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Original Article

D-OPTIMAL MIXTURE DESIGN: OPTIMIZATION, FORMULATION, AND EVALUATION OF BIOSYNTHESIS NANOGOLD GELS

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ABSTRACT

Objective: The aim of this research is the Optimization, formulation, and evaluation of biosynthesis nanogold gels by using experimental design. Investigations were provided to optimize the biosynthesis of nanogold gels on a compound of two gelling agents and develop the biosynthesis of nanogold gels.

Methods: The optimization of the formula of gels utilizing the D Optimum Mixture Design method to discover the optimum result with the ratio of carbopol (X1) and hydroxypropyl methylcellulose/HPMC (X2) as a gelling agent and responses in the form were particle size (Y1), zeta potential (Y2) and spreading capacity (Y3). The 8-run formula assessed the impact of carbopol (X1) and HPMC (X2).

Results: The ANOVA results for particle size showed that the model is highly statistically significant ($P<0.05$). Both Carbopol and HPMC coefficients enhanced the particle size (+118.91 and+594.73). The interaction of Carbopol with HPMC has increased the particle size (+441.73). The HPMC has the most dominant effect in increasing the particle size, and exchanging the two gelling agents will increase the particle size. Both Carbopol and HPMC coefficients are negative (-7.94 and-10.96), which means that these components contribute to a decrease in the zeta potential. The interaction of Carbopol with HPMC does not affect both increasing or decreasing the zeta potential HPMC has the most dominant effect in reducing the zeta potential, and the exchange of the two gelling agents will not increase and decrease the zeta potential. Carbopol and HPMC coefficients are positive (+6.41 and+4.58), meaning these components enhance the spreading capacity. The interaction of Carbopol with HPMC has decreased (-1.58). The prediction value was obtained from a solution that has the highest desirability. The chosen desirability of certain balances was 0.572.

Conclusion: This study has shown that a well-balanced mixture of matrix ingredients could improve nanogold biosynthesis gel. Optimization was possible by applying D optimal mixture design, which confirmed efficiency in designing the nanoparticles gels of gold dosage forms.

Keywords: D-optimum mixture design, Optimization, Nanogold gels, Carbopol, Particle size

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INTRODUCTION

The development of science and technology has guided several studies in nanotechnology, which possible breakthroughs in promising domains such as materials, manufacturing, and medicine [1]. Metallic nanoparticles show immense chemical, physical, optical, and thermal characteristics due to the spatial limitation, the enormous surface area, and fewer imperfections. The deduction of size and shape has revealed impacts on the physical properties significantly different from the identical bulk material [2]. The synthesis of nanoparticles with different crystal shapes, sizes, and morphology is unusual from a practical topic of view [3]. Gold nanoparticles can be synthesized by chemical, physical, or biological processes [4]. Synthesis by physical and chemical methods is non-ecofriendly as dangerous chemicals are involved, and the process is also costly. Researchers are more concerned about the safety of the environment, and finally, the biological method of synthesis has become exceptional. The synthesis of nanoparticles from natural sources is called "green" nanotechnology. The green sources employed for synthesis include bacteria, fungi, or plant parts such as leaves, fruits, and flowers [5]. Among these, plant extracts have been found to be the best opportunity for synthesizing gold nanoparticles from *Muntingia calabura,* L.

Semisolid dosage forms are usually performed in ointments, creams, gels, or pastes. They comprise one or more active ingredients suspended or consistently dispersed in a proper base and appropriate excipients such as antimicrobial agents, viscosity-increasing agents, antioxidants, emulsifiers, or stabilizing agents. The selection of a base for semisolid dosage forms depends on many determinants [6]. Gel formulations mainly deliver faster drug release than creams and ointments [7] Nanogels are enlarged nano-sized particles that have several exceptional characteristic features like suitable swelling behavior, superb loading capacity even for natural macromolecules, simple scale-up manufacture, and continuous colloidal stability [8], Nanogels have multiple potentials in herbal formulations as drug carriers include their flexibility and versatility [9].

