International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 15, Issue 1, 2023

Original Article

NOVEL QUERCETIN NANOEMULGEL OPTIMIZATION: GELLING AGENTS EVALUATION AND THE APPLICATION OF RESPONSE SURFACE METHODOLOGY

CHRISTOFORI MARIA RATNA RINI NASTITI[1*], RINI DWIASTUTI[1], FLORENTINUS DIKA OCTA RISWANTO[2]

[1,2]Division of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, Sanata Dharma University, Campus 3 Paingan, Maguwoharjo, Depok, Sleman, Yogyakarta, 55282, Indonesia
*Email: ratnarini@usd.ac.id

Received: 12 Oct 2022, Revised and Accepted: 02 Dec 2022

ABSTRACT

Objective: This current research aimed to examine the profile of a range of gelling agents by applying principal component analysis (PCA) based on certain physical properties and to develop a novel optimized nanoemulgel formulation containing quercetin (QUE).

Methods: A series of gelling agents with different concentrations were grouped and profiled by applying the PCA based on their viscosity and the spreadability. Based on the profile, one of the gelling agents was selected to be formulated in QUE nanoemulgel. The formulation of QUE nanoemulsion was then fabricated using a spontaneous emulsification method involving triacetin as the oil phase, a combination of Kolliphor® RH 40 and Transcutol® as the surfactant-cosurfactant system, and citrate buffer pH 6 as the aqueous phase. QUE nanoemulgel was fabricated by incorporating the gelling agent (sodium carboxymethylcellulose; Na CMC) into the nanoemulsion. The composition of Kolliphor® RH 40, Transcutol®, and Na CMC in the formulation was further optimized by using Box Behnken Design followed by a response surface methodology provided by Minitab®.

Results: The PCA grouped a range of gelling agents into three principal components (PC) based on the concentration, viscosity and spreadability. The results of PCA showed that Na CMC was the most suitable gelling agent for QUE nanoemulgel. To optimize the QUE nanoemulgel formulation, sixteen runs of BBD were successfully fabricated, providing an optimum-validated composition of 21.45 g, 13.96 g, and 4.00 g for Kolliphor® RH 40, Transcutol®, and Na CMC, respectively, with composite desirability of 0.843.

Conclusion: We successfully conducted gelling agent profiling by providing three types of PC using PCA. An optimized and validated formulation of QUE nanoemulgel was also successfully designed as a potential topical diabetic wound healing formulation.

Keywords: Quercetin, Nanoemulgel, Optimization, Principal component analysis, Response surface methodology

DOI: https://dx.doi.org/10.22159/ijap.2023v15i1.46585. Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Foot diabetic wound is one of severe complications of the diabetic patients with uncontrolled blood sugar level, resulted in challenging recovery due to the risk of endothelial growth failure and angiogenesis blocking [1]. Tissue death and gangrene infection ended with amputation surgery of the extremity organs were the major risks and governed up to 50% of the diabetic wound cases [2, 3]. Enormous strategies have been done to promote foot diabetic wound recovery especially the use of phytochemical compounds [4]. Quercetin (2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4H-1-benzopyran-4-one; QUE) is one of the natural compounds which is potential in promoting and recovering diabetic wounds [5, 6]. QUE shows antioxidant activity anti-inflammatory activity and promotes growth factors which are the key factors in regulating the wound healing process [7–9].

A nanoemulgel system is known as a result of the synergetic combination of nanoemulsion and the gelling system in a form of semisolid preparation [10–12]. Nanoemulsion shows excellent dissolving properties for lipophilic agents [13]. Nanoemulsion is also evidenced in maintaining the stability of the natural products in correlation with acidic pH conditioning [14]. The system of surfactant in the nanoemulsion formulation is essential in establishing a stable and good quality of the emulsion. In the previous study [15], we confirmed that an adequate combination of surfactant and cosurfactant supports the micellization of the nanoemulsion in solubilizing the lipophilic compound, showed by the high transparency of resveratrol nanoemulsion. The characteristic of such nanoemulsion has made the formulation [15] is of choice in delivering the QUE topically to the wound effectively. Nevertheless, low viscosity, thus uncontrolled spread-ability of nanoemulsion, may lead to the inconvenience of the application. However, incorporation of gelling agent enhances the viscosity. Various gelling agents have been widely used in the formulations of nanoemulgels ranged from natural and semi-synthetic polymers, such as tragacanth, xanthan gum, sodium alginate (Na alginate), carbomer, hydroxy-propyl methyl cellulose (HPMC) and sodium carboxymethyl cellulose (Na CMC). The difference of the structures, rheological properties, viscoelasticity nature, expanding mechanisms as well as the concentration of gelling agents in the system determines the gelling agents in affecting the consistency of the system [16–18].

Viscosity and the spread-ability are two of the important quality parameters in developing nanoemulgels, as they govern the physical consistency, physical stability and applicability of the preparation [11, 12]. Viscosity plays an important role in determining the rheological property during filling and packaging related to consistency-handling issues [19], whereas in an application, viscosity affects the ability of the product to spread over the intended topical area (spread-ability). The spread-ability leads to the ease of application thus the convenience of use.

