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Original Article

DEVELOPMENT OF SERUM WITH 4-N-BUTYLRESORSINOL IN THE TRANSETOSOMES VESICULAR SYSTEM

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ABSTRACT

Objective: The study aimed to develop a transetosome system as a delivery system of 4-n-Butylresorcinol (4nBR) and evaluate their physicochemical characteristics and skin penetration capacity compared with another vesicles system.

Methods: Transethosomes were prepared through cold methods and the optimization of the formulation was carried out using "Box–Behnken design" approach from Design-Expert software (version 13.0. 3. 0, State-Ease Inc., Minneapolis, MN). The independent variables were soya lecithin, surfactant (Tween 80: Span 80 with a ratio of 1: 3) and Ethanol. The prepared formulations were characterized for vesicle size, polydispersity (PDI), zeta potential using a particle size analyzer and entrapment efficiency. Furthermore, transethosomes were formulated in serum preparations that tested for *in vitro* penetration test compared to serum with ethosomes, transfersomes and non-vesicles system.

Results: Transethosomes formula optimization using box benhken approach produced a formula of 5.53 % soya lecithin, 3 % surfactant (Tween 80: Span 80 with a ratio of 1: 3) and 30 % Ethanol. The optimized formulation obtained particle size result of 197.4 nm; Polydispersity Index 0.421; zeta potential-56.8 mV and entrapment efficiency 98.40 %. Transethosomes serum met physical stability tests and *in vitro* penetration test showed better results compared to serum with ethosomes, transfersomes and non-vesicles system; the percentage of cumulative penetrated amounts of transethosomes serum, transfersomes, ethosomes and non-vesicle serum, respectively, was 41.43%; 23.59%, 19.85% and 2.43%.

Conclusion: Development of 4nBR transethosomes using surfactant as edge activators and ethanol as an enhancers through optimization with box Behnken design resulted in transethosomes composition as ultra-deformable vesicles that fulfiled the physical characteristics, stability and permeability of 4nBR.

Keywords: 4-n-Butylresorcinol (4nBR), Transethosomes, Box benhken approach, Penetration test

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INTRODUCTION

The depigmentation agents or skin-lightening agents is a popular preparation and is in great demand by global and regional markets, including in the Indonesia region. The molecular mechanism of skin-lightening agents is to reduce melanin, which is the main source of skin color, while melanin is an indole substance produced by the enzymatic oxidation of tyrosine, a key pigment that absorbs ultraviolet radiation, serves as a biological defense for the skin, which is produced by melanosomes in the basal cells of the epidermis (melanocytes) [1]. Excessive melanin deposition in the epidermal layer can cause age spots, lentigines, and other pigmentation disorders. Excessive melanin production and reduced melanin excretion, associated with abnormal melanin turnover, can lead to melanin deposition in the epidermis or pigmentation disorders [2].

Various mechanisms of action, including inhibition of enzymes related to melanin production, increased melanin excretion, and reduction of DOPA. Because tyrosinase plays an important role in the production of melanin, there are many depigmenting agents with a mechanism of inhibition of the tyrosinase enzyme, including substances that are widely used in skin-lightening products that have been widely circulated in the market.

Based on research conducted by Kolbe L, *et al.*, 2013 examining the effect of inhibiting the tyrosinase enzye from kojic acid, hydroquinone, arbutin and from the compound 4nBR on human tyrosinase where, the results showed that 4nBR was proven very effective as a human tyrosinase inhibitor with an IC_{50} value 21 lmol/l and 4nBR showing an inhibitory activity 20 times stronger than kojic acid, which IC_{50} 500 lmol/l and maximum inhibition (89 %) was achieved at concentrations 5.6 mmol/l. Arbutin and hydroquinone with an IC_{50} 6500 lmol/l for arbutin and 4400 lmol/l for hydroquinone. However, neither arbutine nor hydroquinone completely inhibited human tyrosinase [3].

Based on the results of the most potent inhibition of the tyrosinase enzyme, the 4nBR compound will be developed in the subject matter to be made into preparations for skin depigmentation agents, where the 4nBR compound is a resorcinol derivative with a butyl group, although 4-n-butylresorsinol is not stable and has skin irritant and bactericidal effect [4]. Compared with arbutin and kojic acid, 4nBR exhibits a strong tyrosinase inhibitory effect at lower doses. In addition, it also exhibits a TRP-1 inhibitory effect, which results in inhibition of melanin production [5].

Due to the unstable nature and irritating effect on the skin of the 4nBR compound and the route of topical administration, a form of delivery is needed which in addition to being able to protect the active ingredient so that it remains stable, also so that it can pass through the skin deeper and penetrate the stratum corneum, so today many drugs have been developed for drug delivery with nanoparticle dosage systems with particle sizes in the range of 1-1000 nm, which consist of active drug substances encapsulated or entangled into a nanoparticle matrix [6, 7]. This nanoparticle drug delivery system is also used to treat problems such as active substances that are difficult to dissolve [8]. Nanoparticle systems can include several types, namely liposome systems, ethosomes, niosomes, transfersomes, and transethosomes (development of ethosomes and transfersomes). The conventional lipid-based vesicular system with an ethanol base demonstrated the ability to traverse the layers of the stratum corneum [9].

