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DEVELOPMENT AND EVALUATION OF POLOXAMER BASED NANOGEL OF RUTIN BY USING SIMPLEX-LATTICE MIXTURE DESIGN

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ABSTRACT

The objective of this study was to identify and optimize formulation of rutin loaded nanogel. Nanogels have emerged as a promising drug delivery system for the delivery of hydrophobic drugs. Poloxamer 188 and tween 20 have been employed as surfactant/co-surfactant. Formulation chart is made with fourteen formulations, evaluated for goluble size, refractive index, pH, viscosity, gel strength, spreadability, biodhesive force. Poloxamer 188 was one of the best binding nanogel, reported in research paper. The Anton Paar-Litesizer 500 evaluations showed the presence of spherical globules in size range of 209-228nm. Release pattern shows at end of 540 min approximately 98% and 99% of drug release in phosphate buffer from formulations F2 and F13, respectively.

Keywords: Rutin, Nanaogels, Poloxamer 188, Refractive Index, HPLC

INTRODUCTION

Rutin (2-(3,4-dihydroxyphenyl)-4, 5-dihydroxy-3-[3,4,5-trihydroxy-6- [(3,4,5-trihydroxy- 6methyl-oxan-2-yl)oxymethyl] oxan-2-yl]oxy-chromen-7-one) also accepted as quercetin-3-rutinoside or sophorin is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose. It is a polyphenolic compound commonly distributed in higher plants. High concentrations for example are found in buckwheat seed, fruits and fruit rinds, especially in citrus fruits (e.g. orange, grapefruit, lemon, lime). Rutin has significant scavenging properties on oxidizing species such as OH radical, superoxide radical, and peroxyl radical [1]. Furthermore it has several pharmacological activities including antiallergic [2], anti-inflammatory and vasoactive [3], antitumor [4], antibacterial, antiviral and anti-protozoal properties [5]. As an outcome of these biological effects, it has been widely used in treating these diseases. Moreover, it has also been reported that rutin has other therapeutic effects such as hypolipidaemic [6], cytoprotective [7], antispasmodic [8] and anticarcinogenic [9]. Rutin offers an advantage over myricetin, quercetagenin and other flavonoids, which on some occasions behave as prooxidant agents and catalyze oxygen radical production [10]. In recent years, there has been significant progress in utilizing nanotechnology for drug delivery. Among different types of nanoscale drug delivery systems, polymer nanogel hold great promise in tumor treatment since they have a cross-linked three-dimensional network structure that offers great water-retaining property and colloidal stability.[11] Nanogel is defined as the nanosized particles formed by physically and chemically cross linked polymer networks that swell in a good solvent. The term "Nanogel" was first introduced to define cross linked bifunctional networks of the polyion and a nonionic polymer for delivery of polynucleotides. [12] The drug can be released from the nanogel as a result of diffusion. This release mechanism is simple and has been successfully employed. There is an increased interest in developing nanogel that can release biological agents in response to environmental cues at the targeted site of action.

Therefore, the present research work was aimed to develop and optimize nanogel using Design-Expert software (DX10).

