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RESEARCH ARTICLE

Response Surface Methodology Assisted Surfactant Optimization on the Novel Resveratrol Self-assembly Nanoemulsions

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ABSTRACT:

Resveratrol, a strong antioxidant and anti-inflammatory phytoalexin is potential to relieve impaired diabetic wound healing. Self-assembly nanoemulsions were developed to formulate good quality of resveratrol topical preparation. The surfactant system consisted of Kolliphor® RH 40 and Transcutol® were further optimized in terms of viscosity, pH and the percentage of transmittance. A central composite design combined with the response surface methodology successfully resulted in surface responses with the desirability value for viscosity, pH, and transmittance percentage were 0.99742, 0.60598, and 0.37137, respectively. These results contributed to the composite desirability of 0.6077. The optimal condition was obtained at a composition 5.70 g and 2.30 g of Kolliphor® RH 40 and Transcutol®, correspondingly.

KEYWORDS: Central composite design, optimization, nanoemulsion, resveratrol, surfactant.

INTRODUCTION:

The impaired wound healing is one of diabetes mellitus complications which usually occurs in patients who fail to control their sugar levels^{1,2}. This diabetic wound is aggravated by immunodeficiency, periferal neuropathy, inflammation and ischemia due to peripheral vascular disease³. Moreover, endothelial malfunction, decrease of macrofage number and functions, and impeded angiogenesis were reported to be the potential reasons of the slow recovery process of wound healing^{2,4}. Strategies to combat the impaired wound healing have been intensively developed, including the use of nitric oxide for the therapy⁵, chemokyne regulation⁶, and exploration of natural products^{7–9}.

Resveratrol (E-5-(4-hydroxystyryl) benzene-1,3-diol; RSV) is a natural polyphenolic compound which has been therapeutically claimed to reduce the risks of cardiovascular diseases¹⁰⁻¹² and diabetes^{13,14}.

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As a natural product with strong antioxidant and antiinflammatory effects, RSV is also potential to alleviate diabetic wounds by activating the SIRT 1 signalling pathway-mediated angiogenesis, diminishing oxidative stress, and promoting fibroblast cell proliferationmigration^{2,15,16}.

As a wound medication, topical application offers opportunities to give the best use of RSV. To provide good quality of medication, a profile of formulation which can incorporate and protect the RSV in the storage and deliver the RSV well onto the wound, is desirable. RSV topical nanocarriers have been widely developed, such as niosomes, transfersomes, ethosomes, and nanoemulsions^{17–19}. Self-assembly nanoemulsions have been proven to be good quality RSV liquid formulations which show clarity, stability, and good skin penetration.¹⁹.

Multivariate analysis combined with a response surface methodology is a reliable formula optimization tool which is frequently used to assist in the analysis as well as formulation area in finding the optimum condition of the composition of the factors studied²⁰⁻²⁴. Nastiti et al.²⁵

reported that the formula optimisation of RSV nanoemulsions could be carried out by using the Box Behnken Design (BBD) followed by a response surface methodology application. On the factors and levels studied, they suggested that for the RSV nanoemulsions, the optimum composition of three factors i.e a combination of triacetin and eugenol as the oil phase, Kolliphor ® RH40, and Transcutol® composition to provide expected drug load and viscosity could be established.

Central Composite Design (CCD), an integral part of response surface methodology, was applied in this current study since there was no need for a three-level factorial experiment to build an accurate predictive model for this design^{26,27}. Hence, the CCD approach is more efficient and promising in optimizing the nanoemulsion formula containing RSV.

The key of the successful self-assembly nanoformulation is an adequate surfactant system which consists of surfactant and cosurfactant^{28–32}. However, the consistency of the surfactant system may affect the viscosity and other physical characteristics of the formulation. This current study focused on the combination of CCD and a response surface methodology to obtain optimum condition of the surfactant system in RSV nanoformulation containing eucalyptol in generating good quality of RSV selfassembly nanoemulsions.

