral bioavailability, low solubility, and is easily degraded, making its clinical application difficult. This study aims to determine the effect of optimum concentration and formula of chitosan and TPP in the preparation of curcumin nanoparticles on particle size, zeta potential, adsorption efficiency, and incision wound healing. The optimisation method used simplex lattice design method. There were 8 draft formulas consisting of a combination of chitosan and TPP. Each formula was tested for zeta potential value, adsorption efficiency, particle morphology, and wound healing ability. The optimum formula was then determined and analysed using the simplex lattice design method. The combination of chitosan and TPP with simplex lattice design gives an influence on curcumin nanoparticles which reduces particle size, gives a negative zeta potential value, increases the sorption efficiency of the active substance (curcumin) and accelerates the healing effect of cut wounds in mice. The proportion of chitosan and TPP that can produce the optimum formula using simplex lattice design on the critical parameters of particle size, zeta potential value, sorption efficiency and wound healing ability is chitosan.

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INTRODUCTION

Curcumin is a compound found in turmeric plants (Curcuma longa Linn) or temulawak plants (Curcuma Xanthoriza). Curcumin also has polyphenol compounds as yellow colour givers in turmeric and temulawak. In curcumin there are pharmacological activities that vary greatly in the form of anti-inflammatory, antioxidant, antiviral, antimalarial (Zorofchian Moghadamtousi et al. 2014). Curcumin has a low oral bioavailability, and is easily degraded, making its clinical application difficult (Yusuf et al. 2021). Therefore, nanoemulsion formulation of curcumin can be effectively developed to improve the bioavailability of curcumin (Yusuf et al. 2021).

According to (Naruki et al. 2010) The choice of carrier material in drug delivery systems is very important as it significantly affects the pharmacokinetics and pharmacodynamics of the drug. A wide variety of materials, such as chitosan, polymers, cyclodextrins, and dendrimers, have been used as carriers to improve bioavailability. These methods can be used in producing a curcumin nanoparticle and chitosan carrier as one of the emulsion cross-linking method, precipitation, spray drying, emulsion droplet coalescence method, ionic gelation, reverse micellar method, and polyelectrolyte complex. In the preparation of nano curcumin in chitosan using an ionic gelation method, this method offers several advantages, namely, a very simple and lightweight preparation in an aqueous environment. The mechanism of this method allows the formation of chitosan nanoparticles based on electrostatic interactions between the positive amino group (-NH) in chitosan and the negative charge group of polyanions such as tripolyphospate (TPP) (Putri, Sundaryono, and Chandra 2019). Due to the complexity of this interaction chitosan undergoes ionic gelation and precipitates to form particles.

Wound healing is the body's attempt to restore structural integrity and normal function after tissue disruption (Gudiño León., Acuña López., and Terán Torres. 2021). This process can be divided into phases of inflammation, debridement, reparation, and maturation. Many factors influence wound healing, including age, nutrition, tissue necrosis, drug administration and infection. These factors influence the duration of wound healing (Martien et al. 2012). Thomas, et al, (2016) conducted an in vivo study of the effect of curcumin on the healing effect of incision wounds on rats with a dose of 2.5mg showing that treatment with curcumin increased the wound healing effect compared to the group that did not receive it and successfully healed the wound in 15 days compared to the control group (18 days). Wulandari et al (2019) conducted research to determine a transethosome diffusion profile of curcumin in vitro using franz diffusion cells, with a 4% curcumin formula having the highest cumulative amount of diffused curcumin, namely 1921.76 µg.

According to research by Mara et al. in 2019, current wound healing therapies generally fail to provide good clinical results, so nanotechnology can be relied upon for wound healing therapy. Utilising the size and electrical charge of nanoparticles, their biochemical features such as hydrophobicity, interactions with biological targets, and deeper levels of tissue penetration can enable them to aid wound healing. Nanoparticles have an ability to penetrate the intercellular space so that it can be penetrated by colloidal particle size, nanoparticles were chosen because they are relatively easy to move to be combined with various other technologies so as to open up broad potential to be developed for various purposes and targets. Another advantage of nanoparticles is that they have a high sorption efficiency which is beneficial regarding the transport of enough drugs in target cells and increasing drug contact time. (Buzea. et al., 2007).