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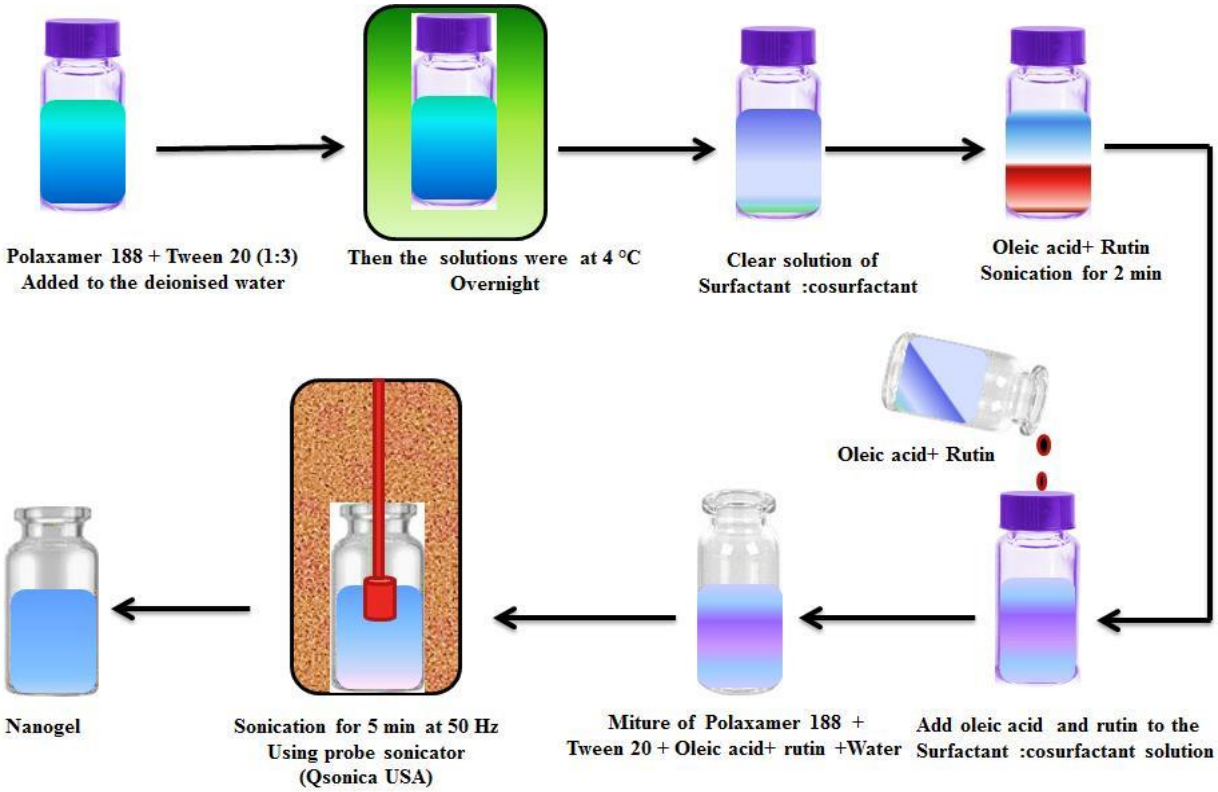
MATERIALS AND METHODS

Materials:

Rutin was purchased from Sigma Aldrich (USA). Tween 20 was obtained from R&M Chemical. Poloxamer 188 was purchased from Merck KGaA (Darmstadt, Germany). All water used in the formulation was of Milli-pore grade.

PREPARATION OF RUTIN NANOGEL

Various concentrations of poloxamer 188 and tween 20 were then added to the water and sonicated for 5 min. This protocol is summarized in Scheme 1.



Scheme 1: Formulation of rutin nanogel

IN-VITRO EVALUATION

Particle size:

The average globule size and PDI of the nanogel droplets were determined by Anton Paar-Litesizer 500, Malaysia.

Refractive indexes:

Refractive indexes were determined for different nanogel formulations by using Lan Optics (LABO LAN, S.L.) at 25 °C. Each sample was repeated three times and standard deviation was calculated.

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 [13].

Measurement of Spreadability:

For the determination of spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000 g weight for 5 min. Weight (50 g) was added to the pan. The time required separating the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (14). S=M×L/T, where M = weight tide to upper slide, L = length moved on the glass slide, T = time taken.

Measurement of gel strength:

A sample of 50gm of nanogel was placed in a 100 ml graduated cylinder. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in the gel. The gel strength, which means the viscosity of the nanogels was determined by the time (seconds), the apparatus took to sink 5cm down through the prepared gel [15].

Determination of mucoadhesive force:

The mucoadhesive force of nanogels was determined as follows, a section of the chicken skin fixed with mucosal side out onto each glass vial using rubber band. The vial with chicken skin was connected to the balance in inverted position while first vial was

placed on a height adjustable pan. Nanogel were added onto the skin 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. Then weight was kept rising in the pan until vials get detached. Mucoadhesive force was the minimum weight required to detach two vials. The chicken skin was changed for each measurement [14].