MATERIALS AND METHODS:

Materials:

Resveratrol (99% purity) was purchased from PCCA (USA). Triacetin, Eucalyptol, Kolliphor® RH 40 (polyoxyl 40 castor oil), and Transcutol® (ethoxydiglycol) were purchased from Sigma Aldrich (Singapore)

Instrumentation and Software:

Instrumentations used were a Merlin VR viscometer (Rheosys, USA), a magnetic stirrer (Thermo Scientific, USA), a pH meter (Ohaus, USA), and a spectrophotometer UV-Vis (Shimadzu, Japan).

Response surface methodology and multiple response optimization were performed by using Minitab® 17 for Windows.

Topical Nanoformulation:

The 2% RSV nanoemulsion was fabricated by the spontaneous emulsification method based on the formulation done by Nastiti et al.²⁵ with some modification. The main formula is presented on the table 1.

Table 1.	The	main	formula	RSV	nanoemulsion

Component	Amount (g)					
Triacetin-eucalyptol	10					
Kolliphor® RH 40	30					
Transcutol®	10					
Citrate buffer pH 6	50					

The oil phase, consists of triacetin and eucalyptol at the same ratio (1:1), was mixed with the aqueous phase (citrate buffer pH 6) and stabilized using the composition of Kolliphor® RH 40 (surfactant) and Transcutol® (cosurfactant) with the ratio S/CoS of 3:1. All components were mixed using a magnetic stirrer at room temperature.

Experimental Design

Formula optimization was performed by applying the response surface methodology. The CCD was applied to observe two different independent variables namely Kolliphor® RH 40 (x_1) and Transcutol® (x_2). Table 2 presents a matrix of independent variables with different levels to generate the CCD model. The levels of factors are coded by - α (extremely low level), -1 (low level), 0 (middle level), +1(high level), and α (extremely high level) This experimental design was executed by observing 16 runs including four factorial points, four central points on factorial points, four-star points, and four central points on star points.

Table 2. Levels of Factors of Experimental Design

Variables	Levels				
	-α	-1	0	+1	$+\alpha$
x ₁ : Kolliphor [®]	4.59	5	6	7	7.41
x ₂ : Transcutol [®]	0.59	1	2	3	3.41

Viscosity Determination

The determination of the viscosity was done 24 hours after nanoemulsion preparation, using a Merlin VR viscometer (Rheosys, USA). The mode of 25 mm concentric (co-axial) cylinders in a rotation speed of 50 rpm was used to set up a cup-and-bob system. The measurement was done twice for each formula at ambient temperature, with a delay time of 10 sec and an integration time of 20 sec. The zero-shear time was set 100 sec in between measurement.

pH Evaluation

The pH of self-assembly nanoemulsions were measured by using a pH meter (Ohaus, USA) after being diluted with the aqueous phase. All formulations were evaluated whether they were in a range of 5.5 - 6.5.

Percentage of Transmittance Evaluation

The measurement of transmittance of the formulations was carried out by placing the nanoemulsion in a cuvette and measuring the percentage transmittance using a spectrophotometer UV-Vis (Shimadzu, Japan) at the wavelength of 660nm. The results of > 80% of transmittance was expected on this study.

Statistical Analysis

Statistical analysis of ANOVA and desirability analysis was applied in a response surface methodology employed by the Minitab software. Comparison of the actual and predicted value were evaluated using the percentage of relative error of prediction (REP) using the following formula:

Actual value

RESULTS:

Topical nanoformulation

The 16 formulas of nanoemulsion were successfully fabricated (Figure 1) based on the matrix of the runs on the experimental design (table 2) with the appearance of transparent brownish-yellow liquids, with the viscosity in the range of 0.1-0.8 Pa.s., pH at around 6, and the transmittance was at approximately 90%.



Figure 1. The RSV nanoemulsions

Experimental Data

Composition of Kolliphor® RH 40 and Transcutol® were selected as the experimental factors, whereas the viscosity, pH, and percentage of transmittance were the responses. Table 3 presents the matrix of run and the results of the experiment.