A simplex lattice design (SLD) is an optimization design in that the total amount of independent variables (ingredients of formulation) have to be persistent [10]. Studies were conducted to optimize nano gold gels on a compound of two gelling agents and develop a gel formulation of biosynthesis nano gold. The Optimization of the formula of gels employing the D Optimum Mixture Design method to discover the optimum result with the proportion of Carbopol (X1) and HPMC (X2) as a gelling agent and responses in the form were particle size (Y1), zeta potential (Y2) and spreading capacity (Y3).

MATERIALS AND METHODS

Material

Leaves of *Muntingia calabura* (voucher specimen no. 1531) were assembled from Indonesian Spice and Medicinal Plants Research Institute (Balitro), Indonesia, Propylene glycol (Brataco, pharmaceutical grade), Carbopol (Merck, pharmaceutical grade), Hydroxy Propyl Methyl Cellulose (Merck, pharmaceutical grade), TEA (Brataco, pharmaceutical quality) and other reagents that applied were analytical grades.

Instrument

Rotary evaporator RE100-pro (Andaru), thermostatic water bath, analytical scales (Mettler Toledo, Swiss), magnetic stirrer (Cimarec, America), one set dispersive power meter, viscometer (Brookfield DV2T), nanoparticle size analyzers series (SZ100 HORIBA, Japan).

Methods

Preparation of *Muntingia calabura,* L

Fresh leaves of *Muntingia calabura* were collected from Indonesian Spice and Medicinal Plants Research Institute (balittro), Indonesia. They were identified by Research Center for Biology (voucher specimen no. 1531) at the Herbarium Bogoriense, Department of Botany, Indonesian Institute of Sciences, Bogor, Indonesia. The leaves were cleaned wholly with ordinary water and sterilized distilled water. The leaves were drained under the shaded condition and were pulverized into particles using the milling machine [11].

Preparation of the plant extract

Leaves of *Muntingia calabura* weighing 5 g each were covered in double distilled water 100 ml and heated at 70 °C for 15 min. The mixtures were then decanted, separated, and filtered through a Whatman filter paper No. 1 for more studies [11, 12].

Preparation of biosynthesis nanogold

Biosynthesis of nanogold was carried out at room temperature by adding *Muntingia calabura* leaves aqueous extract to $HAuCl_4$ 0,002 M in the ratio of 1:9 [12].

D-Optimal experimental design

The synthesis experiment used the D-optimal experimental design with two numerical factors. The main effects and interactions were assessed by analysis of variance (ANOVA) on particle size, zeta potential, and dispersion capacity as a quantitative response to the experiment. The responses were interpreted using the Design-Expert method [14].

Evaluation

Particle size

The mean particle size distribution of nano gels was assessed using a Particle Size Analyzer (HORIBA Ltd., Kyoto, Japan) [15].

Formulation of biosynthesis nanogels

Table 1: Formula for biosynthesis nanogold gel based on software design expert

Ingredients	Formulation (%)							
	F1	F2	F3	F4	F5	F6	F7	F8
Biosynthesis Nanogold	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Extract of *Muntingia*	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol	0.5	2	1	2	0	1.5	1	0
HPMC	1.5	-	1	-	2	0.5	1	2
Propylene Glycol	15	15	15	15	15	15	15	15
Triethanol amine	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Na Meta bisulfit	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Aqua demineralisasata	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Table 1 exhibits the design of the formula for the biosynthesis of nanogold gel based on the software Design Expert with method D-Optimum. In this study, a fixed amount of biosynthesis nanogold (0.5 g), extract of *Muntingia* (0.5 g), and excipients such as propylene glycol (15 g), triethanolamine (0.05 g) and Na meta bisulfite (0.05 g) was used.

Zeta potential

Zeta potential represents the electrical potential in the double layer of ions enclosing a particle at the boundary of the particle surface and the adsorbed ions in the diffuse layer. Zeta potentials were determined with an SZ100 model HORIBA nanoparticle analyzer [12].

Spreading capacity

The spreading capacity of the gel formulations was measured 48 h after preparation by calculating the spreading diameter of the gel (1.00 g) within two glass plates after 1 minute [16].