Particle size analysis of Nanogels:

Particle size of nanogels was determined using Anton Paar-Litesizer 500.

In-vitro drug release:

Release experiments were carried out according to the paddle method [16] using phosphate buffer at pH 5.5 containing 15% methyl alcohol to maintain sink conditions. The medicated nanogel (250 mg) were spread evenly on the surface of a watch glass of 5 cm^2 surface area and covered with a stainless steel mesh screen [17], the assembly was placed at the bottom of the USP dissolution tester containing 250 ml release medium (ELECTROLAB TDT–08L). The temperature was adjusted at 32°C and the speed at 50 rpm. An aliquot of 2 ml of sample was withdrawn from receiver compartment through side tube at time intervals of 0.15, 30, 60, 120,180, 240, 300, 360, 420, 480 and 540 min. Fresh medium was replaced each time to maintain constant volume. Samples were analyzed by RP 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 phase that is constituted acetonitrile, 10mm A.A : ACN (pH 4.5) (50:50, v/v), and detection was made at 370nm. 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.

EXPERIMENTAL DESIGN

The criteria for optimizing the nanogels formulation with adequate globule size, refractive index, pH, viscosity, gel strength, spreadability and bioadhesive force. The experimentally measured input parameters were analyzed by using Design-Expert software (DX10) software. The analysis provided mathematical relationships between the independent variables and the dependent responses in the form of polynomial equations. These polynomial equations represent the quantitative effect of the process variables (C1, C2 and C3) and their interaction on the designated responses and can be graphically visualized through trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response plots.

In this study, we narrate the successful effect on the formulation of rutin nanogel. Through preliminary experiments the Water (C1), Oil (C2) and S:CoS (C3) were identified as the most significant variables influence the globule size (R1), refractive index (R2), pH (R3), viscosity (R4), gel strength (R5), spreadability (R6) and bioadhesive force (R7). The final optimal composition of the rutin nanogels formulation obtained from the analysis by using the 14 factorial design model based on the simplex lattice model. Among various design approaches, the simplex lattice has good design properties, rotatable or nearly rotatable; some have quadratic blocks, insensitive to outliers and missing data. Based on the experimental design, the build information, mixture components, design constraints and factorial design of rutin nanogel are presented in Table 1, 2, 3 and 4. 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 runs. This optimized formulation was further characterized by FT-IR spectroscopy, SEM, HPLC and finally evaluated with *in vitro* drug release studies.

Table-1: Build Information

File Version	9.0.6.2		
Study Type	Mixture	Subtype	Randomized
Design Type	Simplex Lattice	Runs	14
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	3437.00		

Table-2: Mixture Components

Component	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	Water	W/W	Mixture	5	6	+0 ↔ 5	+1 ↔ 6	5.35	0.3608
B	Oil	W/W	Mixture	1	2	+0 ↔ 1	+1 ↔ 2	1.35	0.3608
C	S:CoS	W/W	Mixture	2	3	+0 ↔ 2	+1 ↔ 3	2.31	0.3690
				Total =	9.00	L_Pseudo Coding			

Table-3: Design Constraints

Low Limit		Constraint		High Limit
5.000	≤	A:Water	≤	6.000
1.000	≤	B:Oil	≤	2.000
2.000	≤	C:S:CoS	≤	3.000
		A+B+C	=	9.000

