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DEVELOPMENT AND CHARACTERIZATION OF QUERCETIN NANOGELS BY USING SIMPLEX-LATTICE MIXTURE DESIGN

Shirley Wong Jing Wen, Jaya Raja Kumar

1Research student, Asian Institute of Medicine, Science and Technology (AIMST) University, Bedong 08100, Kedah, Malaysia
2Unit of Pharmaceutical Technology, Faculty of Pharmacy, Asian Institute of Medicine, Science and Technology (AIMST) University, Bedong 08100, Kedah, Malaysia

ABSTRACT

Quercetin is a flavonoid with antioxidant/anti-inflammatory properties, quercetin nanogels are developed as one of the most promising carriers for topical drug delivery. In this study, quercetin loaded poloxamer based nanogels were utilized as a potential drug carrier for topical delivery. Fourteen runs of nanogels were composed of water, oleic acid and poloxamer 407. A simplex-lattice mixture design was used to optimize the process parameters including water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C). Seven dependent variables globule size, pH, refractive index, viscosity, gel strength, spreadability and bioadhesive force was measured as responses. The particle size of nanogels was found to be 223.6-247.7nm. Gel strength was found to be in the range of 40.31-299.52 seconds, and viscosity of formulations found to be in the range of 3939-25500 cps.

Keywords: Quercetin Nanogels, HPLC, Poloxamer 407, Simplex-lattice mixture design

INTRODUCTION

Quercetin (3,30,40,5,7-pentahydroxyflavone) is a flavonoid present in a large number of edible vegetables and fruits [1,2]. This molecule has many health-promoting effects, including improvement of cardiovascular health, reducing risk for cancer, and coping with inflammatory disorders, mainly related to its strong antioxidant action, by which it upregulates the endogenous free radical defenses [3,4]. However, quercetin is extremely lipophilic in nature, so there is need to enhance the solubility of Quercetin [5]. The main purpose of this present work was to expand the application of mixed solvency technique to influence the solubility of quercetin. In this present investigation, we have objective that design and develop new suitable formulation with increasing solubility and bioavailability. Quercetin containing topical preparation has ability to inhabit oxidative stress and inflammation which is induced due to UVB exposure. Anticancer activity of quercetin is not preferable clinically due to low absorption when administered orally. To achieve targeted therapeutic level of quercetin in systemic circulation, high dose is required which is practically not beneficial [6]. So that we prepared topical preparation of drug to avoid first pass metabolism and thereby improving the permeation rate of formulation. Various nanotechnological approaches have been used to enhance its solubility, dissolution rate, and hence, bioavailability, including solid dispersions, nanosuspensions, microemulsions, solid lipid nanoparticles and prodrugs [7–11]. Therefore, a critical need exists to develop alternative formulative strategies to overcome the shortcomings of quercetin nanogels and enhance its penetrability.

Address for correspondence:
Shirley Wong Jing Wen,
Research student,
AIMST University,
Bedong- Semeling, Kedah,
Malavsia 08100

MATERIALS AND METHODS

Materials:

Quercetin and poloxamer 407 were obtained from Sigma-Aldrich. Oleic acid and tween 80 was procured from R&M marketing, Essex, UK and the other chemicals were used in the experiment are analytical grade.

Methods:

Formulation of quercetin nanogels:

The preparation of nanogels as shown in (figure 1)

Determination of pH:

The pH of the nanogels was determined by using a calibrated PH meter (HANNA INS PH211). Measurements were considered after reaching equilibrium. The reading of all runs was noted.

Viscosity studies:

The viscosities of the various formulations were determined by using Brookfield programmable DVII +Model pro II type (USA). The viscosity was noted in Centipoise [12].

Determination of mucoadhesive force:

The mucoadhesive force has been derived from a previously published method [13,14]. A section of sheep nasal mucosa was cut from the slaughter house and instantly fixed with mucosal side out onto each glass vial using rubber band. The vial with nasal mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. The formulations were added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given to the vials. Then, the switch of the infusion apparatus was opened to make the water drop into the glass vial with a constant flow rate of 5 mL/min. The weight of the water in the glass vial kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dyne/cm^2), was determined from the minimal weights that detached the gel. The sheep nasal mucosa pieces were changed for each measurement.

Determination of gel strength:

Gel strength was measured by placing 50 g of formulation in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A piston of weight 27 g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink 5 cm was measured [15].

Spreadability:

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1000g weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability [16].

S= ML/T

Where, M = weight tide to upper slide (g)

L = length moved on the glass slide (cm)

T = time taken (sec)

In-vitro drug release:

Quercetin loaded nanogels (0.05 g) were dispersed in flasks containing 100 mL phosphate buffer solution (PBS) (pH 7.4). The dissolution medium was kept under stirring at 100 rpm min−1and at 37◦C. At predetermined time intervals, 2 mL release medium was taken out from flask and replaced with 2 mL fresh PBS to keep the volume constant [17]. The concentration of drug in the medium was determined by using HPLC method.