Therefore, based on research conducted by EtikMardliyati, et al (2012), it can be seen that the preparation conditions of chitosan concentration of 0.2%, TPP concentration of 0.1% and the best chitosan TPP volume ratio of 5:1. This study aims to obtain optimal conditions for the preparation of curcumin nanoparticles with variant concentrations of chitosan-TPP carriers at a size of less than 100 nm, with a good level of size uniformity and stabiility and based on the inflammatory activity of curcumin, researchers want to know the effect of wound healing on the optimal conditions of preparation of curcumin nanoparticles with chitosan carriers. The parameter that has been used in determining a level of uniformity to measure is the index value on polydispersity, while a parameter to determine stability is the zeta potential value.

THEORETICAL REVIEW

Curcumin

Curcumin is an unsaturated α-β-diketone compound with a slight electrophilic focus (Martono, 1996). Curcumin consists of two ferulic acid molecules joined at the carboxyl group carbon atom through a methylene bridge.

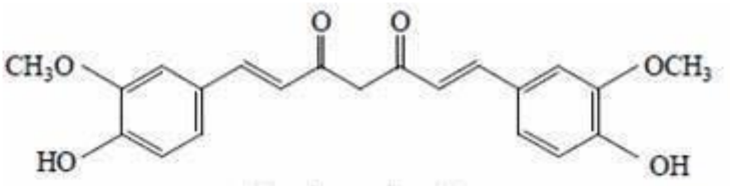


Figure 1. Curcumin Winarto (2003)

Curcumin crystals are rod-shaped or prism-shaped and are orange-yellow in color. The number of carbon atoms in curcumin is less than 40 but can be grouped into carotenoids (tetraterpenoid-structured pigments that are fat-soluble) by giving a yellow to red color. Due to the uniqueness of its structure, this compound is said to come from the decomposition of carotenoids and is not formed from smaller units (Robinson, 1995). Curcumin is rod-shaped or crystal-shaped and varies in orange-yellow color. Curcumin has less than 40 carbon atoms, but can be classified as a carotenoid (tetraterpenoid-structured pigment that is soluble in fat) and produces a yellow to red color. Due to the uniqueness of its construction, this compound is said to come from the decomposition of carotenoids and is not framed from smaller units (Robinson, 1995).

a. *Simplex Lattice Design*

The profile of the influence of a mixture on a parameter can be determined using Simplex Lattice Design (Bolton, 1997). This technique is applied to a recipe for moment grains that uses at least two mixtures, with the easiest combination using two parts, sugar and filler. The premise of this technique is that there are two independent factors A and B. This plan is made by choosing 3 mixtures and observing the reactions obtained. The reactions obtained must approach the objectives that have just been completed, both the most extreme and the least (Bolton, 1997).

The reaction and part relationship are described as follows: Y = a (A) + b (B) + ab (A). (B) Y is the desired parameter, namely the material content, and (A) and (B) are component fractions with the following conditions: The relationship between the reaction and its parts is interpreted as follows: Parts with accompanying conditions:

$0 \leq (A) \leq 1$

$0 \leq (B) \leq 1$ $(A) + (B)$

$= 1$

A, b, and ab are coefficients that indicate the actual quality limits. To find out the excess a, b, ab, 3 recipes are needed as follows; A = 1 part or taken 100 percent without B, B = 1 part or taken 100 percent without A, and the combination of A and B is half each. By entering the reaction obtained from the exploration results with the results above, the benefits of the coefficients a, b, and ab can be determined. The Y value (response) for each variation of the mixture A and B can also be calculated using the coefficient values to explain its profile (Bolton, 1997).

b. *Nano Particles*

1. Nanoparticle Carrier System

   1.1. Nanoliposomes are spherical vesicles formed by hydration of surfactants such as phospholipids when mixed with water using low shear forces. Phospholipids arrange themselves in sheets by joining their tails together, these sheets form a bilayer membrane containing water inside the phospholipid vesicles. Liposomes are flexible carriers, can carry both water-soluble and water-insoluble compounds simultaneously and provide targetability but the drawbacks of this system are high cost, low payload, drug leakage and rapid release are the major drawbacks of this delivery system. Using these nanocarriers may pose stability issues during storage (Jain, Rahi, Pandey, Asati, & Soni, 2017; Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013).

