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# Development and Charecterization of Caffeine and Quercetin Loaded Nasal Niosomal In-Situ Gel for Treatment of Depression

Shilpa p. Chaudhari[1], Sneha U. Gaikwad[2]

[1](Professor, Head of Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy)
[2](PG Student, Department of Pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune, India)
[2]Corresponding Author: snehagaikwad2797@gmail.com

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***Abstract:*** *Caffeine and Quercetin both are used in combination for the treatment of patient with depression as they are adenosine antagonist due to which they elevate the level of neurotransmitters. The study was designed with two aims. First, is to enhance the solubility and bioavailability of BCS Class II i.e. Quercetin; secondly to ease administration of the formulation to the patient with depression. The culmination of this study, the caffeine and quercetin loaded niosomal in-situ gel for nose-to-brain delivery was formulated. Niosomes were prepared and optimized by using definitive screening design whereas, the Niosomal in-situ gel were prepared and optimized using central composite design. The vesicle size of the optimized batch was found to be 0.281±0.26µm. The % EE of all niosomal batches was found to be in a range of 81.52±0.21% to 98.72±0.16% for Caffeine and 94.3±0.31 to 99.73±0.23 for Quercetin and the cumulative % release was found to be in a range of 72.09±0.18% to 103.3±0.26% for Caffeine and 10.9±0.31% to 37.06±0.15% for Quercetin at 4hrs. DSC, FTIR studies were performed for pure drug and optimized niosomal batch. All the gels as per design were formulated, where the Spreadability was found to be in range of 5.1±0.26cm to 7.9±0.16cm and viscosity after gelation in range of 1800±0.11cps to 4780±0.26cps. The % drug permeated was found to be in range of 85.86±0.015% to 98.61±0.024% for Caffeine and 22.65±0.19% to 33.23±0.34% for Quercetin at 6hrs. These results indicated that niosomal In-situ gel can be used to enhance the bioavailability of drug by directly delivering the drug to the brain by avoiding first pass effect.*

***Keywords****:Targeted drug delivery, Depression, Caffeine, Quercetin, Niosomes, Insitu-gel*

## I. Introduction

Depression (according to WHO) is a common mental disorder, characterized by sadness, loss of interest or pleasure, feelings of guilt, disturbed sleep or appetite, feelings of tiredness. Depression may be mild or severe. Neurotransmitters are chemical messengers in the brain by which nerve cells communicate with each other. There are three neurotransmitters, chemically known as monoamines they are, serotonin, dopamine and norepinephrine[1]**.** Depression has been linked to problems or imbalances in the brain with respect to the neurotransmitter's serotonin, norepinephrine, and dopamine[2]**.**

Caffeine is a well-known central nervous system stimulant, and the most widely consumed psychoactive substance in the world [3]. Caffeine is a methylxanthine derivative which has various pharmacological activities, such as blockade of adenosine receptors, inhibition of phosphodiesterase, inhibition of 5-nucleotidase, and modulation of intracellular calcium movement, which may affect neuronal functions [4]. Caffeine seems to exert its effects directly on the central nervous system by blocking $A_1$ and $A_{2A}$adenosine receptors[5].Caffeine enhances dopamine (DA) signaling in the brain, which it does predominantly by antagonizing adenosine $A_{2A}$ receptors which is similar with the other wake-promoting drugs[6].

The phenolic flavonoid quercetin has a high In vitro affinity for blocking same A1, and perhaps A2a, adenosine receptor as caffeine, so it has been suggested that it may have In vivo effects similar to those caffeine [7]. Quercetin show poor water solubility, systematic bioavailability and therapeutic efficiencies. And also have high

first pass metabolism and limited absorption [8].As quercetin has low solubility and low oral bioavailability studies were carried out to formulate caffeine and quercetin loaded niosomes to enhance solubility.

Niosomes are a novel drug delivery system, in which the drug is encapsulated in a vesicle. The niosomes are very small and microscopic in size [9]. Niosomes can entrap both hydrophilic and lipophilic drug, either in aqueous layer or in vesicular membrane made up of lipid material. It can prolong circulation of entrapped drug, because presence of nonionic surfactant with lipid. Cholesterol is included in most niosomes in order to increase rigidity of the niosome membranes and subsequently improve stability of the vesicles and to reduce permeability of the encapsulated material. The in vivo behavior of niosomes might be similar to that of liposomes, and can extend the circulation of entrapped drug in the blood causing an alteration in organ distribution and metabolic stability of the encapsulated material [10].