The solution was determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC-20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of acetonitrile, 20mm AA: 20mm ACN (pH 5.5) (20:80, v/v), and detection was made at 354nm. The mobile phase was prepared daily, filtered through a 0.45µm membrane filter (Millipore) and sonicated before use. A Thermo C18 column (25cm × 4.6mm i.d., 5µ) was used for the separation.

Optimization data analysis and validation of optimization model

For the studied design, the simplex lattice design type was applied to fit full mixture component in terms of L_Pseudo equation with added interaction terms to correlate the studied responses with the examined variables using Design Expert software version 10 (Stat-Ease, Minneapolis, MN). A simplex-lattice mixture design of degree m consists of m+1 points of

equally spaced values between 0 and 1 for each component. If m = 2 then possible fractions are 0, 1/2, 1. For m = 3 the possible values are 0, 1/3, 2/3, 1. The points include the pure components and enough points between them to estimate an equation of degree m. This design differs from a simplex-centroid design by having enough points to estimate a full cubic model.

These equations represent the quantitative effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) and their interaction on globule size (R1), pH (R2), Refractive Index (R3), Viscosity (R4), Gel Strength (R5), Spreadability (R6) and Bioadhesive Force (R7). The values of the coefficient A, B and C are related to the effect of these variables on the responses R1, R2, R3, R4, R5, R6 and R7. Coefficients with more than one factor term and those with higher order terms represent interaction terms and quadratic relationship respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. A backward elimination procedure was adopted to fit the data to the quadratic model. Both the polynomial equations were found to be statistically significant ($P<0.01$), as determined using ANOVA, as per the provision of Design Expert software (DX10).

During the quecertin nanogels development, a three-level 14 full factorial experimental design was used to identify and estimate the main and interaction effects of three different formulation factors water phase (A), oil phase (B), and surfactant: Co-surfactant ratio (1:3) (C) on critical quality attributes of the developed nanogels. Based on the experimental design, the factor combinations yielded different responses as presented in Table 1. These results clearly indicate that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 14 batches. Mathematical relationship generated in terms of L. pseudo components analysis for the studied variables are expressed.

Mathematical relationship generated using multiple linear regression analysis for the studied variables are expressed as shown in Table 3.