Transethosomes are ethanol-based lipid vesicular systems modified by ethosomes and transfersomes that can increase skin penetration [10]. Transethosomes were first developed by Song *et al.* in 2012, Transfersomes work by increasing the flexibility of vesicles by redistributing edge activators and lipids in the skin, while ethosomes work by fluidizing the skin and vesicle lipids. So the transetosomes mechanism of action is a combination of the advantages of both transfersomes and ethosomes systems [11, 12].

The transetosomes vesicle system consists of phospholipids, ethanol and edge activators or permeation enhancers [13].

Therefore, in this study, an analysis of the physical and chemical characteristics of the optimal formula for the 4nBR transetosomes system will be carried out using a software program. Box Benhken's Design Expert to obtain a good transetosomes formula that fulfills the requirements. In this research, transethosomal formulations were carried out using the cold method, where this method is a more stable, simpler and most widely used method and has a high success rate [14]. Zeta potential, encapsulation efficiency, stability test and permeation test [13, 15].

MATERIALS AND METHODS

Materials

4-n-butylresorsinol (SHREEJI Pharma International), aqua dest, ethanol (Merck), span 80 (Sigma-Aldrich), soya lecithin, tween 80 (Sigma-Aldrich), HPMC (Bratachem), TEA (Bratachem), DMDM (Bratachem), phenoxyethanol (Bratachem), Sodium metabisulfite (Bratachem), Phosphate Buffer (Merck).

Methods

4nBR transcetosomes formulation

The transetosomes system in this study was made using the cold methods, which is the simplest and most widely used methods, using 2 phases, namely the lipid phase and the aqueous phase. The lipid phase was obtained by dissolving soya lecithin in ethanol with stirring for 5 min. Then added the surfactant with stirring for 10 min and then added the water phase, namely aquadest and 4nBR, which had been dissolved in the water phase gradually into the lipid phase with stirring at 700 rpm for 60 min until a vesicle suspension was formed, all treatments carried out at 30 ℃. Then sonicated for 25 min using a homogenizer to form transethosomes [16, 17].

Experimental design based on box behnken design approach

The influence of various process factors on the preparation of transethosomes was investigated using the Design of Experiment (DOE) approach. To evaluate the interaction effect of soy lecithin, ethanol and surfactants in formulations; 3 3-factor design [18,19], with Independent and Dependent variables, can be seen in table 1.

Table 1: 4nBR trancetosomes variables

Variable l	Composition	Lower limit %	Upper limit %
Independent Variable	Soya Lecithin	5	10
	Ethanol	30	40
	Surfactant	2	3
	(Tween 80: Span 80 = 1:3)		
Dependent Variable	Particle Size (nm)		
	Polydispersity Index (PDI)		
	Zeta Potential (mV)		
	Entrapment Efficiency (%)		

A total of 17 trials were produced by Design-Expert software (version 1 3.0. 3. 0, State-Ease Inc., Minneapolis, MN) Box–Behnken in table 2.

Table 2: Composition of transethosomes 4Nbr in the box benhken design

Std	Run	Factor 1	Factor 2	Factor 3
		A: soya lecithin	B: ethanol	C: surfactant
		%	%	%
17	1	7,5	35	2,5
1	2	7,5	40	2
8	3	7,5	35	2,5
4	4	10	35	2
13	5	7,5	35	2,5
15	6	7,5	40	3
12	7	5	40	2,5
14	8	7,5	35	2,5
11	9	5	35	3
10	10	7,5	30	2
5	11	7,5	30	3
9	12	10	35	3
3	13	7,5	35	2,5
16	14	5	35	2
6	15	10	40	2,5
7	16	5	30	2,5
2	17	10	30	2,5

Testing of particle size and particle size distribution (PDI)

By using the Particle Size Analyzer (PSA). Samples of 4nBR transethosomal suspension were put into the PSA apparatus and characterized using dynamic light scattering methods. The test was carried out by dissolving the sample in distilled water (1:30) [20, 21].

Measurement of zeta potential

The zeta potential measurement was carried out by diluting the transetosomes suspension sample into distilled water (1:30), then placing it in the cuvete and reading the measurement results [22].

Measurement of entrapment efficiency

4nBR transethosomes dissolved in 2.00 ml of ethanol were placed in a centrifugation tube and centrifuged at 6,000 rpm for 15 min, then the absorbance was measured at 279 nm [23]. The supernatant from centrifugation was taken using an UV spectrophotometer at 279 nm. Encapsulation efficiency (EE) of 4nBR is expressed as a percent of drug entrapped [6, 24]. EE % calculation is done using the following formula:

$$EE\ \% = \frac{\text{Total 4nBR} - \text{Free 4nBR}}{\text{Total 4nBR}} x100\%$$

Serum formulation and optimization

Optimization of the serum base formula refers to the formula from [28], which can be seen in the table. 3 as follow:

Preparation of serum preparations by dispersing Carbopol 940 in demineralized water, then homogenizing, and then adding Triethanolamine (TEA) and homogenizing again to obtain serum

base, DMDM and Phenoxyethanol added to the serum base mixture. Sodium metabisulfite is dissolved in distilled water and added to the serum base. After serum was formed, 4nBR transethosomes were added and homogenized for 15 min.