Formulation by design (FbD) approach has addressed to develop such formulation in a rational, systematic and cost-effective manner [20]. Principle component analysis (PCA) can be applied as the first endeavor in profiling the prime components of formulation based on the expected physical performance prior to formula optimization [21]. Formula optimization using Box Behnken Design (BBD) in association with response surface methodology allows the formulators to build a rigid design of experiment with less risks of trial and error, thus enhancing the efficiency in formula design [15, 22]. Systematic formula optimization offers an excellent solution to establish the novel QUE nanoemulgels. This current study aimed to select a gelling agent with intended physical properties by the aid of PCA and to obtain the optimized composition of formulation to develop qualified QUE nanoemulgels.

MATERIALS AND METHODS

Materials

Quercetin, triacetin, Kolliphor® RH 40, and Transcutol® were purchased from SIGMA (Singapore). Tragacanth was purchased from DwiLabmandiri_Scientific (Bandung, Indonesia), Na carboxymethyl cellulose (Na CMC) and Na alginate were purchased from Centra TeknoSains (Yogyakarta, Indonesia), hydroxypropylmethylcellulose (HPMC) was purchased from Aloin Labora (Kediri, Indonesia), and xanthan gum were purchased from Kimia Market (Bandung, Indonesia) whereas the citrate buffer pH 6 was *in house* production. All materials were of pharmaceutical grade.

Methods

Principal component analysis

Various gelling agents including Na CMC, HPMC, xanthan gum, tragacanth, and Na alginate, each at a concentration of 4%, 5%, and 6% were congealed in pH 6 citrate buffer. After 24 h, the congealed gelling agents were measured in terms of their viscosity and spread-ability using the same methods on the nanoemulgel physical characterization. All data were collected and analyzed using PCA algorithm. Several PCA plots, namely scree plot, score plot, loading plot, and biplot were generated to visualize gelling agent profiles.

Quercetin nanoemulgel formulation

The fabrication of QUE NEG was using a spontaneous nano emulsification method followed by the incorporation of gelling agent. The basic formulation is presented in table 1.

Table 1: Basic formulation of QUE nanoemulgels (100g)

Ingredients	Weight (g)
Triacetin	5
Kolliphor ® RH 40	24
Transcutol ®	12
Na CMC	4.5
Citrate buffer pH 6	54.3
Quercetin	0.2

In brief, Kolliphor® RH 40 and Transcutol® were mixed with triacetin. Citrate buffer (pH 6) was further added on the mixture of the oil phase-surfactant-cosurfactant and stirred with a magnetic stirrer at 650 rpm for 5 min until it achieved clarity. Quercetin (0.2%) was then dissolved in the nanoemulsion system. Na CMC was further homogenously mixed with the QUE nanoemulsion at 350 rpm for 5 min. The QUE nanoemulgels were kept at room temperature and protected from light for 24 h prior to physical characterization.

Quercetin nanoemulgel physical characterization

Organoleptic characterization, pH confirmation and transmittance evaluation

QUE nanoemulsion and nanoemulgel were visually characterized by their appearance, colour, and consistency. The pH of nanoemulgel was confirmed by using pH meter (WTW pH 3110 SET 2, Germany). The clarity of nanoemulsion was examined by transmittance measurement using UV-Vis spectrophotometer (Shimatzu, Japan) at 800 nm at room temperature.

Viscosity examination

Viscosity of QUE nanoemulgels was measured at room temperature using a Merlin VR viscometer (Rheosys, USA) with the mode of cone and plate 2 °/30 mm, running at 50 rpm, at room temperature. The measurement was replicated, with a delay time of 20 seconds, zero-shear time of 20 seconds and the integration time of 10 seconds.

Determination of spread-ability

Spread-ability was determined by measuring the spreading diameter of one gram of nanoemulgel, after being placed in between two glass plate horizontally for 1 min, with the upper plate weighed 125g [23]. The spreading diameter was the average of the diameter measured in 4 plots.

Experimental design

Experimental design for optimizing nanoemulgel formulation was developed in order to achieve the optimum composition of Kolliphor® RH 40 (X_1), Transcutol® (X_2), and Na CMC (X_3). The response surface methodology of the Box-Behnken design was generated using three independent variables and three levels as shown in table 2.