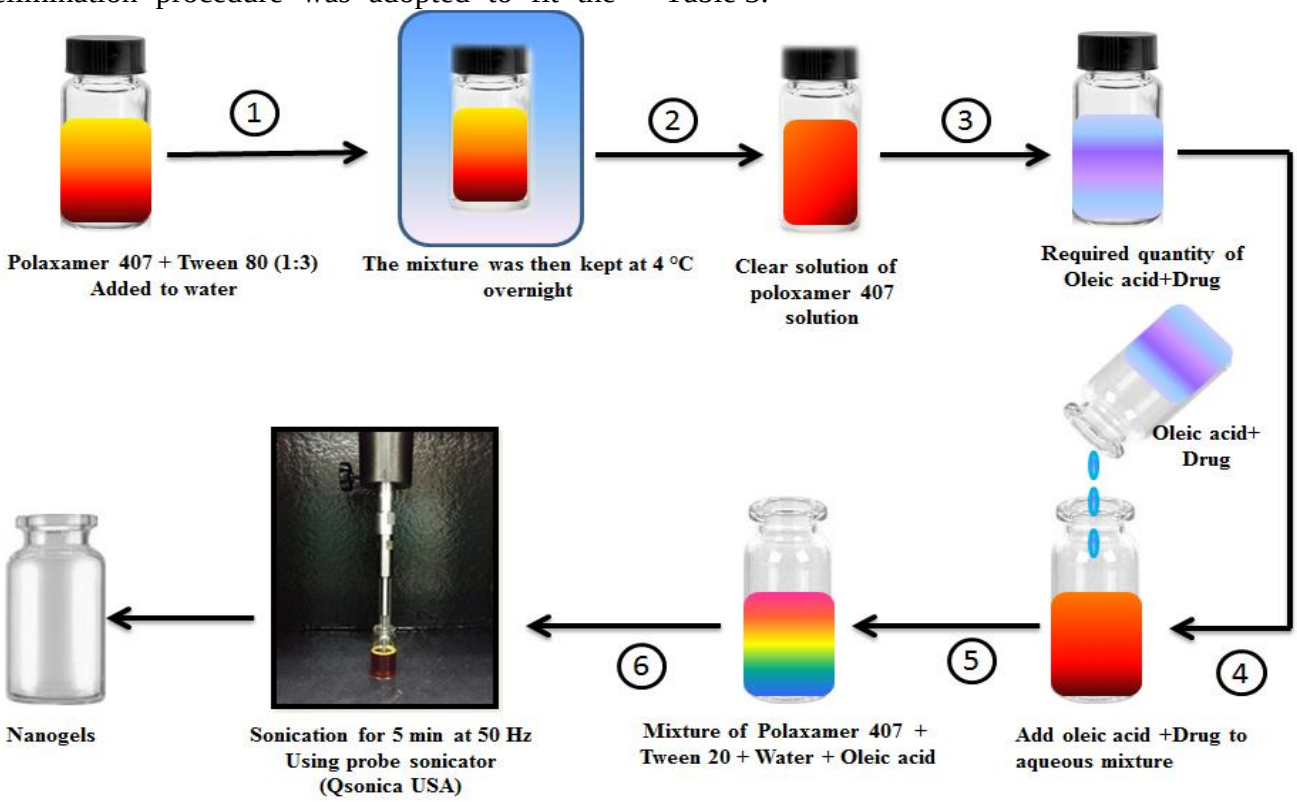

Figure 1: Preparation of quercetin nanogels

Table-1: Factorial design of quercetin nanogels

Run	A:Water w/w	B:OA w/w	C:P 188 :Tween 80 (1:3)	Size nm	pH	Refractive Index	Viscosity cps	Gel Strength seconds	Spreadability gm.cm/sec.	Bioadhesive Force Dynes/cm^2
1	6	1	2	240.1	3.56	1.378	18755	200.11	10.32	20989.3
2	4.6	1.6	2.6	225.4	3.9	1.394	6800	60.21	3.73	21324.3
3	5.3	1.3	2.3	229.3	3.95	1.386	4500	51.44	126.37	30250.3
4	5	2	2	231.6	3.58	1.401	17216	135.27	173.87	23904.5
5	5	1	3	229.6	4.18	1.395	10900	80.46	9.73	21894.9
6	4	1	4	224.8	4.07	1.414	25500	299.52	148.81	22626.8
7	4	3	2	247.7	3.89	1.419	3939	40.31	58.84	17019.7
8	4	3	2	246.7	3.87	1.419	3945	42.22	56.72	17009.1
9	5	2	2	232.4	3.59	1.401	17220	136.56	170.22	23900.3
10	6	1	2	242.3	3.59	1.378	18740	199.12	10.36	20985.1
11	4	2	3	227.4	3.56	1.408	23000	253.28	5.56	15890.1
12	4.3	2.3	2.3	223.6	3.46	1.402	7659	65.33	59.85	18359.4
13	4	1	4	224.2	4.06	1.414	25510	298.31	142.33	22620.3
14	4.3	1.3	3.3	226.5	4.13	1.407	3239	39.15	50.41	18359.4

Table 2: Multiple linear regression analysis

Size = +241.09A +247.09B +224.39C -49.23AB -14.29AC-35.09BC +9.61A^{2}BC +9.61A^{2}BC -742.79AB^{2}C +481.75 ABC^2
pH = +3.57 A +3.88 B +4.06 C -0.57 AB +1.44 AC -1.65 BC +16.89 A^{2}BC-24.69 AB^{2}C+19.60 ABC^2
Refractive Index = +1.38A +1.42 B +1.41C +0.010 AB -3.850 AC -0.034 BC -0.34 A^{2}BC -0.56 AB^{2}C +0.38 ABC^2
Viscosity = +18450.14 A +3644.64 B +25207.64 C +22303.55 AB -48473.35 AC +29537.65 BC-3.542 A^{2}BC +84369.73 AB^{2}C -9.204 ABC^2
Gel Strength = +196.91 A +38.56 B +296.21 C +51.07 AB -707.70 AC +300.28 BC -754.85 A^{2}BC +224.35 AB^{2}C
Spreadability = +12.33 A +59.77 B +147.56 C +559.90 AB -249.02 AC -360.58 BC +4561.09 A^{2}BC -4180.79 AB^{2}C -2660.66 ABC^2
Bioadhesive = +21051.78 A +17078.98 B +22688.13 C +19864.71AB +1133.04 AC -14940.56 BC +7.031A^{2}BC -2.220 AB^{2}C

Experimental design results revealed that the mean glouble size of quecertin nanogels was significantly affected by water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (1:3) (C). Globule size analysis of quecertin nanogels was found to be in the range of 223.6 – 247.7 nm as shown in Table 1 and Figure 3a. The Model F-value of 44.95 implies the model is significant. There is only a 0.03% chance that an F-value this large could occur due to noise. Values of “Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC, AB^{2}C, ABC^2 are significant model terms. The "Lack of Fit F-value" of 10.84 implies the lack of fit is significant. There is only a 3.02% chance that a "Lack of Fit F-value" this large could occur due to noise. All the three variables having the positive effect on

the globule size, which means these factors, are directly proportional to the response. The influence of the main and interactive effects of independent variables on the globule size was further elucidated using the trace graph (piepel), 2D contour, 2D real contour and 3D response surface plots are shown in Figure 3, 4, 5 and 6.