   1.2. Solid Lipid Nanoparticles were introduced in 1990 as selective transporters for liposomes or other polymeric nanoparticles. Solid Lipid Nanoparticles are a type of particulate carrier system made of solid fat that has been dispersed in water and emulsified by surfactants with an average size of 501000 nm. However, Strong Lipid Nanoparticles also have disadvantages due to their low dispersion rate resulting in longer delivery time, with high water content causing crystallization in the framework thereby reducing the solvency of bioactive formation and unpredictable delivery (burst release). Due to the weakness of the developed lipid carrier nanostructure (NLC), the drug loading capacity will be limited because the particles will crystallize and form high energy modifications during storage (Ekambaram, Sathali, & Priyanka, 2012; Ramadon & Im, 2016).

1.3. Transferosomes are lipid vesicles that have the best deformability among other nanovesicles. The difference between liposomes and transferosomes is the use of edge activators which are basically surfactants. Surfactants are used in readiness to remove the lipid layer of the vesicle. This deformity is caused by the additional surfactant which helps better infiltration into the skin. However, transferosomes have constraints because they are made for large scale, their carrier system is not strong against oxidation and cannot carry drugs in high daily doses. (Ramadon & Im, 2016).

1.4. Niosomes are vesicular systems similar to liposomes but use cholesterol and nonionic surfactants to form a bilayer. The advantage of involving nonionic surfactants in the bilayer arrangement is to build up the penetration and bioavailability of drug adsorption. Disadvantages: actual susceptibility, accumulation, combination, spillage of drug adsorption, typical drug hydrolysis that limits the safety of capacity (Md. Rageeb Md. Usman, 2017).

1.5. Polymeric nanoparticles are divided into nanocapsules and nanospheres. Nanocapsules consist of polymers that form walls surrounding the internal center where the drug is trapped. Nanospheres are produced using a strong polymer framework and drug compounds dispersed in it (Delie, 2005). Commonly used polymers include poly lactic acid (PLA), poly glycolic acid (PGA), poly alkylcyaniacrylate (PACA), and others. Some normal polymers contain chitosan.

One of the benefits of nanoparticles is their ability to infiltrate the intercellular space that colloidal particles can enter. In addition, nanoparticles can be combined with various other technologies. With these capabilities, there is a lot of room to develop for various needs and purposes. Another benefit is the expansion of the framework's alignment due to the expansion of the contact surface area by the same amount (Buzea et al., 2007).

c. *Wound Healer*

Administration of curcumin concentrate can increase neutrophils during the injury recovery process. Neutrophils are the main fire cells produced after injury. Neutrophils provide an immune reaction by sending proteolytic catalysts to process foreign particles and kill microscopic organisms through phagocytosis and hydrogen peroxide production. Neutrophils will undergo apoptosis after 24 to 48 hours and are supported by macrophages (Stroncek and Reichert, 2008). Administration of gel in portions can reduce fire interactions (calming), can accelerate the development of fresh blood vessels (neovascularization), re-epithelialization and connective tissue. The ethyl acetate derivative section shows an impact on the wound healing cycle. This may be related to the auxiliary metabolite enhancer found in the ethyl acetate derivative section. The results of phytochemical screening of the ethyl acetate derivative section contain flavonoid, quinone, polyphenol compounds. One of the polyphenol contents found in turmeric rhizomes is curcumin (Andersen and Markham 2006). Curcumin has a mitigating effect by inhibiting cyclooxygenase-2 (COX-2) and lipoxygenase (LOX) proteins, which are important compounds in the provoking interaction. Re-epithelialization, cell proliferation, and collagen synthesis are accelerated by curcumin. (Tangapazham. *et al*. 2007).

METHODOLOGY

Material

Curcumin (PT Gansu Yasheng Hiosbon Food Group), chitosan (PT Brataco), TPP (PT Brataco), acetic acid (PT Brataco), ethanol (PT Brataco), aquadest, white rats.

Instrumen

UV-Vis spectrophotometer (Shimadzu 1780), magnetic stirrer (Thermo Cimarec+), particle size analyzer (Malvern 3000E), centrifuge (Biobase 80-2), and glassware.

Prosedur

Determine the lower and upper limits of the two polymers before entering them into Design Expert Software version 11.1.2.0 to get the formula design based on the simplex lattice design method in Design Expert Software version 11.1.2.0.