Run	Factors		Responses			
	Kolli	Trans	Viscosity	pН	Transmittance	
	phor®	cutol®	(Pa.s)		(%)	
1	5	1	0.493	6.2	88.867	
2	7	1	0.772	5.9	86.316	
3	5	3	0.102	6.0	87.122	
4	7	3	0.239	6.0	87.549	
5	6	2	0.270	6.0	87.622	
6	6	2	0.270	6.0	87.109	
7	6	2	0.283	6.1	92.383	
8	6	2	0.282	6.1	90.979	
9	4.59	2	0.193	6.1	89.856	
10	7.41	2	0.466	6.1	93.542	
11	6	0.59	0.875	6.0	91.931	
12	6	3.41	0.160	6.1	91.479	
13	6	2	0.296	6.1	89.368	
14	6	2	0.303	6.1	91.797	
15	6	2	0.304	6.1	90.906	
16	6	2	0.297	6.1	92.664	

Table 3. Matrix of Factors and Experimental Responses

Response Surface Methodology

Response surface methodology was performed in this study to optimize the composition of Kolliphor® RH 40 and Transcutol ® as the surfactant system. Viscosity, pH, and transmittance percentage of nanoemulsions were investigated as the responses. Figure 2 depicts the response surface plots whereas Figure 3 illustrates contour plots obtained from the response surface analysis.



Figure 2. Response surface plots of Kolliphor® RH 40 (Koll) versus Transcutol® (Trans) for viscosity (a), pH (b), and transmittance percentage (c)



Figure 3. Contour plots of Kolliphor® RH 40 (Koll) versus Transcutol® (Trans) for viscosity (a), pH (b), and transmittance percentage (c)

Multiple Response Optimization

Aided by the Minitab software, the multiple response optimization was conducted using a response optimizer menu. The response of transmittance percentage was set to the maximum condition whereas the viscosity and pH were set to the target of 0.20 Pa.s and 6, respectively. The optimization plot for viscosity, pH, and transmittance percentage is presented in Figure 4.



Figure 4. Optimization plot for viscosity, pH, and transmittance percentage

No	Viscosity	REP of Viscosity	pH	REP of pH	Transmittance	REP of transmittance
	(Pa.s)	(%)		(%)	percentage (%)	percentage (%)
1	0.195	2.498	5.96	1.993	96.094	6.281
2	0.204	2.024	5.95	2.165	95.947	6.138
3	0.201	0.562	5.93	2.509	94.397	4.596
4	0.199	0.438	5.93	2.509	95.898	6.090
5	0.207	3.444	5.91	2.856	95.337	5.537
6	0.203	1.541	5.91	2.856	93.542	3.724

Table 4. Responses of optimum formula on viscosity, pH, and transmittance percentage

Optimum Formula Evaluation

The percentage of the relative error of prediction (REP) was calculated in term of viscosity, pH, and transmittance percentage. Table 4 presents the results of optimum formula responses.

DISCUSSION:

Self-assembly nanoemulsion was selected as topical formulation to deliver the RSV onto diabetic wounds as it offers benefits related to: ease of application, good skin penetration, stability of RSV, simplicity of fabrication, and attractive clarity appearance. Establishing self-assembly nanoemulsions of solubilized RSV with high thermodynamic stability was the prominent strategy of this formulation^{33,34}.

In this study, the CCD approach was implemented to achieve the optimum condition of Kolliphor® RH 40 and Trancutol® composition in terms of viscosity, pH and percentage of transmittance in RSV nanoemulsion containing eucalyptol. Kolliphor® RH 40 is a non-ionic surfactant with an excellent emulsifying and solubilizing effect and it has been widely used to support the stability of a variety of oral and topical formulations^{19,35–37}.