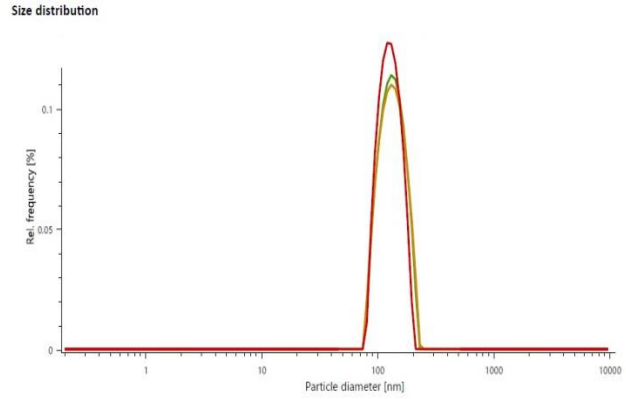

Figure-3a: Size distribution of nanogel

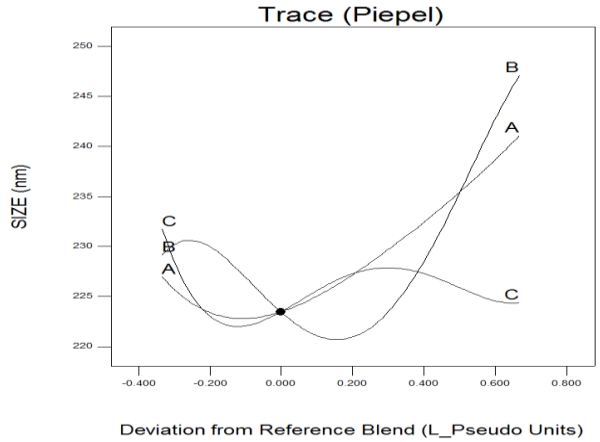

Figure-3: Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on globule size

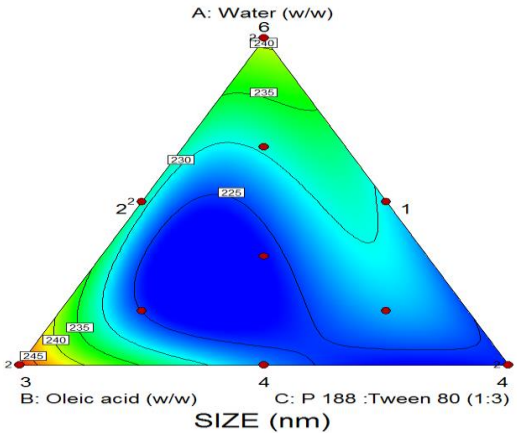

Figure-4: Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size

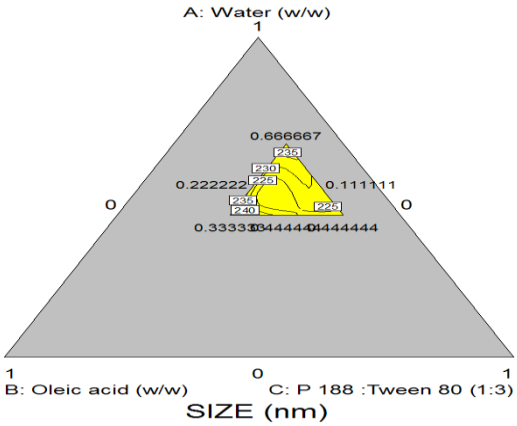

Figure-5: Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size

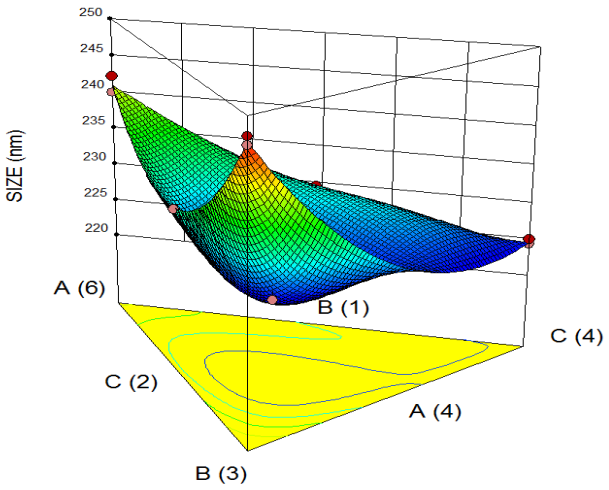

Figure-6: 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size

Rheological behavior of quecertin nanogels was found to be in the range of 3239-25510 cps as shown in Table 1.The factorial equation for viscosity exhibited a good correlation coefficient (1.000) and the Model F value of 7.44 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, C, AC are significant model. All the three variables having the positive effect on the viscosity,

which means these factors, are directly proportional to the response. The influence of the main and interactive effects of independent variables on the viscosity was further elucidated using the perturbation and 3D response surface plots. It is found that all the variables are having interactive effects for the response (viscosity). The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response (viscosity) are shown in figure 7, 8, 9 & 10 to depict the interactive effects of independent variables on viscosity.