Table 1. Upper and lower limits of chitosan-TPP

Kode Nilai	**Nilai sebenarnya (persen dalam formula)**	
	Kitosan	TPP
0	0,1	0,1
1	0,2	0,2

Table 2. Formula Design Based on Simplex Lattice Design Method

Run	**Kitosan %**	**TPP %**
1	0.175	0.125
2	0.125	0.175
3	0.15	0.15
4	0.1	0.2
5	0.2	0.1
6	0.1	0.2
7	0.15	0.15

8	0.2	0.1

The optimised components were zeta potential value, particle size, absorption efficiency and wound healing ability. The formulation steps can be seen in the table 2.

Preparation of Chitosan Solution

Chitosan with a concentration of 0.1 - 0.2% was dissolved in 100 mL of 1% acetic acid solution using a magnetic stirrer. 1% acetic acid was made by mixing 10.0 mL of glacial acetic acid with distilled water to reach a volume of 1000.0 mL (Mardliyati *et al*., 2012).

Preparation of TPP

Solution Sodium tripolyphosphate with a concentration of 0.1 - 0.2% was dissolved in 40 mL of aquademineralised using a magnetic stirrer (Mardliyati, et al, 2012).

Curcumin Nanoparticle

Solution Curcumin powder weighed 0.12 mg was dissolved in 100 ml chitosan solution using a magnetic stirrer. Sodium tripolyphosphate solution of 20 ml was poured directly into the mixed solution at room temperature (25°C) in a homogenizer with a rotation frequency of 5000 rpm for 30 minutes until a nanoparticle suspension was formed (Suryani *et al*., 2016).

Characterisation of Curcumin Nanoparticles

a. Particle Size and Zeta Potential In this study, particle size testing was carried out by sending samples to Pt. DKSH Indonesia which is located in Jakarta. Samples are tested with the suitability that has been applied in the test. The sample is inserted into the tool for examination so that the results of the average particle size and zeta potential will be obtained and recorded. Evaluation of zeta potential was carried out as much as 1.5 ml of nanoparticle dispersion determined zeta potential by Laser Droppler Electrophoresis (LDE) method using Zetasizer Nano tool Akbari B (2011).
b. Binding Efficiency
   Quoted from research conducted by Guan et al in 2011 with minor modifications. The adsorption efficiency of curcumin nanoparticles was determined by separating the free drug from the adsorbed drug nanoparticles using a centrifugation technique. The chitosan nanoparticle dispersion was centrifuged for 30 minutes at 13,000 rpm with the aim of separating the unadsorbed drug. The amount of free drug (F) is called supernatant. The centrifuged supernatant was determined using UV-Vis spectrophotometry.
   (%EP) calculated by the formula: $\%EP = \frac{T-F}{T} \times 100\%$

Wound Healing Activity Test

The test animals in this study used as many as 24 white rats (Rattus Novergicus) Wistar, male, in healthy condition, 2-3 months old, unmarried condition (Marice, 2010). Each white rat was divided into 21 groups for concentration variation and positive control. The test animals were first given ether anaesthetic, then the fur of the test animals was shaved until smooth on the back and cleaned with 70% alcohol. Wounds were made on the back of rats with a length of 2 cm and a depth of 0.2 cm.

Group I is a negative control group with treatment using chitosan-TPP, group II is a positive control with gentamicin ointment treatment, group III is a group treated using curcumin with a concentration of 0.1%, groups IV-VIII are groups treated using variant concentrations of chitosan-TPP nanocurcumin. Treatment was carried out for 14 days by smearing twice a day at 09.00 and 17.00 WIB as much as 1 ml. Observations of the macroscopic wound condition based on the Nagaoka criteria were carried out every day (Irwanda *et al*,. 2020).