Statistical and data analysis

The objective value of response was arranged by applying the linear, quadratic, and cubic models. ANOVA at P<0.05 was used to evaluate the statistical significance of the equation [17].

RESULTS

D-optimal design is used to optimize compounds when restrictions about the experimental setup are unusual. Parameters limits for a variable level of a gelling agent are exhibited in table 2.

Eight experimental runs were conducted using design expert. The particle size, potential zeta, and spreading capacity of each product were determined as responses and outcomes of the research carried out during the D-optimal design, showed in table 3.

The statistical parameters applied in selecting and evaluating the best-fitted model are regression data (p value and F value), lack-of-fit, coefficient of determination ($R2$), adjusted coefficient of determination (adjusted $R2$), and predicted coefficient of determination (predicted $R2$). The statistical analysis also builds an equation of the best-fitted model (table 4).

Table 2: Parameters limits for a variable level of gelling agent

No	Variables level (code)	Low (%)	High (%)
1	Carbopol	0	2
2	HPMC	0	2

Table 3: The design and results of each response

Run*	Component 1 A: carbopol %	Componen 2 B: HPMC %	Response 1 particle size (nm)	Response 2 zeta potential (mv)	Response 3 spreading capacity (cm)
1	25	75	567.2	-10.9	4.9
2	100	0	147.6	-6.8	6.5
3	50	50	467.9	-9.7	5
4	100	0	127.4	-8.6	6.35
5	0	100	596.1	-10.6	4.5
6	75	25	224.4	-8.9	5.63
7	50	50	532.4	-9.5	5.1
8	0	100	578.1	-10.6	4.6

*Run: The number of experiments that must be accomplished according to the selected experimental design

Table 4: Data from ANOVA

Response	Model	p-value	Lack of fit (p-value)	F value	R2	Adjusted R2	Predicted R2
Y1	Quadratic	0.0006	0.0674	46.42	0.9489	0.9285	0.8973
Y2	Linear	0.0028	0.0674	23.88	0.7990	0.7655	0.5686
Y3	Quadratic	0.0001	0.033	184.46	0.9866	0.9813	0.9675

Notes: $p<0.05$ is significant, $p>0.05$ is not significant

The formulation variables consist of the following:

X1: HPMC

X2: Carbopol

The dependent variables of the research include:

Y1: Particle size

Y2: Zeta potential

Y3: Spreading capacity

The general form of the quadratic model from Particle Size (Y1) is presented as Eq 1 [14].

$$Y1 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots\ (Eq.1)$$

$$Y1 = 118.91\ A + 594.73\ B + 441.77\ (A)(B)$$

Wherein:

Y1: Response of particle size

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

The general form of the Linear model from Zeta Potential (Y2) is presented as Eq 2.

$$Y2 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots\ (Eq.2)$$

$$Y2 = -7.94\ A - 10.96\ B$$

Wherein

Y2: Response of zeta potential

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

The general form of the quadratic model from dispersive ability (Y3) is presented as Eq 3.

$$Y3 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots\ (Eq.3)$$

$$Y3 = 6.41\ A + 4.58\ B - 1.58\ AB$$

Wherein:

Y3: response of spreading capacity

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

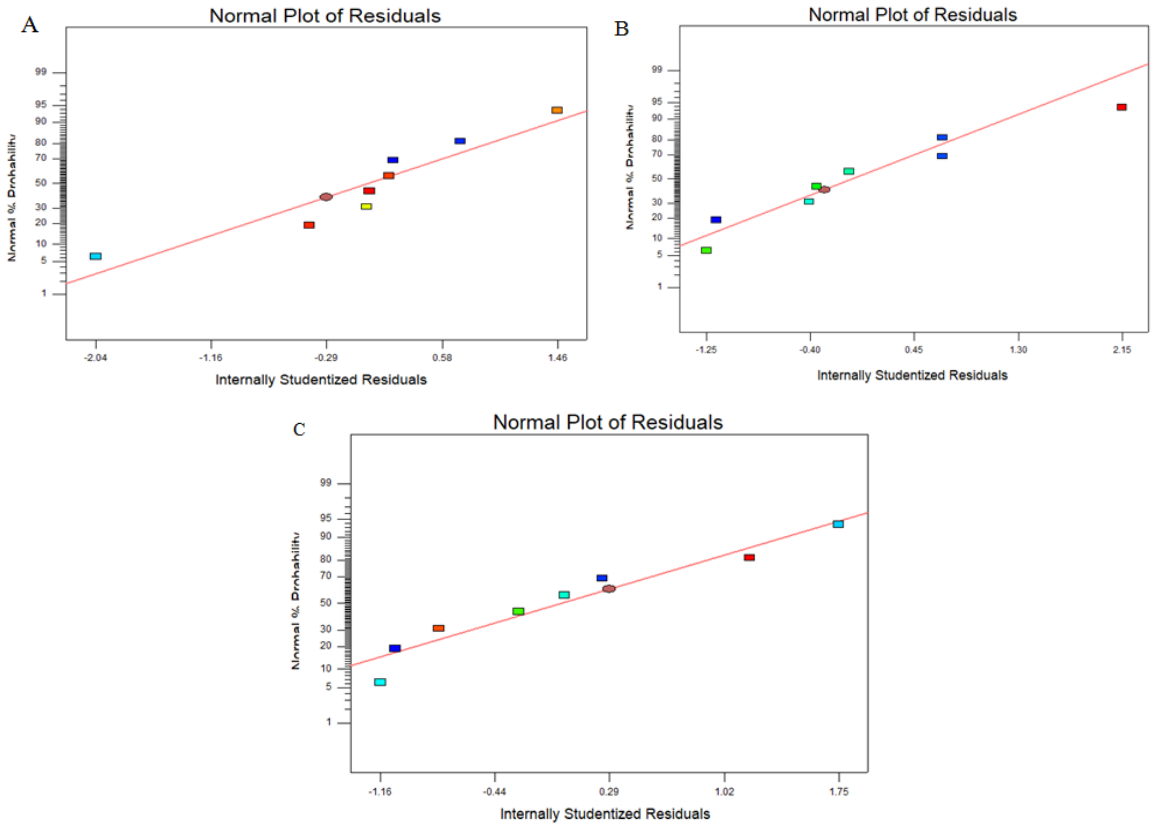

Fig. 1: Normal probability plot the particle size (A), zeta potential (B), spreading capacity(C)