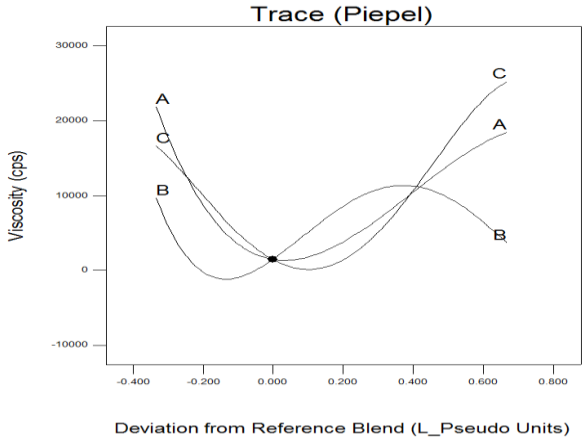

Figure-7: Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on viscosity

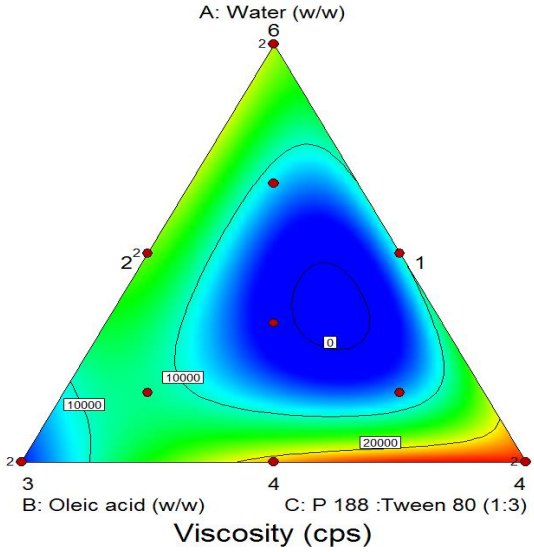

Figure-8: Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity

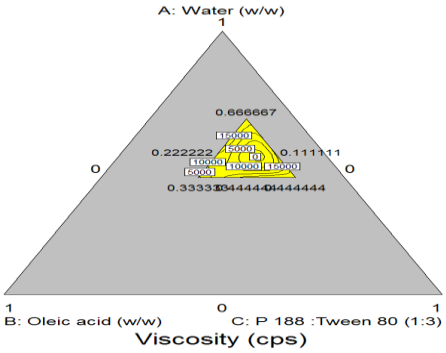

Figure-9: Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity

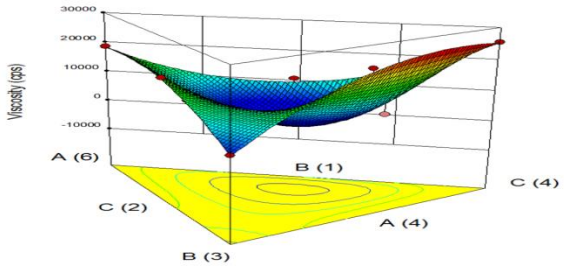

Figure-10: 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity

Gel strength of quecertin nanogels was found to be in the range of 39.15- 299.52 seconds as shown in Table 1. The factorial equation for gel strength exhibited a good correlation coefficient (1.000) and the Model F value of 12.47 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, C, AC, ABC^2 are significant model. All the three variables having the positive effect on the gel strength, which means these factors, are directly proportional to the response. The influence of the main and interactive effects of independent variables on the gel strength was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on gel strength are as shown in Figure 1. It is found that all the variables are having interactive effects for the response (gel strength) The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response (viscosity) are shown in figure 11, 12, 13 & 14 to depict the

interactive effects of independent variables on gel strength.