Table 3. Nagaoka Macroscopic Criteria

Parameter dan Deskripsi	skor
Waktu penyembuhan luka	
Dibawah 7 hari	3
Antara 7 - 14 hari	2
Diatas 14 hari	1
Infeksi lokal	
Tidak ada infeksi	3
Infeksi lokal disertai pus	2
Infeksi lokal tanpa pus	1
Reaksi alergi	
Tidak ada alergi	3
Reaksi alergi bintik merah diarea sekitar luka	1

From the test data of characteristics and wound healing time, the price for each response will be obtained and the equation model will be obtained. The simplex lattice design model will produce the optimum formula obtained after entering the test values of particle size, zeta potential, absorption efficiency and wound healing time test using Analysis Of Variance (ANOVA) in Design Expert software version 11.1.2.0. The optimum formula was obtained based on each parameter value obtained and a contour plot was made. Contour plot of particle size test parameters, zeta potential, absorption efficiency and incision wound healing time test were superimposed so that the optimum area could be determined. Then the test results of the optimum formula were compared with the prediction results obtained from the design expert using SPSS 25 with One Sample T-test at 95% confidence level. Wound healing time was then statistically analysed using one-way ANOVA with 95% confidence level. If the results were not normally distributed ($p<0.05$) the Mann-Whitney test was continued.

RESULTS AND DISCUSSION

Characterisation of Curcumin Nanoparticles

The stages of making curcumin nanoparticles begin with chitosan dissolved in 1% acetic acid, the solvent used to dissolve TPP is aquademineralisata in different containers. Curcumin was dissolved in the chitosan solution that had been made, then mixed with TPP solution drop by drop in a ratio of 5 : 1, stirred using a stier magnet for 3 hours at 1000 rpm. The nanoparticles were then characterised by particle size, zeta potential, absorption efficiency and wound healing test.

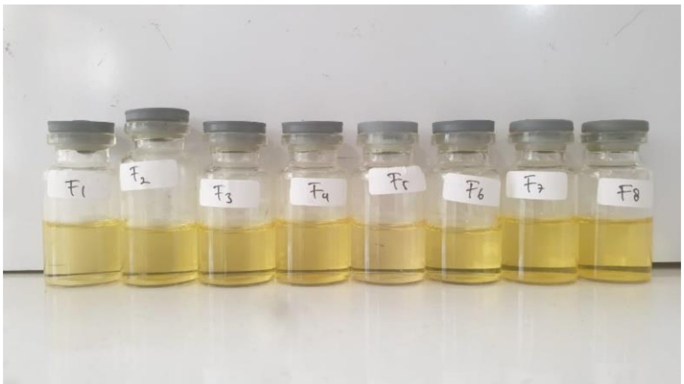


Figure 2. Sample of Curcumin Nanoparticles

Particle size testing was carried out using the PSA tool, the observation showed the results of varying particle sizes for each formula, respectively 1241 nm, 1012 nm, 540.9 nm, 596.2 nm, 1743 nm, 605.7 nm, 1085 nm, 1652.4 nm. From the results obtained, it shows that formula 3 has the smallest particle size of all formulas. From the results of zata potential testing, it shows that all formulas have a negative charge of -19.07 mV, -13.39 mV, -15.53 mV, -12.22 mV, -25.1 mV, -12.17 mV, -15.79 mV, and -24.72 mV. This value shows that curcumin nanoparticles as a stable suspension because it is close to the value of -30 mV. From the zeta potential test results, it shows that all formulas have a negative charge. Zeta potential is a measure of electrical charge on the surface of dispersionparticles and is an important indicator in evaluating dispersion stability. A negative zeta potential value on dispersion particles usually indicates the presence of a negative charge on the particle surface. The results showed high values of trapping efficiency in all formulas 90.041%, 80.479%, 85.915%, 74.586%, 94.718%, 74.446%, 85.451%, and 94.785%. The trapping efficiency corresponds to the amount of drug adsorbed on the nanoparticles. A good nanoparticle system is one that has a high adsorption efficiency. The effectiveness of entrapment depends on the drug and drug-polymer interactions. Curcumin is a partially negatively charged compound that can weakly interact ionically with the positive charge of chitosan. Therefore, curcumin can be adsorbed into the polymer matrix and provide high adsorption efficiency (Savitry and Wathoni 2018).

Table 4. Particle Size, Zeta Potential, and Sorption Efficiency Test Results

Sample	Particle Size	Zeta potential	Sorption efficiency
F1	1241	-19,07	90.041
F2	1012	-13,39	80.479
F3	540,9	-15,53	85.915
F4	596.2	-12,22	74.586
F5	1743	-25,1	94.718
F6	605,7	-12,17	74.446
F7	1085	-15,79	85.451
F8	1652,4	-24,72	94.785

Wound Healing Test

The results of the observation of incision wound healing in formula 4 gave the fastest effect of incision wound healing on day 4. Smaller particles have a better ability to penetrate tissue and reach deeper areas of the wound, small particles can help stimulate new tissue growth and collagen formation which is important for wound healing (Garini 2021). Smaller particles have a greater surface to mass ratio than larger particles of the same mass. This means there is more surface area available to interact with cells and healing factors in the body. This can increase the likelihood of faster and more effective wound healing (Abdassah 2017).