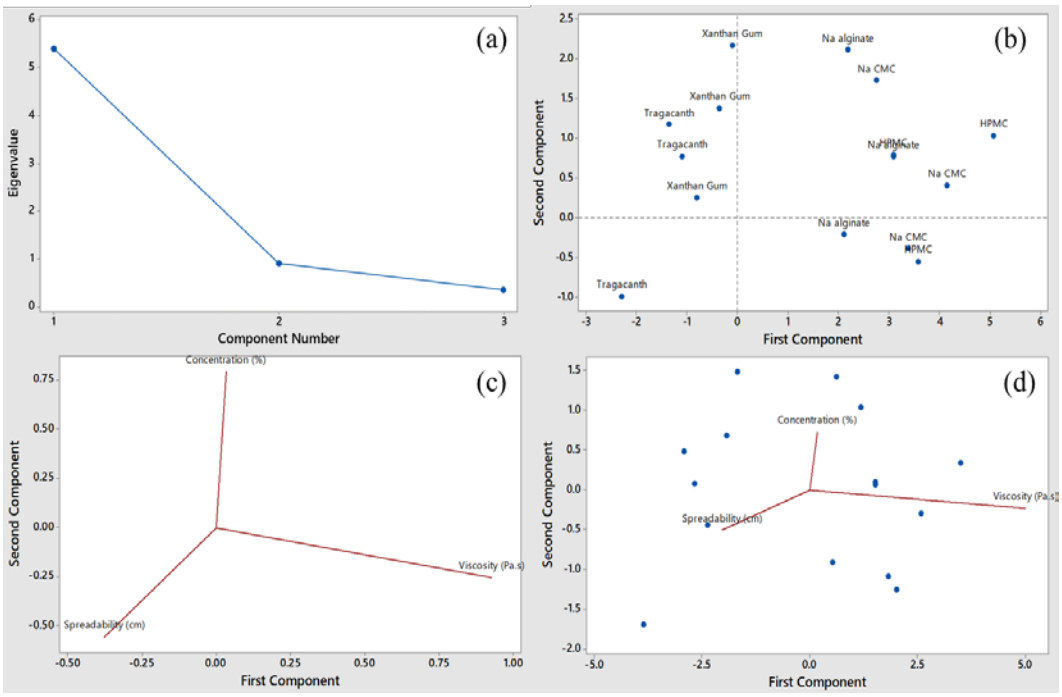

Fig. 1: Visualization of PCA model of gelling agents including scree plot (a), score plot (b), loading plot (c), and biplot (d)

Table 2: Experimental variables with three levels for response surface methodology

Variables	Levels		
	Low	Medium	High
X_1: Kolliphor® RH 40	20	24	28
X_2: Transcutol®	10	12	14
X_3: Na CMC	4.0	4.5	5

Statistical analysis

The establishment of the BBD model, as well as the RSM, were executed using the Minitab® 17 statistical software (Pennsylvania, USA). Multiple response optimization along with the desirability analysis was carried out to gain the optimum condition of independent variables. Sixteen runs were conducted with Kolliphor® RH 40, Transcutol® and Na CMC as the independent variables, whereas viscosity (Pa. S), spread-ability (cm), and transmittance (%), were investigated as the dependence variables.

RESULTS

Principal component analysis

Principal component analysis was applied in order to evaluate gelling agent profiles due to their concentration, spread-ability, and viscosity properties. Four useful plots were generated to visualize the results of PCA evaluation, namely scree plot, score plot, loading plot, and biplot (fig. 1). It was found that two first components resulted eigenvalues of 5.4104 and 0.9138 with 94.6% variances explained. The score plot and loading plot illustrated the two dimensions projection of the principle component and the strength of each variable to influence PCA model, respectively. Combination of the score plot and loading plot was depicted as the biplot.

Design of experiment

The Box-Behnken design was applied in this study to generate the matrix of runs. Kolliphor® RH 40, Transcutol®, and Na CMC were stated as factors, whereas the viscosity, spreadability, and percentage of transmittance were stated as responses. Results of the experimental design observation was presented in table 3.

Table 3: The box-behnken design and experimental responses for Kolliphor® RH 40, Transcutol ®, and Na CMC optimization

Runs	Factors			Responses		
	Kolliphor® RH 40 (g)	Transcutol® (g)	Na CMC (g)	Viscosity (Pa. s)	Spreadability (cm)	Transmittance (%)
1	20	10	4.5	1.104	8.100	97.095
2	28	10	4.5	0.672	4.963	98.376
3	20	14	4.5	0.617	7.463	98.328
4	28	14	4.5	0.185	8.438	99.438
5	20	12	4	1.087	8.300	98.572
6	28	12	4	0.317	5.475	98.650
7	20	12	5	0.7756	6.938	98.828
8	28	12	5	0.406	8.763	98.755
9	24	10	4	0.516	8.225	99.878
10	24	14	4	0.547	5.175	99.255
11	24	10	5	0.365	7.388	99.707
12	24	14	5	0.411	8.400	99.779
13	24	12	4.5	0.51	8.013	99.927
14	24	12	4.5	0.523	7.450	99.609
15	24	12	4.5	0.519	7.113	99.841
16	24	12	4.5	0.501	8.438	99.670

Response of viscosity was evaluated using RSM. Contour plot and response surface were generated and depicted in fig. 2. This model resulted the R^2 value of 84.05% with the regression equation in uncoded units:

$$\text{Viscosity} = 11.5-0.765\ X_1+0.093\ X_2-0.44\ X_3+0.00993\ X_1^2-0.0069\ X_2^2$$

$$-0.103\ X_3^2-0.00000\ X_1*X_2+0.0501\ X_1*X_3+0.0037\ X_2*X_3 \ldots\ldots\ldots (1)$$

Response of spreadability was evaluated using RSM. Contour plot and response surface were generated and depicted in fig. 3. This model resulted the R^2 value of 81.11% with the regression equation in uncoded units:

$$\text{Spreadability} = 127.6-3.60\ X_1-5.85\ X_2-19.2\ X_3-0.0138\ X_1^2-0.073\ X_2^2$$

$$-0.66\ X_3^2+0.1285\ X_1*X_2+0.581\ X_1*X_3+1.016\ X_2*X_3 \ldots\ldots\ldots (2)$$