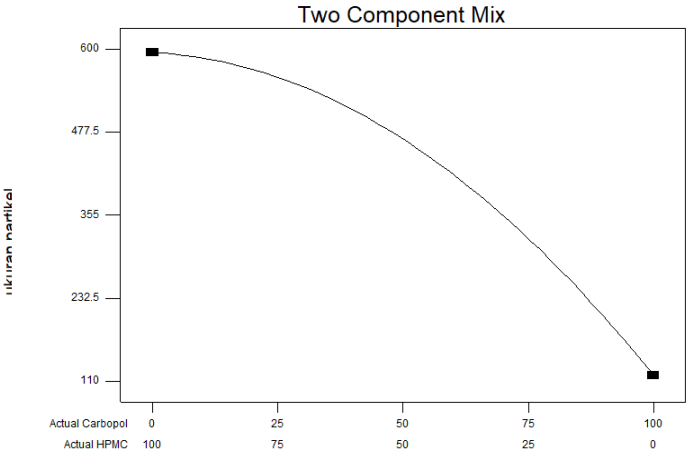

Fig. 2: Contour plot of particle size

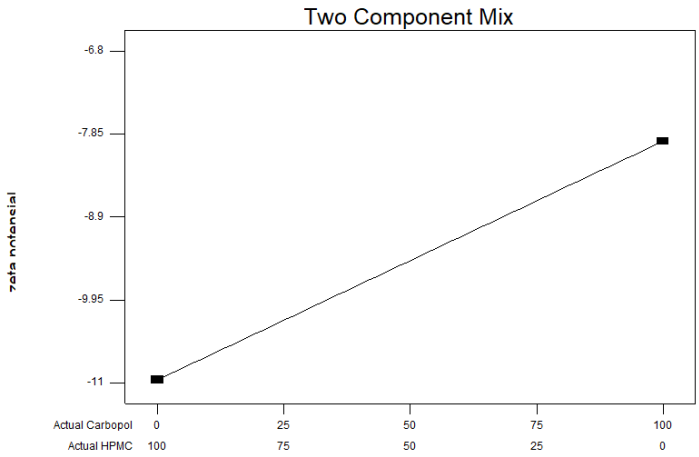

Fig. 3: Contour plot of zeta potential

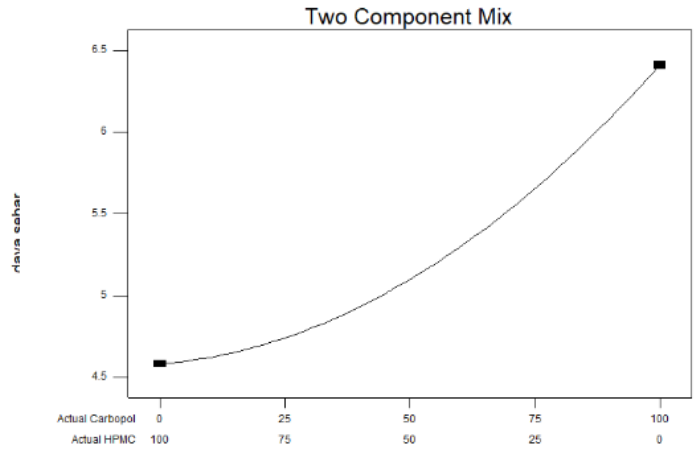

Fig. 4: Contour plot of spreading capacity

Table 5: Lower and upper limit of response

No	Response	(Goal)	Lower limit	Upper limit	Score
1	Particle Size (nm)	Minimum	127.4	596.1	5
2	Zeta potential	Maximum	-6.8	-10.9	4
3	Spreading capacity (mm)	In range	4.5	6.5	3

This mixed desirability was achieved according to the desirability of carbopol and HPMC, as shown in fig. 5.

Table 6: Desirability and formula solution from DX software

No	Carbopol	HPMC	Particle size	Zeta potential	Spreading capacity	Desirability
1	97.875	2.9215	144.365	-8.02389	6.31549	0.572
2	100	0	-6.8	-7.93889	6.41023	0.566

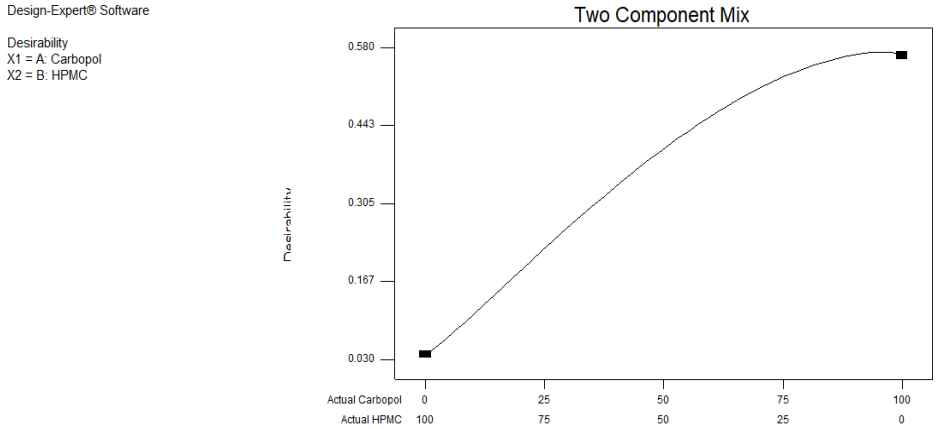

Fig. 5: Overlay contour plot of two-component mix

Table 7: Optimized values obtained by the confirmation

No	Response	Predicted	Observed
1	Particle size	144.365	127.6
2	Zeta potential	-8.02	-7.8
3	Spreading capacity	6.31549	5.35