Table 1. Wound Healing Time Test

Group	Time (days)			Mean Healing Time (days) ± SD
	Replication 1	Replication 2	Replication 3	
F1	7	8	6	7 ± 1
F2	5	6	7	6 ± 1
F3	3	7	5	5 ± 2
F4	5	4	3	4 ± 1
F5	8	8	8	8 ± 0
F6	4	5	6	5 ± 1
F7	5	7	3	5 ± 2
F8	9	7	8	8 ± 1
K+	3	3	3	3 ± 0
K-	15	15	15	15 ± 0
Curcumin	14	13	15	14 ± 1

The results of the wound healing time were analysed using SPSS, because the normality test results obtained did not meet the requirements of the normality test, namely the siq value <0.05, then the nonparametric test using mann-whitney was continued.

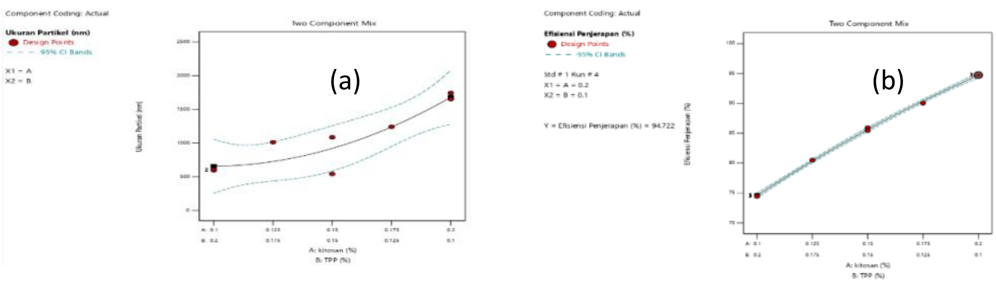
Based on the results of the nonparametric test analysis using mann-whitney, the F3, F4 and F7 groups did not have a significant difference from the positive control which was indicated by the siq value> 0.05 because F3, F4 and F7 had smaller particle sizes. Smaller particles have a better ability to penetrate tissue and reach deeper areas of the wound, small particles can help stimulate new tissue growth and collagen formation which is important for wound healing (Garini 2021). Smaller particles have a greater surface to mass ratio than larger particles of the same mass. This means there is more surface area available to interact with cells and healing factors in the body. This can increase the likelihood of faster and more effective wound healing (Abdassah 2017).

Table 6. Nagaoka Criteria Score

Group	Average			Total Nagaoka average score
	Healing (Score)	Infection (Score)	Allergy (Score)	
F1	2.333	3	3	8.333 ± 0.31
F2	2.667	3	3	8.667 ± 0.16
F3	2.667	3	3	8.667 ± 0.16
F4	3	3	3	9 ± 0.00
F5	2	3	3	8 ± 0.47
F6	3	3	3	9 ± 0.00
F7	2.667	3	3	8.667 ± 0.16
F8	2	3	3	8 ± 0.47
K+	3	3	3	9 ± 0.00
K- placebo	1	3	3	7 ± 0.94
Curcumin	1.667	3	3	7.667 ± 0.63

Macroscopic observations of the Nagaoka criteria score obtained results in F1, F5, F8 and curcumin had a score of 8 which means wound healing healed between day 7 and day 14, there was no infection and no allergies. Group F2, F3, F4, F6, F7, and positive control get a total score of 9 which means wound healing heals under 7 days, there is no infection and no allergies. Meanwhile, the negative control received a relatively low score of 7, which means that the wound healing in the negative control healed for more than 14 days. The higher the total score of Nagaoka, the better the wound healing, with the characteristics of fast wound healing time, no infection, and no allergies.

The results of nonparametric testing using mann-whitney showed F1, F2, F3, F4, F6, F7 there was no difference with control + marked with siq value> 0.05, while F5 and F8 there was a difference with control + marked with siq value <0.05.