Table 3: Serum bases optimization formula

Materials	Concentration (%)					
	FS1	FS2	FS3	FS4	FS5	FS6
HPMC	0.5	1	1.5	-	-	-
Carbopol 940	-	-	-	0.1	0.3	0.5
TEA	-	-	-	0.4	0.4	0.4
DMDM	0.5	0.5	0.5	0.5	0.5	0.5
Phenoxyethanol	0.9	0.9	0.9	0.9	0.9	0.9
Sodium metabisulfite	0.075	0.075	0.075	0.075	0.075	0.075
Aquade st	ad 100	ad 100	ad 100	ad 100	ad 100	ad 100

Description: FS = Serum formulas

Evaluation of serum preparations

Organoleptic observation, homogeneity observation and pH measurement

Organoleptic observation of, color, odor and homogeneity as well as measurement of the pH of the serum preparation [25].

Viscosity determination

Measurements were made with a Brookfield viscometer. The spindle that corresponds to the serum is dipped into the container containing the serum preparation in the beaker for approximately 5 min, then the number is read on the instrument [23].

Stability test

The stability test of the 4nBR transetosomes system was carried out using the accelerated freeze-thaw method. 4nBR transethosomal samples stored at 2 temperature conditions, namely 4 °C in the refrigerator and 45 °C in the oven, for 6 cycles; each cycle is 24 h. Each formulation was prepared in triplicate for testing [16, 26].

***In vitro* penetration test using franz diffusion cells**

Penetration test of serum preparations was carried out using a 0.45 µ PTFE filter with Franz diffusion cell (diffusion area 1.77 cm^2, compartment volume 13 ml, receptor compartment filled with phosphate buffer pH 7.4 and temperature 37±0.5 °C). Serum transetosomes 4nBR and serum without transetosomes 4nBR were weighed as much as 1 gram each and applied to the membrane. A total of 0.5 ml of sample was taken from the receptor compartment periodically for 8 h using a syringe and replaced with an equal amount of phosphate buffer pH 7.4. The samples obtained were measured for their absorption with an UV spectrophotometer at a wavelength of 249 nm [27].

RESULTS AND DISCUSSION

Transethosomes optimization design 4nBR based on box behnken design approach served by table 4 as follow

Table 4: Response results prediction optimization of transetosomes 4nBR from design expert software

Run	A: soya lecithin %	B: ethanol %	C surfactant %	Particle size (nm)	Particle size distribution	Zeta potential (mV)	Entrapment efficiency (%)
1	7.5	35	2.5	267.6±0.43	0.911±0.013	70.9±0.80	97.813±0.60
2	7.5	40	2	352±0.93	0.504±0.08	31.2±0.29	99.97±0.82
3	7.5	35	2.5	267.6±0.85	0.900±0.023	70.9±0.08	97.813±0.20
4	10	35	2	353.2±0.36	0.5±0.051	48.9±0.22	97.32±0.48
5	7.5	35	2.5	267.6±0.36	0.911±0.012	70.9±0.07	97.813±0.20
6	7.5	40	3	267.6±0.71	0.900±0.024	70.9±0.62	97.813±0.44
7	5	40	2.5	180.2±0.43	0.700±0.045	47.5±0.64	98.01±0.18
8	7.5	35	2.5	267.6±0.29	0.911±0.005	70.9±0.14	97.813±0.56
9	5	35	3	277.9±0.88	0.355±0.006	-45±0.39	97.87±0.66
10	7.5	30	2	201.6±0.59	0.552±0.006	;54.9±0.45	98.32±0.24
11	7.5	30	3	287.8±0.80	0.611±0.019	60.9±0.51	98.813±0.60
12	10	35	3	303.2±0.22	0.48±0.016	50.9±0.59	97.83±0.74
13	7.5	35	2.5	267.6±0.24	0.911±0.009	70.9±0.50	97.813±0.71
14	5	35	2	289.2±0.57	0.256±0.016	-45±0.62	97.96±0.26
15	10	40	2.5	364.5±0.51	0.597±0.042	;56.1±0.73	96.11±0.56
16	5	30	2.5	247.9±0.73	0.225±0.023	-45±0.78	97.76±0.26
17	10	30	2.5	332.6±0.36	0.410±0.050	48.9±0.70	95.32±0.67

*Values [mean±SD (n = 3)]

Based on table 4, variations in transetosomal formulations with independent variables, soy lecithin as a phospholipid, ethanol and surfactant produce 17 running formulas. All formulas were prepared and evaluated from the aspects of particle size, particle size distribution, zeta potential and percent entrapment from all of them showed a particle size range of 202-353 nanometers, with the particle size still meeting the required portion size. The size distribution ranges from 0.2-0.9; with this value, some formulas do not meet the requirements, whereas the permitted requirement is<0.5, sizes below 0.5 indicate a homogeneous particle size. The zeta potential value ranges from-45-70 mV, the permissible values for the zeta potential value are<-30 mV and>30 mV; this zeta potential value is related to the stability parameters of the transetosomes, the greater the repulsion value indicates that aggregation is less likely to occur. The percent entrapment value shows a value of 95-99%, the allowable value is>85%, all running formulas meet the requirements.