Table 8: Verification of optimal conditions

Result	Particle size	Potential zeta	Spreading capacity
Prediction value	144,365	-13,2	6,31
Laboratory value	226,100	-7,8	5,17
P value	0,393	0,004	0,010
T value	4,475	4,475	4,475
Sample size	3	3	3
Standard error of Mean	75,806	0,033	0,115
Confidence interval	-113.68,56	-7,949	4,653

The contour plot (fig. 2, 3, and 4) for respective responses were produced by design-expert software version 7.0 for a more desirable order.

Formula nano gels were developed according to determined optimized levels. Observed responses were in near agreement with the predicted values of the optimized process, as illustrated in table 7, by confirming the feasibility.

The optimal formula is verified to ensure that the results predicted by the model are not significantly different from the results of research observations [18]. This process was performed using the open stat app (table 8).

DISCUSSION

Preparation of biosynthesis nano gels was applying D-optimal Mixture Design. Two independent variables (Carbopol and Hydroxy Propyl Methyl Cellulose concentration), accompanied by three response variables composed of nano gel particle size, zeta potential, and spreading capacity, were selected for the conclusive optimization studies. There has been significant interest in using Carbopol in various pharmaceutical excipients in recent decades. Carbopol is a polymer of acrylic acid cross-linked, efficient, and valuable rheology modifiers. They supply excellent thickening and stabilization advantages [19]. D-optimal design is used for optimizing compounds when restrictions about the experimental setup are unusual. Parameters limits for a variable level of a gelling agent are exhibited in table 2.

Table 1 displays the design of the formula for the biosynthesis of nanogold gel. Eight experimental runs were conducted using Design Expert, and the particle size, zeta potential, and spreading capacity of each product were determined as responses and results of the experiments carried out during the D-optimal design displayed in table 3.

The formulation variables consist of the following:

X1: HPMC

X2: Carbopol

The dependent variables of the research include:

Y1: Particle Size

Y2: Zeta Potential

Y3: Spreading Capacity

The statistical parameters applied in selecting and evaluating the best-fitted model are regression data (p value and F value), lack-of-fit, coefficient of determination ($R2$), adjusted coefficient of determination (adjusted $R2$), and predicted coefficient of determination (predicted $R2$). The statistical analysis also builds an equation of the best-fitted model (table 4).

Tables of ANOVA can be applied to restrict how great the model and each parameter fits the data by analyzing the mean least square error estimates to the mean pure experimental error and by seizing

that the errors are normally distributed. Tests such as the F test can then be used to evaluate the significance of the fit for both the model and the individual parameters [20]. F-tests can ascertain significant or non-significant parameters. This investigation applied a 95% confidence interval to assess the signification parameter. Therefore, a probability value (P-value) of 5% would be the significant level in F-tests for the description of the outcomes [14]. The research used ANOVA to discover which factors significantly impact the particle size, zeta potential, and spreading capacity. Table 4 shows the variation analysis for all the reduced models; p values<0.05 signify that these models were significant. During whole cases, the lack of fit tests proved that it was not significant relative to the unproved mistake [21].

Table 4 presents the Results of ANOVA for particle size. As can be seen from table 4, the model is highly statistically significant (P<0.05); also, the overall model F-value of 46.42 and P-values<0.05 show that the model is considerable model terms [22]. The p-value of the quadratic model is 0.00006 (<0.05), which means that the results are significant. The lack of fit value for particle size is 0.0674 (>0.05). The little lack of fit value is good because there is no particle size variation for each replication in the optimization run [23]. The general form of the quadratic model from particle size (Y1) is presented as Eq 1 [14].

$$Y1 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots. (Eq.1)$$

$$Y1 = 118.91\ A + 594.73\ B + 441.77\ (A)(B)$$

Wherein:

Y1: Response of particle size

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

Y is the studied response of particle size, A and B are the variables considered in this research, and a and b are the estimated coefficients. A combination of factors describes interactions between the individual elements in that term. ANOVA was utilized to assess the influence of independent variables on the response. The optimized states were designed and matched the predicted values [24].