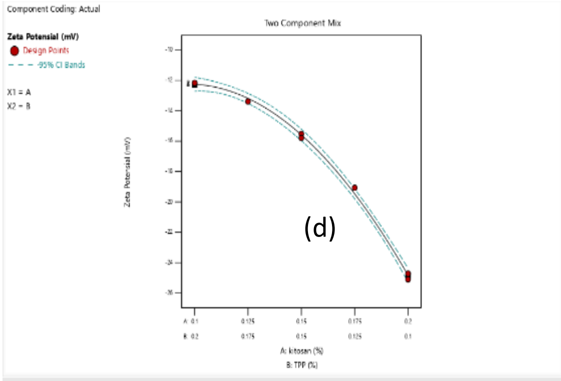


Figure 3. ANOVA test, Particle size (a), Zeta potential (b), Sorption efficiency (c)

ANOVA analysis with Design Expert application version 11 simplex lattic design method

The results of the testing of particle size, zeta potential, absorption efficiency and wound healing time were carried out by ANOVA analysis with the Design Expert application version 11 simplex lattic design method. In the particle size analysis, the p-value of the model is <0.0120, indicating that the value is <0.05, which means that the model used is significant. In the Fit Statistic results, the R2 value of 0.9331 indicates a good fit generated for the response data because the R2 value is close to the value of 1. Design expert suggests a quardratic model for particle size with a value of F = 12.15 meaning this model is significant. The Adjusted R2 value is 0.7610 and predicted R2 with a value of 0.6415, which shows that the difference between the two values is < 2 so it is said that this model is suitable and can be used. For the Adeq precision value obtained of 7.2957, it is said to be appropriate because this value is> 4. Based on the graph in Figure 2a, it can be seen that chitosan can affect the particle size of curcumin nanoparticles, this shows an increase in particle size when the concentration of chitosan is also increased.

Analysis of zeta potential The p-value of the model is < 0.0001 indicating that the value is < 0.05 which means that the model used is significant. In the Fit Statistic results, the R2 value of 0.9982 indicates a good fit generated for the response data because the R2 value is close to the value of 1. The design expert suggested a quardratic model for zeta potential with a value of F = 1405.23, meaning that this model is significant. The Adjusted R2 value is 0.9975 and predicted R2 with a value of 0.9959, which shows that the difference between the two values is < 2 so it is said that this model is suitable and can be used. For the Adeq precision value obtained of 78.6034, it is said to be appropriate because this value> 4. Based on the graph in Figure 2b, it can be seen that TPP can affect the zeta potential of curcumin nanoparticles, this shows a decrease in zeta potential when the concentration of TPP also decreases.

The results of the analysis of sorption efficiency The p-value of the model is <0.0001 indicating that the value is <0.05 which means that the model used is significant. In the Fit Statistic results, the R2 value of 0.9994 shows a good fit produced for the response data because the R2 value is close to the value of 1. Design experts suggest a quardratic model for zeta potential with an F value = 3944.88, meaning this model is significant. The Adjusted R2 value is 0.9991 and

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predicted R2 with a value of 0.9987, which shows that the difference between the values of both < 2 so that it is said that this model is suitable and can be used. Adeq precision value is 136.5217, said to be appropriate because this value is 4. Based on the graph in Figure 2c, it can be seen that chitosan can affect the sorption efficiency of curcumin nanoparticles, this shows an increase in sorption efficiency when the concentration of chitosan is also increased.

Determination of Optimum Formula

Determination of optimum region of Curcumin nanoparticles using design expert 13 software in this study a numerical approach was used to determine the optimum formula. The data entered as parameters are particle size, zeta potential, sorption efficiency and incision wound healing test. Determination of the optimum formula using weights and goals is in the following table.