Table-4: Factorial design of rutin Nanogel

	C 1	C 2	C 3	R 1	R 2	R 3	R 4	R 5	R6	R 7
Run	Water W/W	Oil W/W	C:S:CoS W/W	Globule Size (nm)	Refract-ive Index	pH	Viscosit-y (cps)	Gel Strength (sec)	Spreadabilit-y (gm.cm/s)	Bioadhesive Force (dynes/cm)
1	5.5	1	2.5	222	1.39	4.2	17000	87	22.43	20741.3
2	5.5	1.5	2	228	1.381	3.83	18816	93	54.47	25517.2
3	5	1	3	210	1.393	4.12	19000	264	6.31	15890.9
4	5	1	3	209	1.394	4.13	19015	259	20.45	19381.5
5	5.6	1.1	2.1	226	1.384	3.66	18140	97	30.97	20766.1
6	5	2	2	211	1.384	3.87	10418	407	50.79	22378.7
7	5.1	1.6	2.1	226	1.386	4.18	18850	102	48.78	19513.2
8	5	2	2	213	1.383	3.87	10450	401	49.23	22369.1
9	5	1.5	2.5	215	1.389	4.01	17960	92	5.94	16932.9
10	6	1	2	216	1.377	3.81	2700	181	60.27	15903.3
11	5.3	1.3	2.3	230	1.383	3.59	16690	77	13.56	15890.9
12	5.1	1.1	2.6	225	1.392	4.01	18760	101	4.33	19252.7
13	5.5	1.5	2	227	1.382	3.81	18800	95	52.14	25510.1
14	6	1	2	217	1.378	3.79	2900	178	58.77	15909.7

Optimization of process variables for the rutin nanogel

The effects of the three factors (water, oil and S:CoS) on the globule size, refractive index, viscosity, gel strength, spreadability and bioadhesive force were tested and reported in Table 4.

Establishment and evaluation of the fitted model a standard simplex lattice design was applied to design the experiment for identifying the relationship between the response function and the process variables.

Globule size of rutin nanogels were found to be in the range of 209 – 228 nm as shown in Figure 2a,b&c. The globule size (R1) was found to be significant with F-value of 66.74 implies that the model is significant. There is only 0.01% 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, AC, BC, ABC^2 are significant model terms. All the variables are having interactive effects for the response (globule size). The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response are shown in Figure 3a, 3b, 3c and 3d to depict the interactive effects of independent variables on globule size.

The Model F-value of refractive index **(**28.35) implies the model is significant. There is only 0.01% 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, AC are significant model terms. The "Lack of

Fit F-value" of 9.19 implies that the Lack of Fit is significant. There is only 2.70% chance that a "Lack of Fit F-value" this large could occur due to noise. The "Pred R-Squared" of 0.8782 is in reasonable agreement with the "Adj R-Squared" of 0.9132; *i.e*. the difference is less than 0.2. All the variables are having interactive effects for the response (refractive index). The interactive effects of independent variables on refractive index are shown in Figure 4a, 4b, 4c and 4d.

Regression equation for the response of refractive index= +1.38 a+1.38b+1.39c+1.88ab+0.015ac-1.19bc

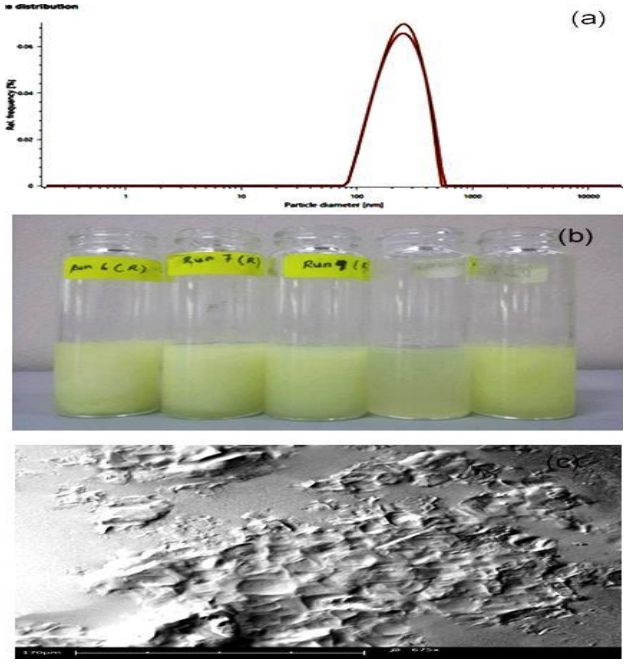


Figure-2: (a) Particle size distribution, (b) Appearance of rutin nanogels. (c) SEM image of rutin nanogel