Based on the equation, the positivity of the coefficient presents a positive increase in the response. In this study, both carbopol and HPMC coefficients are positive (+118.91 and+594.73), which means that these components enhance the particle size. The interaction of carbopol with HPMC has increased the particle size (+441.73). These equations show that HPMC has the most dominant effect in increasing the particle size, and the interaction of the two gelling agents will increase the particle size.

Zeta potential was applied for particle size analysis to investigate the surface charge and size distribution of drug-loaded nanoparticles of gels. Zeta potential will help determine the *in vivo* portion of the nanocarriers and the impact of varying parameters on the nanocarrier's size [25]. Table 4 displayed the ANOVA results for Zeta Potential as the response. As can be observed from table 4, the F-value of 23.88 and P-values<0.05 indicates that the model is significant model terms. The general form of the linear model from zeta potential (Y2) is presented as Eq 2.

$$Y2 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots. (Eq.2)$$

$$Y2 = -7.94\ A - 10.96\ B$$

Wherein:

Y2: Response of zeta potential

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

In this study, both carbopol and HPMC coefficients are negative (-7.94 and-10.96), which means that these components contribute to a decrease in the zeta potential. The interaction of carbopol with HPMC does not affect increased or decreased zeta potential. These equations show that HPMC has the most dominant effect in reducing the zeta potential, and the interaction of the two gelling agents will not increase or decrease the zeta potential.

Table 1 displays the results of ANOVA for spreading capacity. Based on table 4, the model is highly statistically significant (P<0.05), with a model F-value of 184.86 and P-values<0.05, indicating that the model is considerable model terms. The general form of the quadratic model from dispersive ability (Y3) is presented as Eq 3.

$$Y3 = a(A) + b(B) + ab\ (A)(B) \ldots\ldots.(Eq.3)$$

$$Y3 = 6.41\ A + 4.58\ B - 1.58\ AB$$

Wherein:

Y3: response of spreading capacity

a: Regression coefficient of factor A

b: Regression coefficient of factor B

A: Carbopol

B: HPMC

The positivity of the coefficient presents a positive contribution toward the response. In this study, both carbopol and HPMC coefficients are positive (+6.41 and+4.58), which means that these components enhance the spreading capacity. The interaction of carbopol with HPMC has decreased the (-1.58). The conclusion shows that carbopol has the most dominant effect in increasing the spreading capacity, and the interaction of the two gelling agents will decrease the spreading capacity. Current researchers concerned with topical semisolid dosage forms quality assessment utilize a fixed batch of methods to test the spreadability [26].

Average probability plot residuals for particle size, zeta potential, and spreading capacity are represented sequentially in fig. 1A, 1B, and 1C. The outcome mainly received comes on a direct line, showing that the results gained are normally distributed and properly equaled with the regression pattern [27].

The contour plot (Fig. 2, 3, and 4) for respective responses were produced by Design-Expert Software Version 7.0 for a more desirable order. According to table 6, statistic analysis was obtained using one-sample test analysis for the optimum nano-gels from the prediction value from DX 10 software. The prediction value was obtained from a solution that has the highest desirability. The chosen desirability of certain balances was 0.572. This mixed desirability was achieved according to the desirability of carbopol and HPMC (fig. 5). Formula nano gels were developed according to determined optimized levels. Observed responses were in near agreement with the predicted values of the optimized process, as illustrated in table 7, by confirming the feasibility.

The verification results for the response in the form of particle size (table 8) show that there is no significant difference between the predictions of the model and the results of research observations (p-value>0.05). The verification results for the zeta potential response and dispersion showed that the p-value was less than 0.05 (table 8), which means a significant difference between the model prediction results and the results of research observations. There was a possibility from the desirability value, which is less than 0.7

CONCLUSION

This study has shown that a well-balanced mixture of matrix ingredients could improve nanogold biosynthesis gel. Optimization was possible by applying D optimal mixture design, verified valuable in producing nanogold gels dosage forms.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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