Table 7. Optimum Parameter Value and Goal of Curcumin Nanoparticles

Name	Goal	Lower Limit	Upper Limit	Importance
A: Chitosan	*is in range*	0.1	0.2	3
B: TPP	*is in range*	0.1	0.2	3
Particle Size	*minimize*	540.9	1743	3
Zeta potential	*is in range*	-25.1	-12.17	3
Sorption efficiency	*maximize*	74.449	94.781	3
Wound Healing Time Test	*minimize*	4	8	3

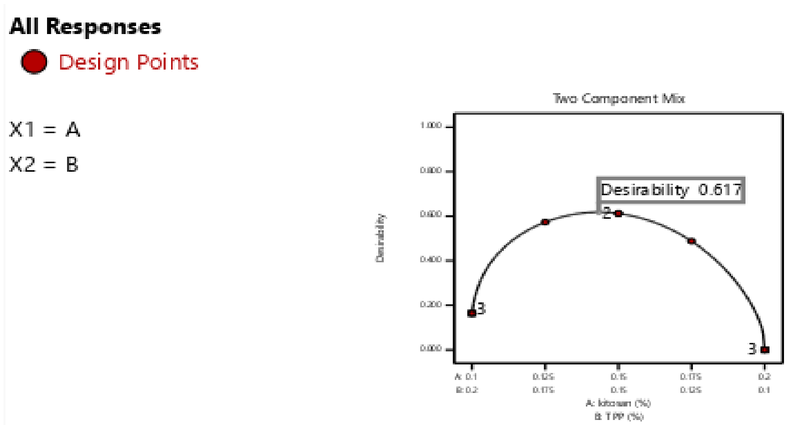

Figure 4. Desirability Counter Plot

Based on the picture 3, it is illustrated that the desirability curve is said to be normal and the optimum desirability value obtained from data processing is 0.617. The most optimal formula is the one with the maximum desirability value. The desirability value also has a function value with an optimization goal that shows a program's ability to fulfill what is desired based on the criteria specified in the final product. Desirability values range from 0 to 1. Where when the desirability value increases and approaches 1, it indicates that the results are increasingly optimal in producing the desired product. The optimization score itself is not always considered good to reach the value of 1, but to find the best condition in uniting all functions (Raissi and Farsani, 2009).

Table 8. Prediction of optimum formula with simplex lattice design

Components of Curcumin Nanoparticles	Optimum Concentration	Parameters			
		Particle Size	Zeta potential	Sorption efficiency	Wound Healing Time Test
Chitosan	0.143	856.649	-14.799	84.242	5.35
TPP	0.157				

Optimum formula prediction was obtained by simplex lattice design on chitosan (0.143%) and TPP (0.157%) components. Three replicates of the optimum formula were made and tested to obtain the values of particle size, zeta potential, absorption efficiency, and incision wound healing of the optimum formula which will then be verified by comparing with the predicted values obtained from each test parameter.

Verification of Optimum Formula Testing Results

Comparing the prediction value with the test value of the optimum formula, an analysis was carried out using SPSS 25 with the One Sample T-test test at the 95% confidence level to see whether the prediction value and the test value had no significant difference or there was a difference between the two values. The results of One Sample T-test obtained sig value > 0.05 for all parameters, there is no difference between the predicted value and the test value of the optimum formula.

Table 9. Comparison of Predicted Value with Testing Value of Optimum Formula

Parameters	Predicted Value	Testing Value	Value *Sig.*	Description
Particle Size (nm)	856.649	855.266	0.623	not different
Zeta potential (mV)	-14.799	-14.48	0.183	not different
Sorption efficiency (%)	84.242	84.434	0.326	not different
Wound Healing Time Test (days)	5.35	5	0.606	not different

Comparison of the prediction value with the test value of the optimum formula was analyzed using SPSS 25 with One Sample T-test at 95% confidence level to see whether the prediction value and the test value had no significant difference or there was a difference between the two values. The results of the One Sample T-test test obtained a sig value of 0.05 for all parameters, there is no difference between the predicted value and the test value of the optimum formula.

CONCLUSIONS AND RECOMMENDATIONS

Curcumin nanoparticles with ionic glycation technique give an influence on curcumin nanoparticles which reduce particle size, provide a good zeta potential value of (+- 30 mV), increase sorption efficiency, accelerate incision wound healing. the proportion of chitosan 0.143% and TPP 0.157% obtained by simplex lattice design as the optimum formula reduces particle size, provides good zeta potential close to (+- 30 mV), increases sorption efficiency and better incision wound healing ability. In the future, it is necessary to review the rotation speed and the falling speed of the cross-linker.

FURTHER STUDY

It is necessary to conduct research by adding SEM (Scanning Electron Microscope) morphology testing parameters.

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