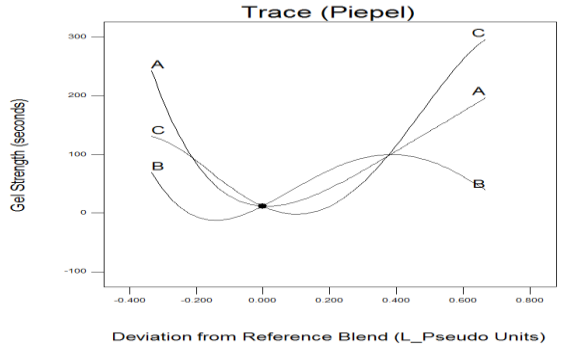

Figure-11: Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on gel strength

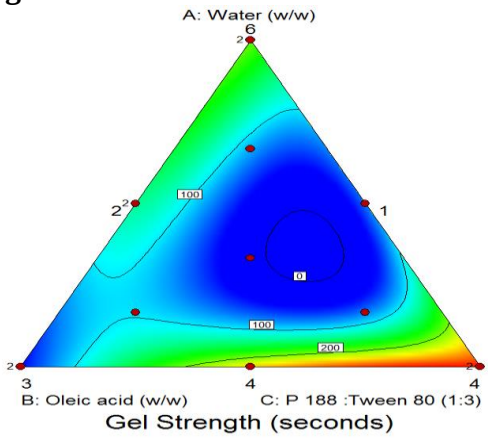

Figure-12: Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the gel strength

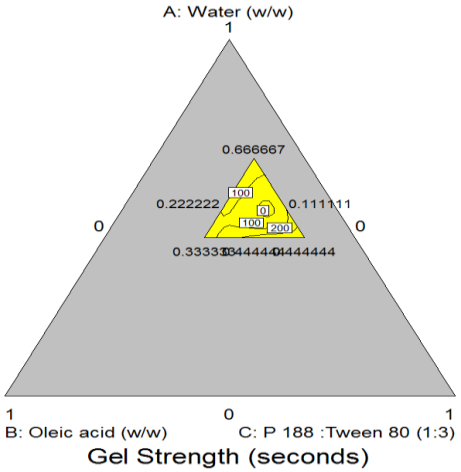

Figure-13: Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the gel strength

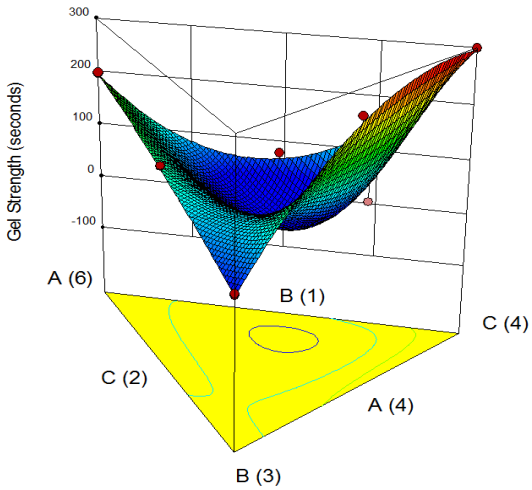

Figure-14: 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the gel strength

The spreadability was found to be significant with F-value of 10.17 implies the model is significant. There is only a 1.03 % chance that an F value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B, C, AB, BC are significant model terms. The influence of the main and interactive effects of independent variables on the spreadability was further elucidated using the trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response shown in figure 15, 16, 17 and 18.