Effect of independent variables on transethosomes 4nBR for particle size can be observ in fig. 1 as follow

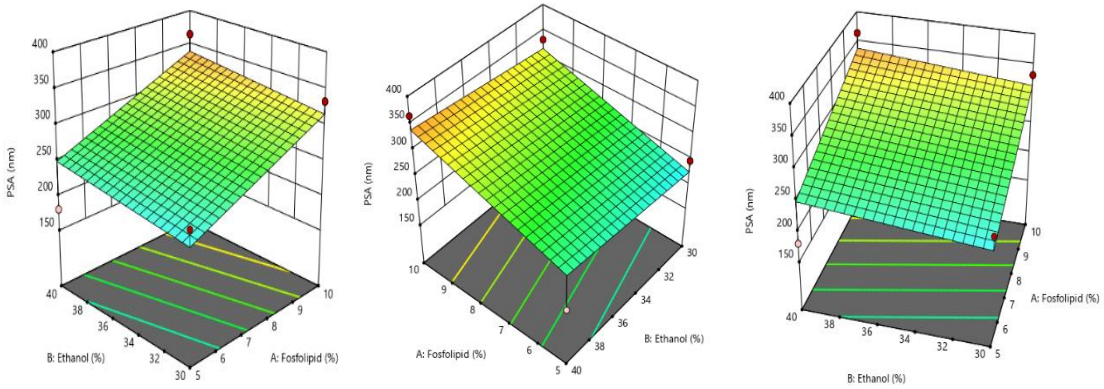

Fig. 1: Plot a response surface showing the influence of an on particle size

The resulting model for particle size has a p value<0.05 and an F value of 3.55, which indicates that the model is significant. Based on the coefficient table, the following equation is obtained:

Y =+2 82.1+44.7875A+11.8B–7.4375C. (Equation 1)

Description: Y = Particle size (nm)

A = Soya lecithin

B = Surfactant

C = Ethanol

Based on equation 1, the particle size through the Box Behnken Design approach is that the addition of soya lecithin has a significant effect on particle size. According to Singh P, *et al.*, 2019 that, the increase in the vesicle size of transethosomes is concomitant with increasing phospholipid concentrations [29]. The use of surfactants with high concentrations allows the formation of micelles to produce particles with a larger size.

In the results above, the particle size is in the range of 100–400 nm, which is appropriate according to Ghasemiyeh P and Mohammadi-Samani S, 2020 that the particle size for nanocarriers is below 500 nm [30], where according to Danaei *et al.*, 2018 that vesicles with an average diameter of 300 nm can penetrate deep into the skin layers, while vesicles with an average diameter of more than 600 nm cannot penetrate deep skin layers and most of it remain on the surface of the stratum corneum [31].

Effect of independent variables on transethosomes 4nBR for particle size distribution, showed by fig. 2 as follow

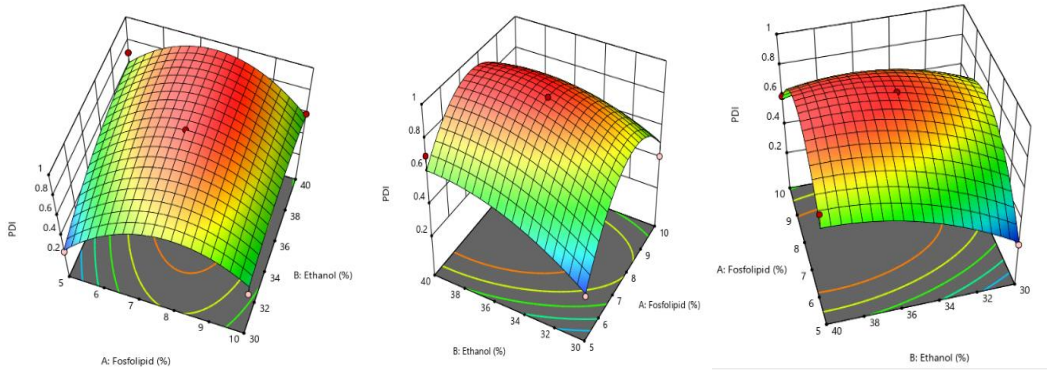

Fig. 2: Plot a response surface particle size sistribution

The resulting model for the particle size distribution has a p value<0.05 and an F value of 13.88, which indicates that the model is significant. Based on the coefficient table, the following equation is obtained:

Y=+0.9088+0.05525A+0.112875B+0.065625C-0.072AB-0.0275AC+0.08425BC-0.333775A^2-0.092025B^2-0.175025C^2…. (Equation.2)

Description: Y = particle size distribution

A = soya lecithin

B = surfactant

C = ethanol

Based on the above equation, the particle size distribution profile shows that the addition of surfactants shows a significant effect on the particle size distribution. The requirement of the particle size distribution is that for a perfectly uniform sample, the numerical value of the particle size distribution is between 0.0 and 1.0 for a highly polydispersed sample with multiple particle size populations [31].