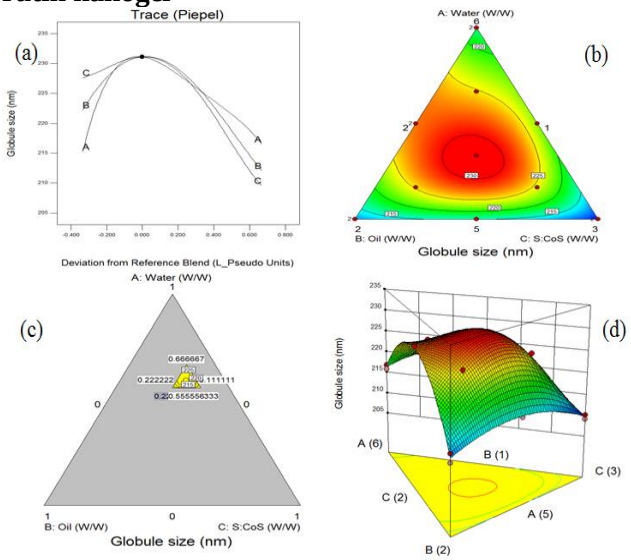


Figure-3: (a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on globule size. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the globule size.

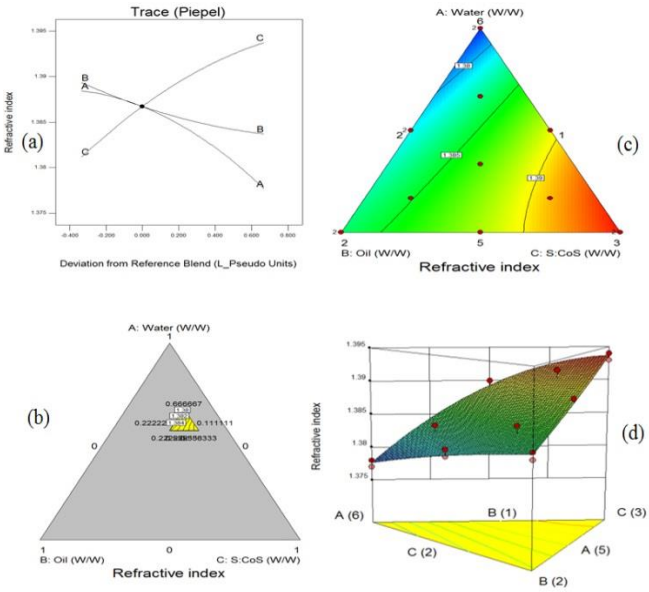


Figure-4(a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on refractive index. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the refractive index. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the refractive index. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the refractive index

The mathematical model generated for pH was found to be significant with F-value of 1.17 implies that the model is not significant relative to the noise. There is a 39.95 % chance that F-value this large could occur due to noise.

Regression equation for the response of pH = +3.78 A+3.93 B+4.13 C-0.33 AB+0.18 AC-0.24 BC

The viscosity was found to be significant with F-value of 28.09 implies that the model is significant. There is only 0.10% 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, AC are significant model terms. The influence of the main and interactive effects of independent variables on the viscosity was further elucidated using the trace graph (piepel) and response surface plots. The trace graph (piepel) plot showing the main effects of A, B and C on the viscosity of nanogels. This figure clearly shows that A, B and C has the main and the major effect on viscosity. The relationship between the dependent and independent variables was further elucidated using trace graph (piepel), 2D contour, 2D real contour and 3D response surface plots shown in figure 5a to 5c.