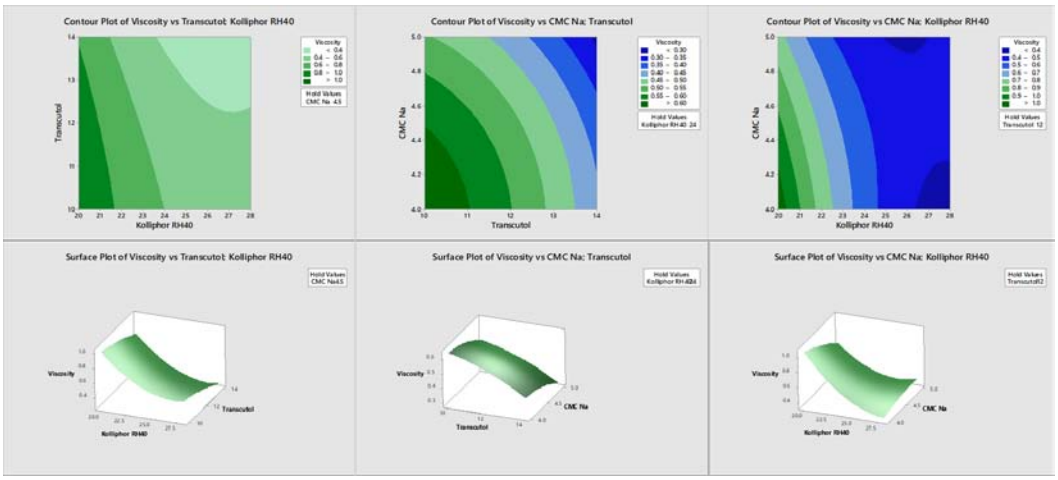

Fig. 2: Contour plot and response surface plot for viscosity

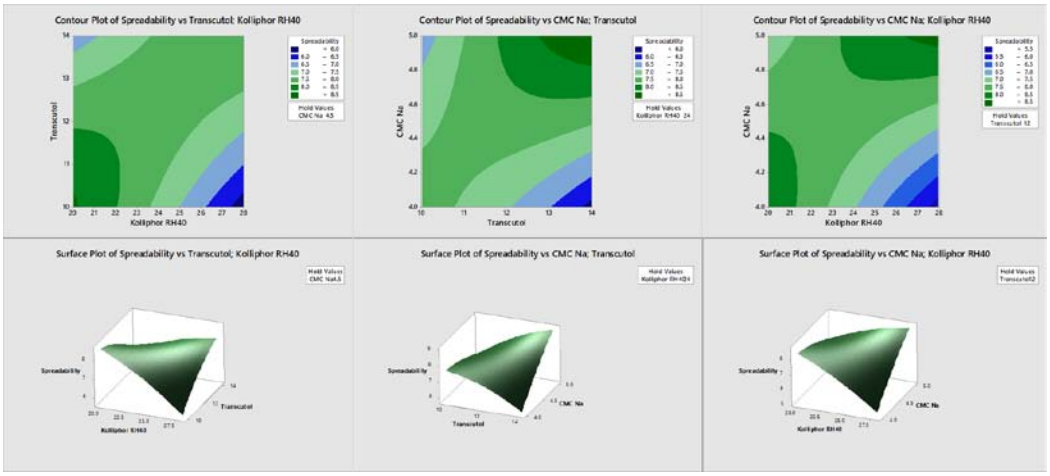

Fig. 3: Contour plot and response surface plot for spreadability

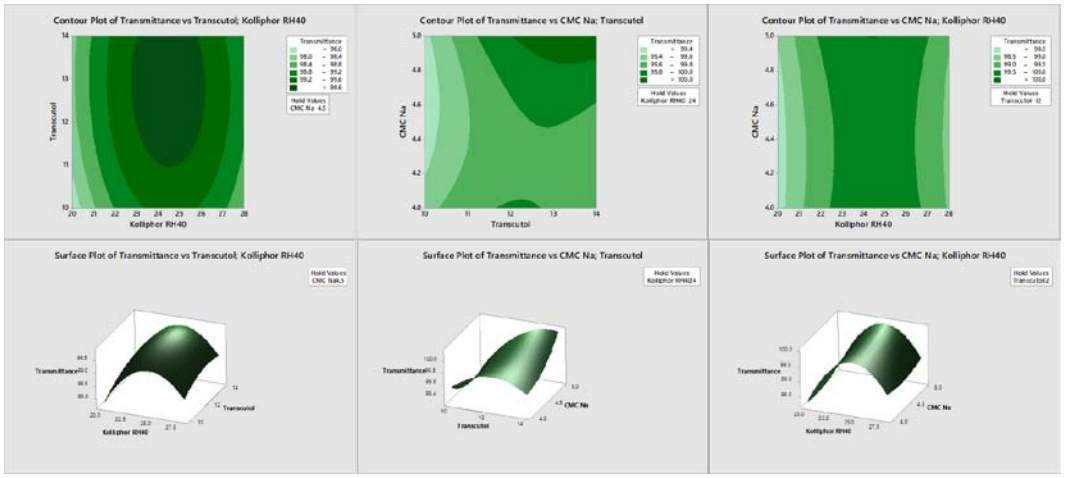

Fig. 4: Contour plot and response surface plot for the percentage of transmittance