Kolliphor® RH 40 shows semisolid consistency at 25°C³⁸. The semisolid consistency of Kolliphor® RH 40 was reported to impact the viscosity of the RSV nanoemulsions²⁵. Transcutol® as a cosurfactant adds the value of Kolliphor® RH 40 in establishing adequate surfactant system with an excellent solubilizing effect without confronting the skin integrity³⁹.

CCD was applied in this study due to its accuracy and capability to build a second-order quadratic model without performing the three-level factorial experiment. The star points represented lower and higher extreme values. It is possible to extend two level independent variables, which have been widely applied in response surface both for modelling and optimization. In CCD approach, the central points were eventually augmented with the group of "star points" in order to estimate of curvature²⁶. Sixteen experimental runs were obtained from the model including four factorial points, four central points on factorial points. These experimental

runs were formulated and fabricated in the laboratory followed by nanoemulsion characterisation and evaluation on viscosity, pH, and percentage of transmittance. The observational results were exploited to build a response surface model for viscosity, pH and percentage of transmittance. Response surface equation obtained for the responses were: (1) viscosity = 0.728 - $0.0110 x_1 - 0.4656 x_2 + 0.01519 x_1^* x_1 + 0.10919 x_2^* x_2$ - 0.03550 $x_1^* x_2$ (R²=0.998), (2) pH = 7.090 - 0.187 x_1 - $0.345 \ x_2 \ \text{-} \ 0.0000 \ x_1 \ \ x_1 \ \text{-} \ 0.0250 \ x_2 \ \ \ x_2 \ + \ 0.0750 \ \ x_1 \ \ x_2$ $(R^2=0.632)$, and (3) transmittance percentage = 81.8 + 3.54 x₁ - 3.08 x₂ - 0.387 x₁* x₁ - 0.384 x₂* x₂ + 0.74 x₁* x_2 (R²=0.511). It was found that viscosity resulted the best model accuracy compared to other responses since the R^2 value of viscosity was 0.998. Viscosity is the important factor to consider in manufacturing liquid and semisolid preparation. Viscosity determined the consistency and the spread-ability of formulation associated with the ease of topical application. Determination of viscosity is also essential to address the issues related flowability during formulation, mixing process, and primary packaging⁴⁰. However, the response surface model of pH and percentage of transmittance was still considered to build predictive composite model of the optimization. The pH was still considered as the stability of RSV relies on pH (acidic $(5-6)^{41}$. condition with pН Moreover, the nanoformulations aimed for topical administration, therefore to avoid the risk of skin irritation, the formulators must consider that the pH of the products must be closed to pH of the skin⁴². High percentage of transmittance indicates the transparency of the formulation due to the absence of solid particles and liquid crystals in the system. This condition was suggested as a result of high micellar solubilization achieved by spontaneous emulsification^{37,43–45}.

Desirability value for viscosity, pH, and transmittance percentage were 0.99742, 0.60598, and 0.37137, respectively. These results contributed to the composite desirability of 0.6077. Desirability value of 0 represents completely unexpected response, whereas 1 represents the most expected response⁴⁶. The optimum condition resulted from the multiple response optimization suggested the composition of Transcutol® and Kolliphor® RH 40 of 2.30 g and 5.70 g, respectively. These optimum conditions were examined in eight replications of nanoemulsion formulations. It was found that the viscosity, pH, and transmittance percentage of the formulated nanoemulsion were 0.201 ± 0.004 Pa.s, 5.904 ± 0.061 , and 94.730 ± 1.271 %, respectively. The percentage of the relative error of prediction for each replication were also calculated (Table 4) and showed low values.

CONCLUSION:

Surfactant optimization by the response surface methodology model of CCD on the composition of Kolliphor® RH 40 and Transcutol® for the novel RSV nanoemulsions, was successfully developed with a good predictive model. The desirability analysis contributed to the generation of multiple response optimization of viscosity, pH, and percentage of transmittance indicated the composite desirability of 0.6077.

CONFLICT OF INTEREST:

The authors have no conflicts of interest regarding this investigation.

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