Regression equation for the response of viscosity = *+2911.00A +10545.00B +19118.50C +49208.01AB+25717.04AC+14289.04 BC+1.86 A^2BC -46713.01AB2C-3.08ABC^2*

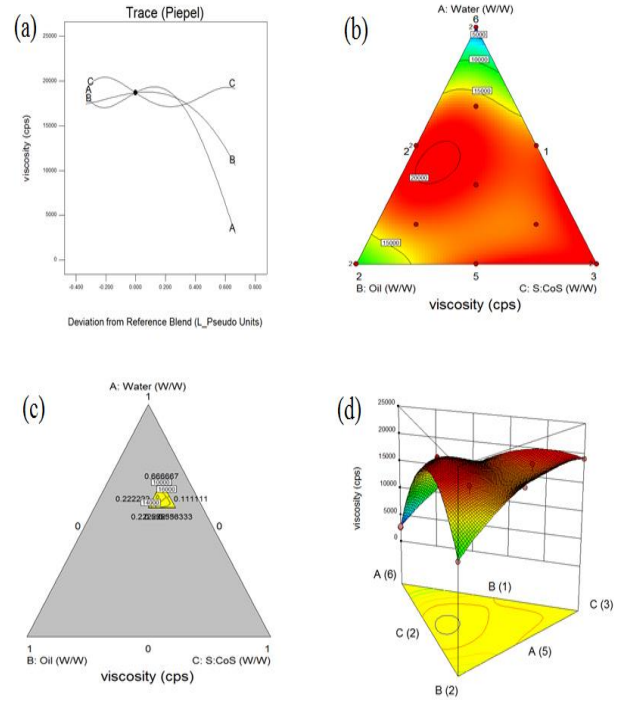


Figure-5: (a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on viscosity. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the viscosity.

Trace plots are similar to perturbation plots for non-mixture designs. They are used parallel with the effects of all the components in the design space. The factors tool is expended to set the reference blend through which the traces are plotted. The goal is to determine how sensitive the response is to deviation from the formulation near the reference blend. The reference blend is best determined by the results of numerical optimization, but defaults to the centroid values. The trace plots can be created using either Piepel's or Cox's direction as shown in Figure 6a. The Model F-value of 247.16 implies that the model is significant. There is only 0.01% chance that an F-value this large could occur due to noise. The relationship between the dependent and independent variables was further elucidated using response surface plots (figure 6b to 6d).

Regression equation for the response of gel strength= +178.79A+403.29B +260.79C -793.83AB -542.50AC -971.50BC +4445.42 A^2BC -3276.58AB^2C +2048.41ABC^2

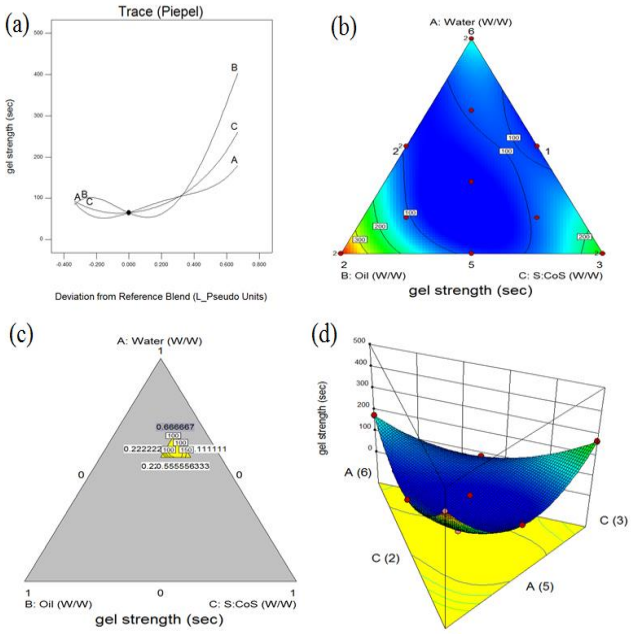


Figure-6: (a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on gel strength. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the gel strength. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant

ratio affecting the gel strength. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the gel strength.

Spreadability of semisolid formulations, that is, the ability of a cream or gel to evenly spread on the skin, plays an important role in the administration of a standard dose of a medicated formulation to the skin and the efficacy of a topical therapy. Table 4 shows the spreading values of 14 runs. In this case A, B, C, AC, BC are significant model terms. The "Lack of Fit F-value" of 3.95 implies that the Lack of Fit is not significant relative to the pure error. There is a 10.58% chance that a "Lack of Fit F-value" this large could occur due to noise. It is found that all the variables are having interactive effects for the response (spreadability). The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response are shown in figure 7a to 7d to depict the interactive effects of independent variables on spreadability.