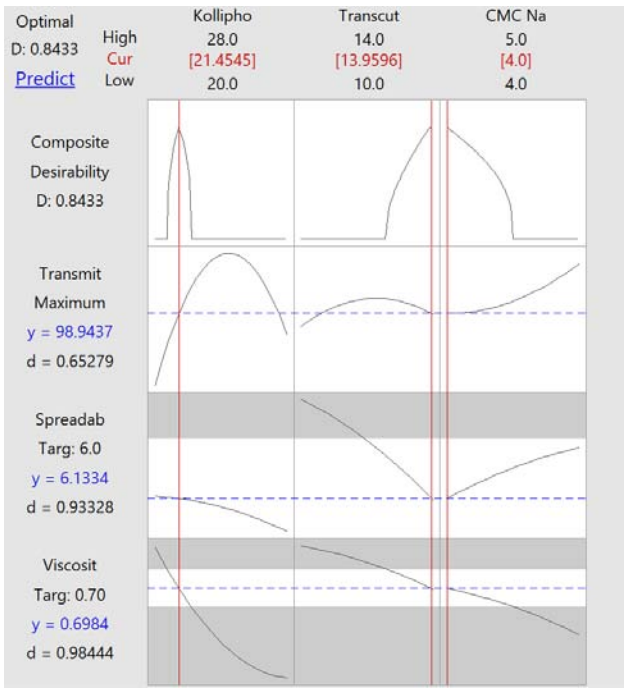

Fig. 5: Optimization plot of Kolliphor® RH 40, Transcutol®, and Na CMC

Response of transmittance percentage was evaluated using RSM. Contour plot and response surface were generated and depicted in fig. 4. This model resulted the R^2 value of 81.11% with the regression equation in uncoded units:

$$\text{Transmittance} = 60.9+3.83\ X_1+0.95\ X_2-6.6\ X_3-0.0752\ X_1^2-0.0624\ X_2^2$$

$$+0.57\ X_3^2-0.0053\ X_1*X_2-0.019\ X_1*X_3+0.174\ X_2*X_3 \quad \ldots\ldots\ldots (3)$$

The target of the optimization was a viscosity of 0.7 Pa. s, spreadability of 6.0 cm, and maximum value of transmittance percentage. The multiple response optimization was further carried out for this study. It was found that the RSM recommendation conditions for Kolliphor® RH 40, Transcutol®, and Na CMC were 21.45 g, 13.96 g, and 4.00 g, respectively. This condition was expected to obtain a composite desirability value of 0.843. Optimization plot was depicted in fig. 5.

Validation of the optimized composition had been successfully carried out with six replications and it shows the promising results of 0.435±0.102 Pa. s of the viscosity, 6.193±1.391 cm of the spread-ability and 99.4±0.409 % of the transmittance. Visual performance of optimized formulation were presented in fig. 6.

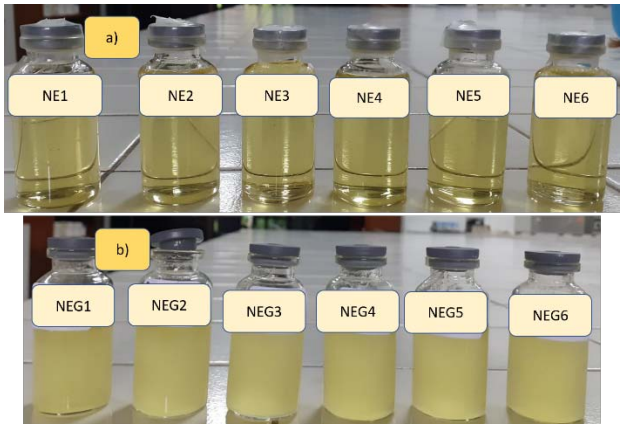

Fig. 6: Visual performance of optimized formulation of a) QUE nanoemulsion (NE) and b) QUE nanoemulgel (NEG)

DISCUSSION

Due to the high potential of quercetin (QUE) in the treatment of diabetic wound, the novel topical formulation of QUE has been emerging [5, 6]. Nanoemulgel offers an excellent opportunity to develop as the novel carrier thanks to the preferable consistency, ease of application and enhanced penetration into the skin [11, 12]. In this current study, gelling agent profiling and formula optimization of a novel QUE nanoemulgel has been well performed. Chemometrics techniques of principal component analysis and response surface methodology were successfully applied to config. the gelling agent profiles and to optimize the formula of QUE nanoemulgel [24]. In terms of quality target of product profile, the QUE nanoemulsions show excellent transparency indicating well solubilization of QUE [25], whereas the QUE nanoemulgels are white-yellowish in color, homogenous, relatively semisolid in consistency and are easy to apply.