The result above has an average particle size distribution of 0.626. When the particle size distribution value of a nanoparticle is less than 0.1, it is regarded to be highly homogenous (highly monodisperse); between 0.1 and 0.4, it is considered to be moderately dispersed (moderately dispersed), and above 0.4, it is regarded to be highly polydisperse (highly heterogenous) [32]. The 4nBR transethosomes were highly polydisperse and, therefore, unstable. Meanwhile, at the other side, Regulatory agencies such as Food and Drug Administration (FDA) have not stated the criteria for an acceptable particle size distribution range for different routes of administration. The criteria for the formulation of the various drug administration methods based on their particle size distribution range must, therefore, be mentioned by regulatory agencies [33].

Effect of independent variables on the transethosomes of 4nBR for zeta potential:

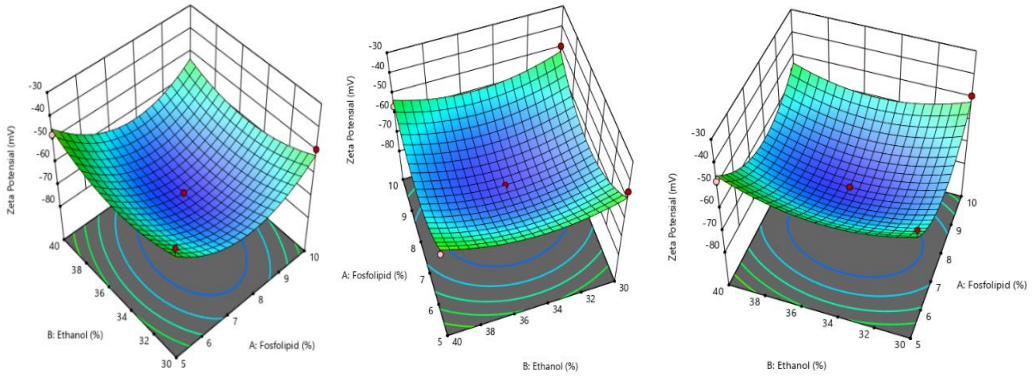

Fig. 3: Plot a response surface showing the influence zeta potensial

The resulting model for particle size has a p value<0.05 and an F value of 5.6 1, which indicates that the model is significant. Based on the coefficient table, the following equation is obtained:

Y =-70.9-2.7875A+0.5B-5.9625C-1.175AB-0.5AC-8.425BC+14.275A ^{2}+7.25B ^{2}+9.175C ^{2}.....(Equation 3**)**

Description: Y = Zeta Potential (mV)

A = soya lecithin

B = surfactant

C = ethanol

Based on the above equation 3. shows that the zeta potential is influenced by ethanol, which show negative interactions by giving a significant effect on the zeta potential. Likewise with the addition of surfactants and ethanol provide a significant effect on zeta potential.

Potential Zeta, which is the main particle surface component and is frequently evaluated using the Zetasizer, is crucial in assessing stability. It has an impact on nanosuspension stability. Extremely positive or negative zeta potential values that greater than+30 mV or less than-30 mV produce more repulsive forces, nevertheless, repulsion between particles with equivalent electric charges prevents the particles from aggregating and promotes straightforward redispersion [34, 35]. The zeta potential value that is close to-30 mV, which is-31.2 mV is a formula that uses 7.5% of soya lecithin, 40% of ethanol and 2 % of surfactant. These results were obtained in experiments with the use of the largest ethanol concentration, which is 40%, where the use of large amounts of ethanol will produce a system with a more negative particle surface so that it will reduce the particle size of the transetosome system [36].

Effect of independent variables on the transethosomes 4nBR for encapsulation efficiency can be observed on fig. 4

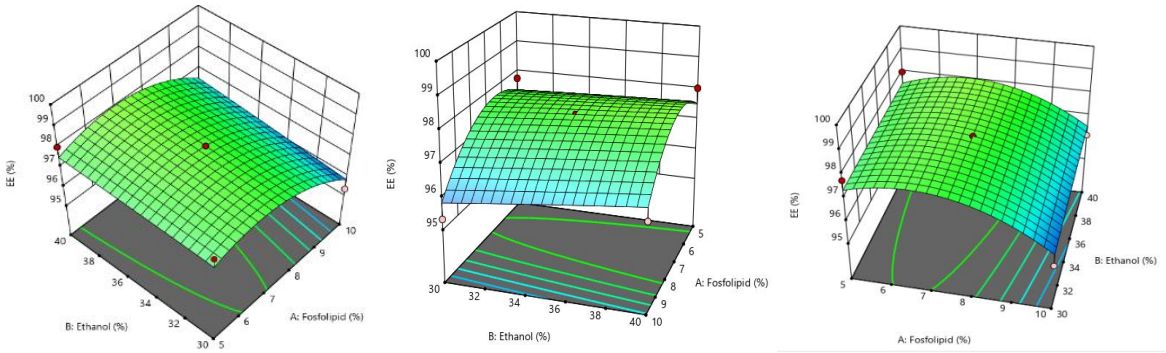

Fig. 4: Plot a response surface showing the influence of entrapment efficiency

The resulting model for particle size has a p-value <0.05 and an F value of 4.54, which indicates that the model is significant. Based on the coefficient table, the following equation is obtained:

Y = 97.8125–0.6275A+0.21125B–0.155625C+0.135AB**+**0.15AC–0.6625BC–0.998125A ^{2}–0.014375B ^{2}+0.930625C ^{2}. . . (Equation. 4)

Description: Y = Entrapment Efficiency (%)

A = Soya lecithin

B = Surfactant

C = Ethanol

Based on equation 4, it shows that the entrapment efficiency through the Box Behnken Design approach shows that with the addition of soya lecithin give a significant effect on the entrapment efficiency. 7,5%, surfaktan 3%, ethanol 30%.