Regression equation for the response of spreadability =+58.45A +52.72 B+12.95C-8.13AB-76.25AC-100.50 BC

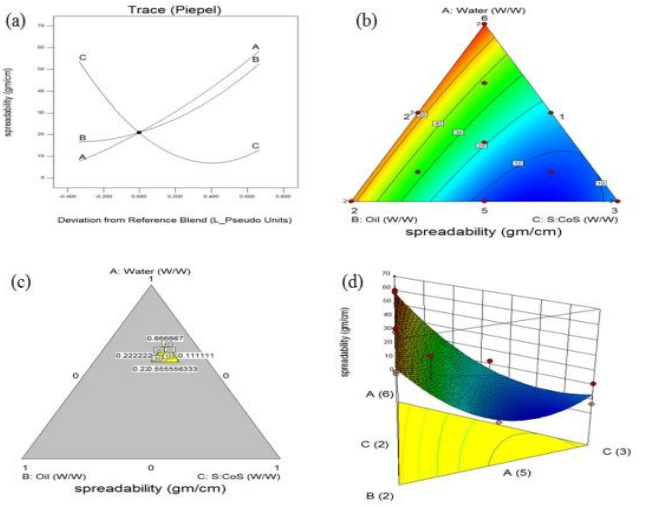


Figure-7: (a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on spreadability. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the spreadability.

Experimental design results revealed that the mean bioadhesive force of rutin nanogel was significantly affected by water phase (A), oil phase (B) and surfactant: S: Co-surfactant ratio. Bioadhesive force analysis of rutin nanogels was found to be in the range of 4.33- 60.27 gm.cm/s as shown in table 4. The Model F-value of 5.36 implies that the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, BC are significant model terms. The influence of the main and interactive effects of independent variables on the bioadhesive force was further elucidated using the trace graph (piepel), 2D contour, 2D real contour and 3D response surface plots are shown in Figure 8a to 8d.

Regression equation for the response of bioadhesive force = +16183.16 18073.28C+20765.55AB+9714.80AC-20287.13 BC

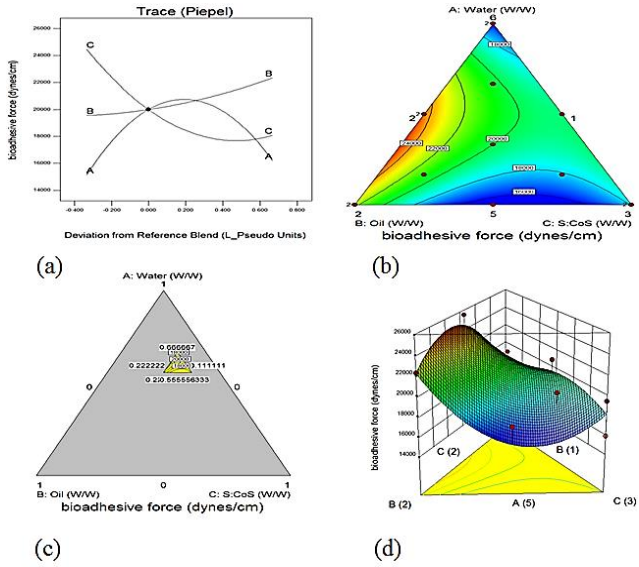


Figure-8: (a) Trace graph (piepel) showing the main effect of water phase (A), oil phase (B) and surfactant: Co-surfactant ratio (C) on bioadhesive force. (b) Response 2D contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the s bioadhesive force. (c) Response 2D real contour plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the bioadhesive force. (d) 3D surface plot presenting the interaction between the water phase, oil phase and surfactant: Co-surfactant ratio affecting the bioadhesive force.