Gelling agent is the key factor in determining the viscosity/consistency and the spread-ability of nanoemulgels. Therefore, it is of importance to provide information based on the profiles of various gelling agents in different concentrations which can be used to decide the type of gelling agent with the most accepted physical performance for QUE nanoemulgels. Principal Component Analysis (PCA) has been widely used to aid the screening of components involved in the formulation and analytical area which can be tedious and time-consuming [26–28]. By classification the components into clusters, the PCA assists the screening process more efficiently. This technique implemented dimensionality reduction followed by data projection onto a two-dimensional plot [29]. The PCA technique was also successfully applied in this study by considering the concentration, spread-ability, and viscosity properties of gelling agents. Score plot or individual plot visualized the projection of each gelling agent due to their principle-component scores. Notably, this projection model was built using 94.6% variances explained. Loading plot or variable plot showed the strong effects of each variable (concentration, spread-ability, and viscosity) on the generation of PCA model [30]. The targeted values of viscosity and spread-ability of were at approximately 4-5 Pa. s for the viscosity and 3-4 cm for the spreading diameter (spread-ability). Among various types and concentrations of gelling agents, Na CMC in the concentration 4-6% has shown the most prominent gelation performance in terms of visual appearance, viscosity and spread-ability. A number of articles also emphasized that Na CMC has generated good-quality of nanoemulgels [31–34].

Response surface methodology (RSM) of the Box-Behnken Design (BBD) has been successfully applied in this study. Three factors with three levels of experimental design of Kolliphor® RH 40, Transcutol®, and Na CMC were modelled using Minitab® 17 statistical software to obtain 16 experimental runs with various compositions (table 3) which were further fabricated as QUE nanoemulgels. Those QUE nanoemulgels were then characterized in terms of viscosity, spreadability, and percentage of transmittance. Those results were analyzed to execute the response surface methodology. From the response surface plots, it can be observed that every dual-factor combination resulted various response surface profiles. Response surface plots of viscosity presents profiles of the valley for Kolliphor® RH40 vs Transcutol®, Transcutol® vs Na CMC, and Kolliphor® RH40 vs Na CMC. Response surface plots of spread-ability present profiles of the rising ridge for Kolliphor® RH40 vs Transcutol®, Transcutol® vs Na CMC, and Kolliphor® RH40 vs Na CMC. Response surface plots of transmittance percentage presents profiles of maximum, sadle, and rising ridge for Kolliphor® RH40 vs Transcutol®, Transcutol® vs Na CMC, and Kolliphor® RH40 vs Na CMC, respectively. Equations of the models have been successfully generated from the results, yielding prominent contour plots and response surface plots. It was found that the R^2 value of three responses was more than 80% indicated the high accuracy of models [35, 36]. Formula equation for each model can be useful for response prediction in the subsequent analysis. Desirability analysis were also performed in this study as an approach to set the selected condition of factor with high score of desirability in optimization purpose [37-39]. After determining the goal setting of each response, the desirability function for viscosity, spread-ability, and transmittance percentage were analyzed and resulted individual desirability of 0.984, 0.933, and 0.653, respectively. Considering the individual desirability of each response, the total or composite desirability of 0.843 was achieved. Desirability value of 0 indicates a completely unexpected response, whereas 1 indicates the most expected response of experimental design [40-43]. Those conditions were contributed to achieve the promising results of QUE nanoemulsion and QUE nanoemulgel.

CONCLUSION

A study on optimization of nanoemulgel containing quercetin has been successfully carried out. Gelling agent profiling and evaluation was performed using PCA resulting in the selection of Na CMC to be incorporated into nanoemulgels. It was found that PCA can be applied for gelling agents profiling onto the 2D visualization of the components scores. Response surface methodology of BBD on formula optimization for Kolliphor® RH 40, Transcutol®, and Na CMC levels was valuable to achieve the optimised conditions of viscosity, spread-ability and percentage of transmittance in the composition of Kolliphor® RH 40, Transcutol®, and Na CMC of 21.45 g, 13.96 g, and 4.00 g, respectively. A total desirability of 0.843 was obtained and indicated the high desirability score for further formulation or formula development.