The highest entrapment efficiency value was the experiment containing 7.5% soya lecithin, 3 % surfactant, and 30 % ethanol 30. These results were obtained in an experiment with the highest surfactant concentration at 3 %. If using lower concentrations, monomers surfactants will combine through a lipid bilayer and inhibit vesicle formation, meanwhile, fluidity of the membrane increase in higher concentration of surfactant, leading to optimizing entrapment efficiency [37]. The concentration of surfactant affects the percent entrapment efficiency if using lower concentration because the surfactant monomers will combine through a lipid bilayer and inhibit vesicles formation meanwhile fluidity of the membrane increase in higher concentration of surfactant leading to optimizing entrapment efficiency [37].

Determination and Characterization of Particle Size, Zeta Potential, Particle Size Distribution, and Zeta Potential of the Transethosomes Optimal Formula 4nBR can be seen in table 5.

Stability test of the vesicular system transetosomes 4nBR

Fig. 5, 6 and 7 showed the results of the physico-chemical evaluation after 6 cycles of the accelerated freeze-thaw methods.

Table 5: Optimal formulation components transethosomes 4nBR based on the box behnken design approach

Soya lecithin	Surfactant	Ethanol
5.53 %	3 %	30 %

Based on the Box Behnken design, the optimal formula components are obtained transethosomes 4nBR with soya lecithin 5.53 %, 3 % surfactant and 30 % ethanol, with the desirability value is 0.755

Table 6: Characterization of the optimal formula for Transethosomes 4nBR

Formulation	Article size (nm)	PDI	Zeta Potential (mV)	Encapsulation efficiency (%)
Blank transethosom	190.5±12.46	0.752±0.05	-40.5±1.60	-
Transetosom 4nBR	197.4±4.94	0.421±0.02	-56.8±4.24	98.40±0.81

*Values [mean±SD (n = 3)]

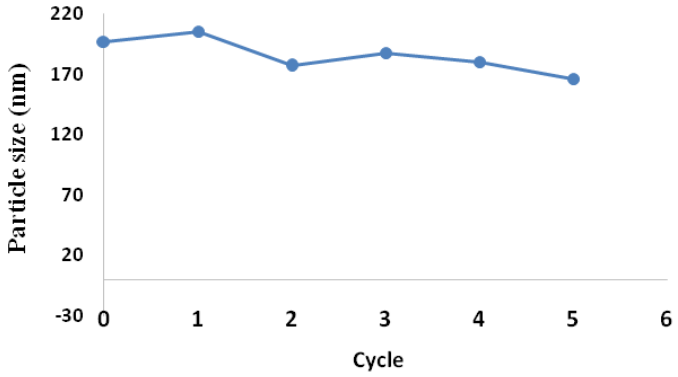

Fig. 5: Stability evaluation of transethosomes 4nBR particle size

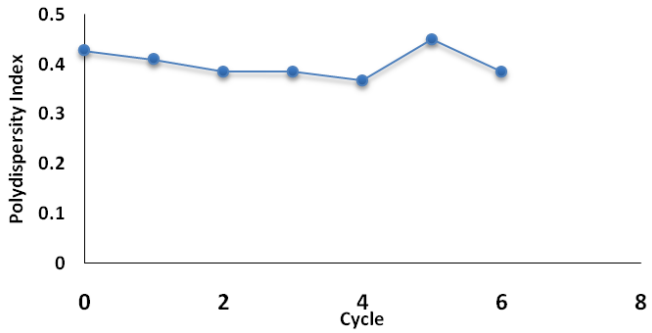

Fig. 6: Stability evaluation of transethosomes 4nBR particle size distribution

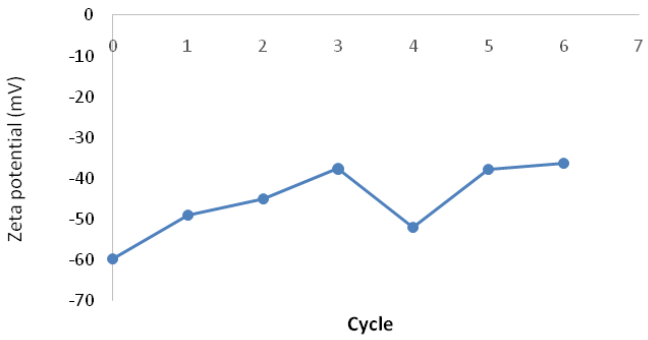

Fig. 7: Stability evaluation of the transetosomes zeta potential of 4nBR

Based on stability test data after 6 cycles. The particle size shows that the particle size is still within 220 nm (100-400 nm), this data shows that the particles are still within the required value range. The particle size uniformity index in 6 cycles is still below the value

of 0.5, this value shows the value that is still required and likewise, the zeta potential value shows a value below-30 mV, this shows particle stability with a high value of repulsion between particles so that the possibility of aggregation is small [10-13].