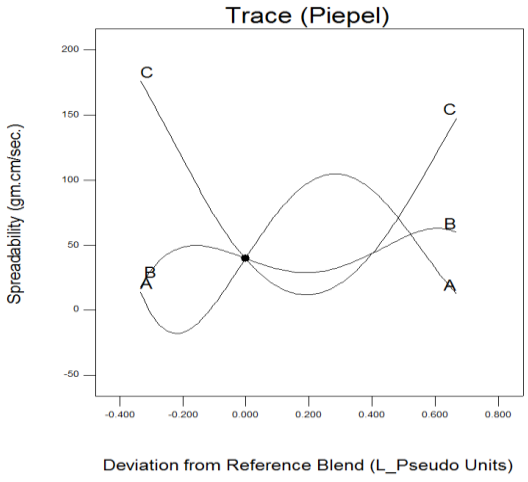

Figure-15: Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on spreadability

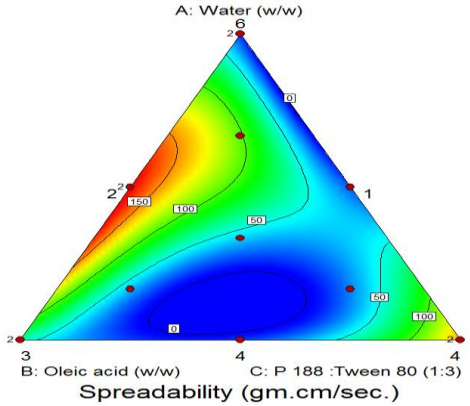

Figure-16: Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

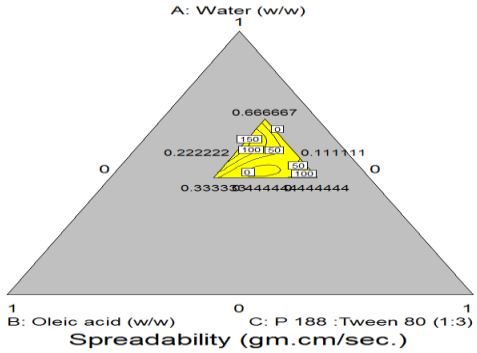

Figure-17: Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

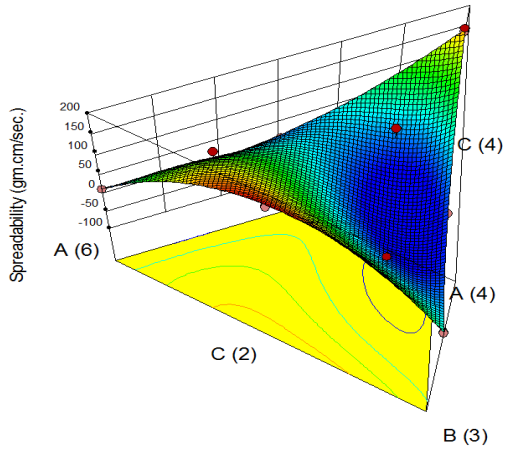

Figure-18: 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

The mathematical model generated for biodhesive force was found to be significant with F-value of 33.07 implies the model is significant. There is only a 0.06% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC, A^2BC, AB^2C, ABC^2 are significant model terms. The relationship between the dependent and independent variables was further elucidated using trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response shown in figure 19, 20, 21 and 22.

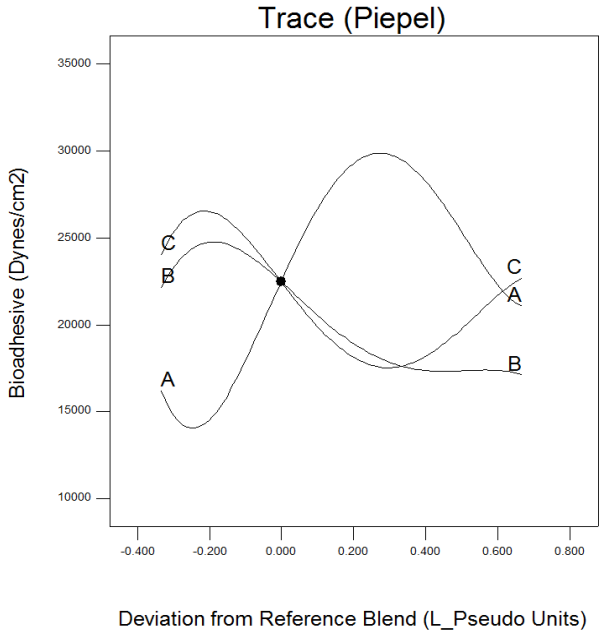

Figure-19: Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on biodhesive force