FUNDING

Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Huang X, Sun J, Chen G, Niu C, Wang Y, Zhao C. Resveratrol promotes diabetic wound healing via SIRT1-FOXO1-c-Myc signaling pathway-mediated angiogenesis. Front Pharmacol. 2019;10:421. doi: 10.3389/fphar.2019.00421, PMID 31068817.
2. McKittrick LS, McKittrick JB, Risley TS. Transmetatarsal amputation for infection or gangrene in patients with diabetes mellitus. Ann Surg. 1949;130(4):826-42. doi: 10.1097/00000658-194910000-00019.
3. Ammendola M, Sacco R, Butrico L, Sammarco G, de Franciscis S, Serra R. The care of transmetatarsal amputation in diabetic foot gangrene. Int Wound J. 2017;14(1):9-15. doi: 10.1111/iwj.12682, PMID 27696694.
4. Prabhakar PK, Singh K, Kabra D. Gupta J. Natural SIRT1 modifiers as promising therapeutic agents for improving diabetic wound healing. Phytomedicine. 2020;76:153252. Available from: https://www.sciencedirect.com/science/article/pii/S0944711320300830.
5. Kant V, Jangir BL, Sharma M, Kumar V, Joshi VG. Topical application of quercetin improves wound repair and regeneration in diabetic rats. Immunopharmacol Immunotoxicol. 2021 Sep 3;43(5):536-53. doi: 10.1080/08923973.2021.1950758, PMID 34278923.
6. Badhwar R, Mangla B, Neupane YR, Khanna K, Popli H. Quercetin loaded silver nanoparticles in hydrogel matrices for diabetic wound healing. Nanotechnology. 2021;32(50):505102. doi: 10.1088/1361-6528/ac2536, PMID 34500444.
7. Polera N, Badolato M, Perri F, Carullo G, Aiello F. Quercetin and its natural sources in wound healing management. Curr Med Chem. 2019;26(31):5825-48. doi: 10.2174/0929867325666180713150626, PMID 30009700.
8. Jee JP, Pangeni R, Jha SK, Byun Y, Park JW. Preparation and in vivo evaluation of a topical hydrogel system incorporating highly skin-permeable growth factors, quercetin, and oxygen carriers for enhanced diabetic wound-healing therapy. Int J Nanomedicine. 2019;14:5449-75. doi: 10.2147/IJN.S213883, PMID 31409998.
9. Yin G, Wang Z, Wang Z, Wang X. Topical application of quercetin improves wound healing in pressure ulcer lesions. Exp Dermatol. 2018;27(7):779-86. doi: 10.1111/exd.13679, PMID 29733461.
10. Alyoussef A, El-Gogary RI, Ahmed RF, Ahmed Farid OAHA, Bakeer RM, Nasr M. The beneficial activity of curcumin and resveratrol loaded in nanoemulgel for healing of burn-induced wounds. J Drug Deliv Sci Technol. 2021;62:102360. doi: 10.1016/j.jddst.2021.102360.
11. Ojha B, Jain VK, Gupta S, Talegaonkar S, Jain K. Nanoemulgel: a promising novel formulation for treatment of skin ailments. Polym Bull. 2022;79(7):4441-65. doi: 10.1007/s00289-021-03729-3.
12. Choudhury H, Gorain B, Pandey M, Chatterjee LA, Sengupta P, Das A. Recent update on nanoemulgel as topical drug delivery system. J Pharm Sci. 2017;106(7):1736-51. doi: 10.1016/j.xphs.2017.03.042, PMID 28412398.
13. Chellapa P, Mohamed AT, Keleb EI, Elmahgoubi A, Eid AM, Issa YS. Nanoemulsion and nanoemulgel as a topical formulation. IOSR J Pharm. 2015;5(10):43-7.
14. Nastiti CMRR, Ponto T, Mohammed Y, Roberts MS, Benson HAE. Novel nanocarriers for targeted topical skin delivery of the antioxidant resveratrol. Pharmaceutics. 2020;12(2):1-15. doi: 10.3390/pharmaceutics12020108, PMID 32013204.
15. Nastiti CMRR, Riswanto FDO. Analytical method validation and formula optimization of topical nanoemulsion formulation containing resveratrol. Indones J Chem. 2021;21(5). doi: 10.22146/ijc.63730.
16. Berdey II, Voyt OI. Rheological properties of emulgel formulations based on different gelling agent. J Pharm Innov. 2016;5(4B):76.
17. Singh MP, Nagori BP, Shaw NR, Tiwari M, Jhanwar B. Formulation development and evaluation of topical gel formulations using different gelling agents and its comparison with marketed gel formulation. Int J Pharm Erud. 2013;3(3):1-10.
18. Rathod HJ, Mehta DP. A review on pharmaceutical gel. Int J Pharm Sci. 2015;1(1):33-47.
19. Pal R. Modeling the viscosity of concentrated nanoemulsions and nanosuspensions. Fluids. 2016;1(2):11. doi: 10.3390/fluids1020011.
20. Gabel J, Desaphy J, Rognan D. Beware of machine learning-based scoring functions-on the danger of developing black boxes. J Chem Inf Model. 2014;54(10):2807-15. doi: 10.1021/ci500406k, PMID 25207678.
21. Setyawan EI, Rohman A, Setyowati EP, Nugroho AK. The combination of simplex lattice design and chemometrics in the formulation of green tea leaves as transdermal matrix patch. Pharmacia. 2021;68(1):275-82. doi: 10.3897/pharmacia.68.e61734.
22. Florentinus DOR, Abdul R, Suwidjiyo P, Sudibyo M. Application of response surface methodology as mathematical and statistical tools in natural product research; 2019.
23. Garg A, Aggarwal D, Garg S, Singla KA. Spreading of semisolid formulations: an update. Pharm Technol. 2002;26(9):84-105.
24. Dwiastuti R, Suhendra PA, Yuliani SH, Octa FDR. Application of the central composite design approach for optimization of the nanosilver formula using a natural bioreductor from Camellia sinensis L. extract. J Appl Pharm Sci. 2022;12(8):48-56. doi: 10.7324/JAPS.2022.120806.
25. Gokhale JP, Mahajan HS, Surana SJ. Quercetin loaded nanoemulsion-based gel for rheumatoid arthritis: *in vivo* and *in vitro* studies. Biomed Pharmacother. 2019;112:108622. doi: 10.1016/j.biopha.2019.108622, PMID 30797146.
26. Bohidar NR, Restaino FA, Schwartz JB. Selecting key parameters in pharmaceutical formulations by principal component analysis. J Pharm Sci. 1975 Jun 1;64(6):966-9. doi: 10.1002/jps.2600640615, PMID 1133752.
27. Armstrong NA. Pharmaceutical experimental design and interpretation. CRC Press; 2006.
28. Oh CM, Heng PWS, Chan LW. A study on the impact of hydroxypropyl methylcellulose on the viscosity of PEG melt suspensions using surface plots and principal component analysis. AAPS PharmSciTech. 2015;16(2):466-77. doi: 10.1208/s12249-014-0204-x, PMID 25370022.
29. Fujiwara T, Kwon OH, Ma KL. Supporting analysis of dimensionality reduction results with contrastive learning. IEEE Trans Vis Comput Graph. 2020;26(1):45-55. doi: 10.1109/TVCG.2019.2934251, PMID 31425080.
30. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. Phil Trans R Soc A. 2016;374(2065):1-16. doi: 10.1098/rsta.2015.0202.
31. Bashir M, Ahmad J, Asif M, Khan SU, Irfan M, Y Ibrahim A. Nanoemulgel, an innovative carrier for diflunisal topical delivery with profound anti-inflammatory effect: *in vitro* and *in vivo* evaluation. Int J Nanomedicine. 2021;16:1457-72. doi: 10.2147/IJN.S294653, PMID 33654396.