Serum preparation formulation

Transethosomes, which is formulated into serum preparations, is the formula yield 1 prediction from the Design of Expert (DOE) software (version 1.0.3.0, State-Ease Inc., Minneapolis, MN) with the Box Behnken Design approach, as shown in table. 5. Transethosomes suspension added to serum equivalent to 0.1 % 4nBR. The formula consist of soya lecithin 5.53 %, surfactant 3 % and ethanol 30%. It composition showed the optimal formula that can be continue to next evaluation base on the required paramaters.

Serum optimization and stability

In table 7 below are the results of observations of the physico-chemical properties of serum preparations in stability testing for 6 cycles to determine and determine the optimal formula to be combined with the transetosome vesicular system.

Table 7: Serum base evaluation results

Formulas	Evaluation						
	Cycle 0	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
FS1	• Color: Clear • Viscosity: 435.0 cPs • pH: 5.22 • Homogeneous	• Color: Clear • Viscosity: 660.0 cPs • pH: 6.21 • Homogeneous	• Color: Clear • Viscosity: 760.0 cPs • pH: 5.63 • Homogeneous	• Color: Clear • Viscosity: 810.0 cPs • pH: 5.22 • Homogeneous	• Color: Clear • Viscosity: 765.0 cPs • pH: 5.56 • Homogeneous	• Color: Clear • Viscosity: 620.0 cPs • pH: 5.36 • Homogeneous	• Color: Clear • Viscosity: 585.0 cPs • pH: 5.32 • Homogeneous
FS2	• Color: Clear • Viscosity: 8280 cPs • pH: 5.75 • Homogeneous	• Color: Clear • Viscosity: 21960 cPs • pH: 6.25 • Homogeneous	• Color: Clear • Viscosity: 16920 cPs • pH: 6.14 • Homogeneous	• Color: Clear • Viscosity: 21000 cPs • pH: 5.93 • Homogeneous	• Color: Clear • Viscosity: 14280 cPs • pH: 6.33 • Homogeneous	• Color: Clear • Viscosity: 6090 cPs • pH: 6.59 • Homogeneous	• Color: Clear • Viscosity: 5690 cPs • pH: 6.56 • Homogeneous
FS3	• Color: Clear • Viscosity: 33040 cPs • pH: 6.21 • Homogeneous	• Color: Clear • Viscosity: 63600 cPs • pH: 6.19 • Homogeneous	• Color: Clear • Viscosity: 54000 cPs • pH: 6.24 • Homogeneous	• Color: Clear • Viscosity: 59700 cPs • pH: 6.30 • Homogeneous	• Color: Clear • Viscosity: 47100 cPs • pH: 6.67 • Homogeneous	• Color: Clear • Viscosity: 27900 cPs • pH: 6.70 • Homogeneous	• Color: Clear • Viscosity: 21800 cPs • pH: 6.74 • Homogeneous
FS4	• Color: Clear • Viscosity: 24.00 cPs • pH: 8.11 • Homogeneous	• Color: Clear • Viscosity: 60.00 cPs • pH: 8.13 • Homogeneous	• Color: Clear • Viscosity: 84.00 cPs • pH: 8.13 • Homogeneous	• Color: Clear • Viscosity: 90.00 cPs • pH: 8.14 • Homogeneous	• Color: Clear • Viscosity: 90.00 cPs • pH: 8.14 • Homogeneous	• Color: Clear • Viscosity: 75.00 cPs • pH: 8.35 • Homogeneous	• Color: Clear • Viscosity: 70.00 cPs • pH: 8.45 • Homogeneous
FS5	• Color: Clear • Viscosity: 7070 cPs • pH: 6.59 • Homogeneous	• Color: Clear • Viscosity: 28520 cPs • pH: 7.00 • Homogeneous	• Color: Clear • Viscosity: 32640 cPs • pH: 7.08 • Homogeneous	• Color: Clear • Viscosity: 45360 cPs • pH: 7.16 • Homogeneous	• Color: Clear • Viscosity: 40140 cPs • pH: 7.06 • Homogeneous	• Color: Clear • Viscosity: 53400 cPs • pH: 7.19 • Homogeneous	• Color: Clear • Viscosity: 71700 cPs • pH: 7.23 • Homogeneous
FS6	• Color: Clear • Viscosity: 64800 cPs • pH: 5.71 • Homogeneous	• Color: Clear • Viscosity: 101200 cPs • pH: 5.84 • Homogeneous	• Color: Clear • Viscosity: 123200 cPs • pH: 5.87 • Homogeneous	• Color: Clear • Viscosity: 14300 cPs • pH: 5.83 • Homogeneous	• Color: Clear • Viscosity: 156000 cPs • pH: 5.74 • Homogeneous	• Color: Clear • Viscosity: 160600 cPs • pH: 5.86 • Homogeneous	• Color: Clear • Viscosity: 170600 cPs • pH: 5.92 • Homogeneous

From the evaluation results it was found that after 6 cycles the most optimal serum base was FS1 serum base, which in terms of viscosity met the requirements for serum, namely in the pH range 4.5-6.5 and viscosity in the range 230-1150 cPs, while for FS1 on the 6th cycle showed a pH of 5.32 and a viscosity value of 585.0 cPs.