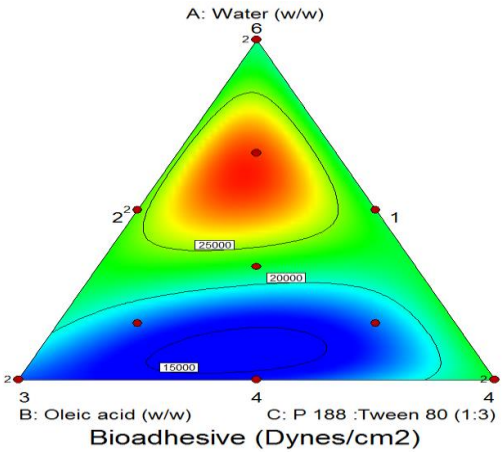

Figure-20: Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

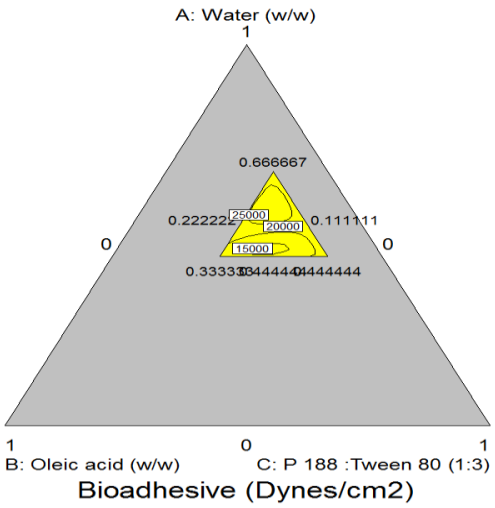

Figure-21: Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

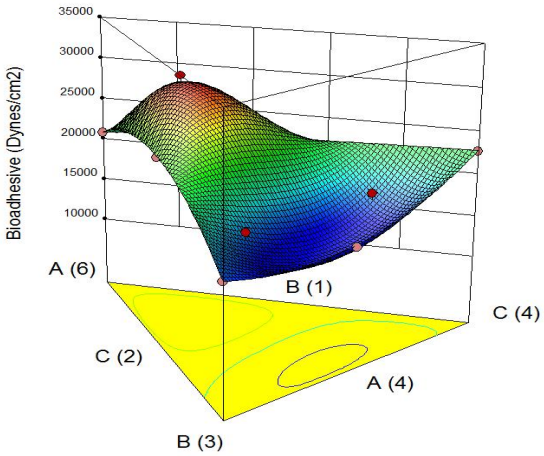

Figure-22: 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability

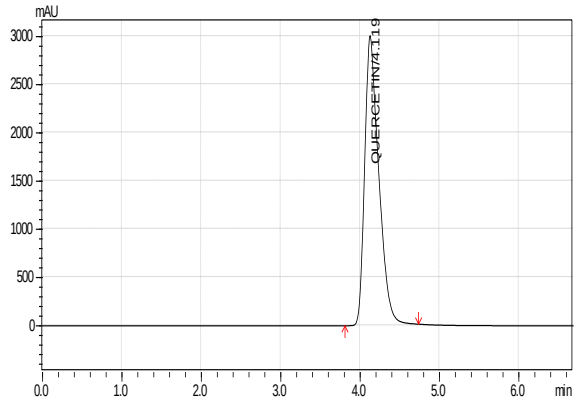

Figure-23: Typical chromatogram of quecertin

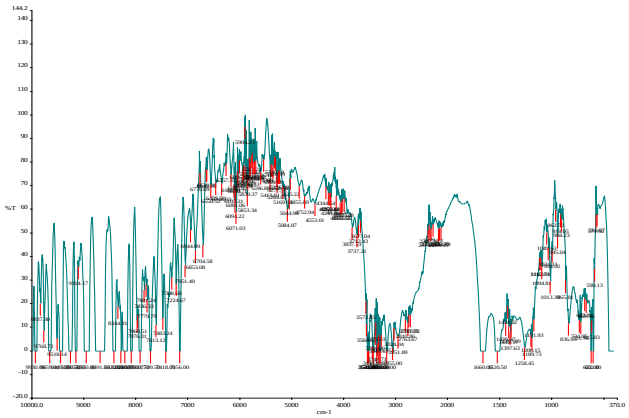

Figure-24: FTIR spectra of quercetin

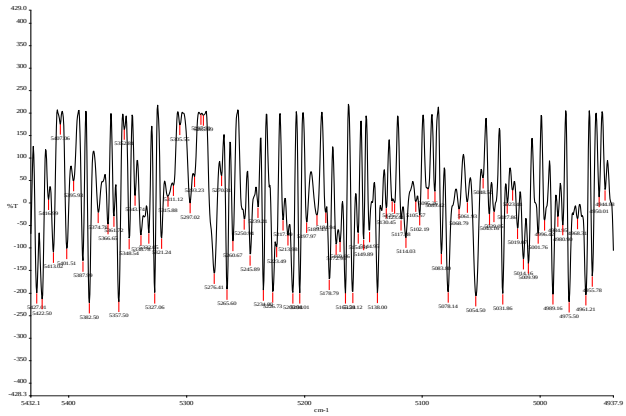

Figure-25: FTIR spectra of poloxamer 407

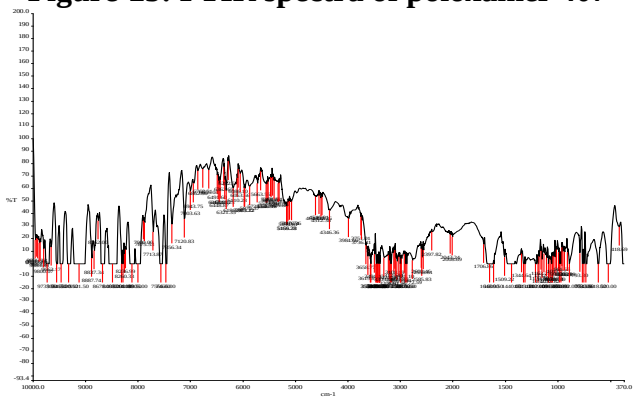

Figure-26: FTIR spectra of quercetin and poloxamer 407

FT-IR analysis was performed to ensure that no chemical interaction between the drug and the polymer had occurred. FT-IR spectra of quercetin, poloxamer 407, quercetin and poloxamer 407 are shown in Fig.24, 25 &26.

CONCLUSION:

In the future, topical drug delivery will be used extensively to impart better patient compliance. Since nanogels is helpful in enhancing spreadability, adhesion and viscosity, this novel drug delivery become popular. Moreover, they

will become a solution for loading hydrophobic drugs in water soluble gel bases for the long term stability. Similarly in the study, topical nanogels of quercetin were formulated and subjected to physicochemical studies i.e. rheological studies, spreading coefficient studies, gel strength and bioadhesion strength. The statistical analysis (analysis of variance, ANOVA) showed that generated models for globule size, pH, refractive index, viscosity, gel strength, spreadability and bioadhesive force were significant ($p < 0.05$), indicating that three listed responses are well described by the proposed models.

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