32. Sapiun Z, Wicita PS, Buloto AS, Kamba V, Imran AK, Buana WAA. Preparation and characterization of butterfly pea (Clitoria ternatea L.) nanoemulgel. Open Access Maced J Med Sci. 2022 Apr 14;10(A):585-9. doi: 10.3889/oamjms.2022.8964.eu/index.php/mjms/article/view/8964.
33. Morsy MA, Abdel-Latif RG, Nair AB, Venugopala KN, Ahmed AF, Elsewedy HS. Preparation and evaluation of atorvastatin-loaded nanoemulgel on wound-healing efficacy. Pharmaceutics. 2019;11(11):609. doi: 10.3390/pharmaceutics11110609, PMID 31766305.
34. Saryanti D, Setiawan I, Dayanto HH. Use of CMC Na as gelling agent in nanoemulgel formulation of methanol extract of sappan wood (Caesalpinia sappan L). J Trop Pharm Chem. 2022;6(1):21-9. doi: 10.25026/jtpc.v6i1.276.
35. Riswanto FDO, Desra A, Sari RM, Thomas V, Rohman A, Pramono S. Employing an R software package rsm for optimizing of genistein, daidzein, and glycitein separation and its application for soy milk analysis by HPLC method. Indones J Chem. 2020;20(5):1184-98. doi: 10.22146/ijc.51669.
36. Prabaningdyah NK, Riyanto S, Rohman A, Siregar C. Application of HPLC and response surface methodology for simultaneous determination of curcumin and desmethoxy curcumin in Curcuma syrup formulation. J Appl Pharm Sci. 2017;7(12):58-64.
37. Dwiastuti R, Putri DCA, Hariono M, Riswanto FDO. Multiple response optimization of a HPLC method for analyzing resorcinol and 4-*n*-butyl resorcinol in lipid nanoparticles. Indones J Chem. 2021;21(2):502-11. doi: 10.22146/ijc.58537.
38. Patel MN, Kothari CS. Multivariate approaches for simultaneous determination of avanafil and dapoxetine by UV chemometrics and HPLC-QbD in binary mixtures and pharmaceutical product. J AOAC Int. 2016;99(3):649-63. doi: 10.5740/jaoacint.15-0259.
39. Muralikrishna P, Babu AK, Mamatha P. Formulation and optimization of ceritinib loaded nanobubbles by box-behnken design. Int J App Pharm. 2022;14(4):219-26. doi: 10.22159/ijap.2022v14i4.44388.
40. Candioti LV, De Zan MM, Camara MS, Goicoechea HC. Experimental design and multiple response optimization. Using the desirability function in analytical methods development. Talanta. 2014;124:123-38. doi: 10.1016/j.talanta.2014.01.034, PMID 24767454.
41. Iftikhar SY, Iqbal FM, Hassan W, Nasir B, Sarwar AR. Desirability combined response surface methodology approach for optimization of prednisolone acetate loaded chitosan nanoparticles and *in vitro* assessment. Mater Res Express. 2020 Nov;7(11):115004. doi: 10.1088/2053-1591/abc772.
42. Modi DM, Modi AD, Parikh RH, Parikh JR. Preparation, characterization, and optimization of mebendazole spherical agglomerates using modified evaporative precipitation in aqueous solution (EPAS). Int J Pharm Pharm Sci 2022;14(9):30-8. doi: 10.22159/ijpps.2022v14i9.44728.
43. Shaikh AA, Chaudhari PD, Holkar SS. A design of experiment approach for optimization and characterization of etodolac ternary system using spray drying. Int J Pharm Pharm Sci 2017;9(2):233-40. doi: 10.22159/ijpps.2017v9i2.16087.