The incorporation of the transethosomes formula into the serum formula. After the serum base is available, 0.1 % 4nBR transethosomes suspension is added to the serum base with stirring using a homogenizer with a speed of 500 rpm for 30 min.

In vitro penetration, testing of serum preparations using Franz diffusion cells aims to determine the amount of 4nBR that can penetrate through the skin during a certain time interval. The cumulative amount of 4nBR that penetrated, the percentage of penetration and its flux value for 3 h (180 min) can be seen in fig. 9, 10 and fig. 11 below:

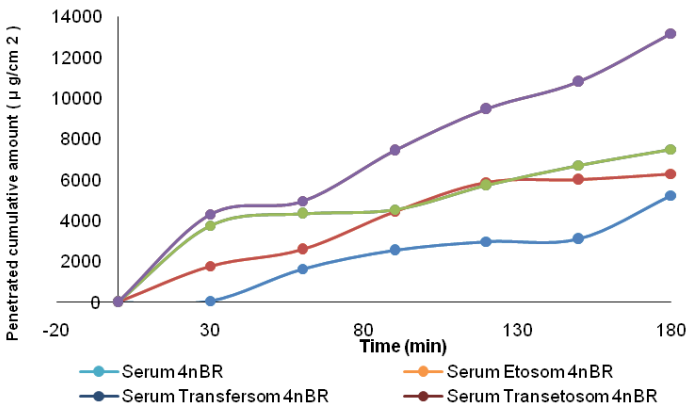

Fig. 9: The result of the cumulative amount of the active substance 4nBR penetrated from various forms of vesicles in the serum

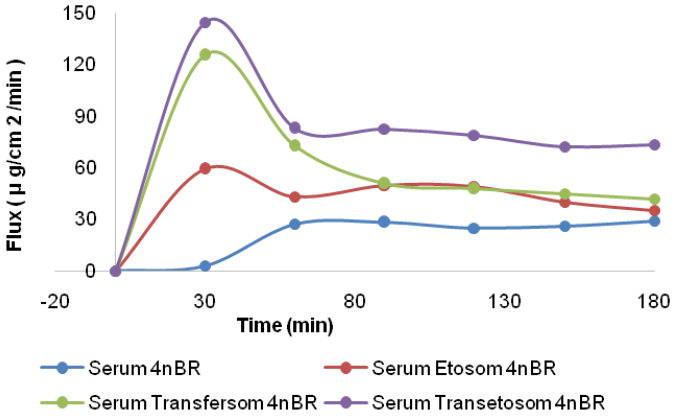

Fig. 10: Flux of active substance 4nBR from various forms of inner vesicles serum various vesicle forms

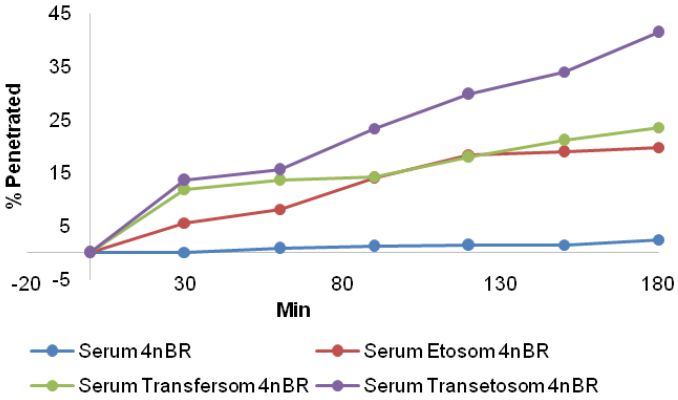

Fig. 11: Percentage of active substance 4nBR penetrated from various forms of vesicles in serum

Base on result on fig. 9, 10 and 11, it can be seen that the serum transethosomes 4nBR has a better cumulative amount penetrated, percentage and flux value compared to serum preparations without transethosomes, as well as in the form of other vesicles, namely serum in the form of ethosomes and transfersomes. Transetosomes vesicles can penetrate better into the skin; this is related to the deformability of transetosom vesicles 4nBR so that it provides better penetration ability compared to other vesicular systems, while the graph shown in fig. 10 shows the value transethosomes 4nBR serum flux has the highest drug penetration rate, so do in fig. 11 Serum with the 4nBR transethosomes vesicle system exhibits better than serum with ethosomal, transfersomal and non-vesicle form serum with the percentage of total penetration was 41.43%; 23.59%, 19.85% and 2.43% respectively. That results is because in the transetosomes system there are enhancers and also edge activators at the same time, where enhancers help penetrate the skin layers better and edge activators help vesicles adjust their shape when they pass through the skin pores and return to their original shape when successful penetrated the skin layer, or what is known as deformability properties.

CONCLUSION

Development of 4nBR transethosomes using surfactant as edge activators and ethanol as an enhancers through optimization with box Behnken design resulted in transethosomes composition as ultra-deformable vesicles that fulfiled the physical characteristics, stability and permeability of 4nBR.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors declare no confilct of interest in this manuscript

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