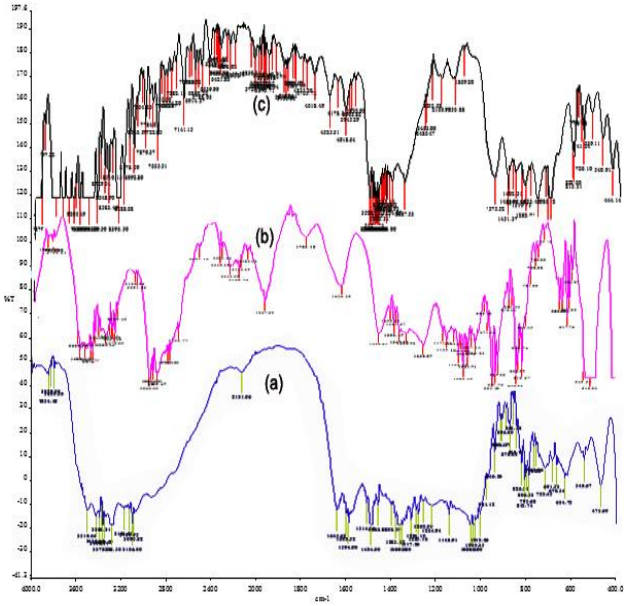


Figure-8: (a) FTIR spectra of rutin and poloxamer 407, (b) FTIR spectra of poloxamer 188, FTIR spectra of rutin and poloxamer 188

The FTIR spectra results showed that there was no chemical interaction or changes in the physical mixture and rutin was compatible with polymers shown in figure 8.

The percentages of rutin released from nanogel (F2 and F13) are presented. An initially rapid release during the first 2 h (burst effect) can be seen for all the release profiles showed in Figure 9.The release of rutin from nanogel was considerably slow after 200 min of release study period. At end of 500 min approximately 98% and 99% of rutin was found to be released in phosphate buffer from formulations F2 and F13, respectively.

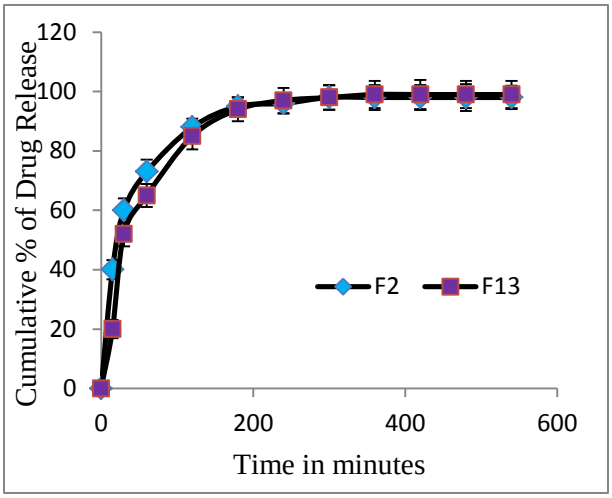


Figure-9: Cumulative % of drug release

Table-5: Optimized values obtained by the Confirmation

Response	Predicted	Observed	
		F2	F13
Globule size	231.12	230.11	227
Refractive Index	1.38	1.38	1.382
pH	3.72	3.73	33.81
Viscosity	18688.1	18687	18800
Gel Strength	64.25	94.13	95
Spreadability	20.8326	53.22	52.14
Bioadhesive Force	19999.7	25517.2	25510.1

F2 and F13 batches code of rutin nanaogels were prepared according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process as shown in Table 5, thereby demonstrating the feasibility.

CONCLUSION

Poloxamer 188 based rutin nanaogels were successfully developed and optimized with the use of stat-ease design-expert software (DX10). It is evidence that the statistical methods based on experimental designs of tests, regression analysis and optimization techniques can be used to carry out this task more effectively and efficiently. Rutin nanaogels showed adequate globule size, refractive index, pH, viscosity, gel strength, spreadability and bioadhesive force. Moreover observed responses were also found in close agreement with the predicted values of optimized process, which demonstrate the feasibility.

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