The most desirable and convenient method of drugadministration is the oral route because of their ease of administration. However, in many instances oral administration is not desirable when the drug undergoes degradation via first pass effect in liver. Hence, lack of systemic absorption through the gastrointestinal tract led to research on alternate routes of drug delivery such as parenteral, intramuscular, subcutaneous, intranasal, transdermal, etc. In case ofdepression, direct delivery to the brain is necessary and in such a case, the nasal route can be considered as an alternate route to central nervous system, as it offers some advantages like fast absorption and bypass hepatic first pass metabolism. Intranasal administration is needle free and hence an ideal alternative to the parenteral route for systemic drug delivery.Drug administration through thenasal cavity is easy and convenient. Avoidance of first pass metabolism is the main advantage of nasal route of drug delivery; it also eases administration, patient comfort, and compliance[11]. By using the niosomal in-situ nasal gel formulation, an attempt was made to increase its permeation as well as solubility so as to increase its bioavailability.

The aim of this study was to formulate caffeine and quercetin loaded niosomal in-situ-nasal gel in treatment of depression for lowering the dosage, improving patient compliance and to provide prolonged action.

## II. Materials and Methods

***Materials***

Caffeine was purchased from Vijay Chemicals Pvt. Ltd (Pune) and Quercetin was purchased from Otto Chemie Pvt. Ltd (Mumbai, Maharashtra). All the reagents used in this assay were of analytical grade.

***Methods***

***Preparation Of Niosomes*:**

The hand shaking (thin film hydration) and ether injection method has been chosen for the preparation of niosomes.

***Hand Shaking (Thin Film Hydration) Method:***

The mixture of vesicle forming ingredients like surfactant and cholesterol with different molar ratios was dissolved in a volatile organic solvent (chloroform) in a round bottom flask. Then the organic solvent is removed at room temperature ($60^0$C) using rotary evaporator at different rpm leaving a thin layer of solid mixture deposited on the wall of the flask. The dried surfactant film was rehydrated with10ml of phosphate buffer pH 7.4 at $60^0$Cwith gentle agitation. This process forms multilamellar niosomes. Then the formed suspension was sonicated for 10 min using bath sonicator to convert the multilamellar vesicles (MLV) into small vesicles [12].

***Ether Injection Method:***

Different concentration of cholesterol and surfactant dissolved in 6ml of diethyl ether and ix with 2ml of methanol which previously containing weighed quantity of Caffeine and Quercetin dehydrate. Then, the resultant solution was slowly injected using micro syringe at a rate 1ml/min into 15 ml of hydrating solution (phosphate buffer pH 7.4) with continuous stirring and temperature maintained at 60-$65^0$C. As the lipid solution injected slowly into aqueous phase, the difference in temperature between phases cause rapid vaporization of ether. After that this formulation were sonicated and result in the formation of niosomes [13].

***Experimental Design for Niosome:***

Optimization studies for the given procedure was performed by using Definitive screening design (Design Expert Software Trial version 12.0), 18 runs were generated. Concentration of surfactant, concentration of cholesterol, drug ratio (where concentration of caffeine kept constant), Sonication time, RPM, Type of Surfactant and Method of preparation was selected as independent variables. Particle size ($Y_1$), % Entrapment Efficiency (% Entrapment of Caffeine=$Y_2$, % Entrapment of Quercetin=$Y_3$), and % Drug release(% Drug release of Caffeine=$Y_4$, % Drug release of Quercetin=$Y_5$) were selected as dependent variables.

| **Independent variables** | | | **Level(-1)** | **Level(+1)** |
|---|---|---|---|---|
| Formulation Variables | A | Concentration of Surfactant | 1 molar conc. | 3 molar conc. |
| | B | Concentration of cholesterol | 1 molar conc. | 3 molar conc. |
| | C | Drug Ratio | 1:1 | 1:3 |
| | D | Sonication time | 5 | 30 |
| Process Variables | E | RPM | 100 | 500 |
| | F | Type of surfactant | Tween60 | Tween80 |
| | G | Method | Ether injection | Rotary evaporator |
| **Dependent variables:** | | | | |
| Particle size | | Entrapment Efficiency | % Drug Release | |

Table 1: Independent and dependent variables selected for optimization studies of niosome.

| **Run** | **A** | **B** | **C** | **D** | **E** | **F** | **G** |
|---|---|---|---|---|---|---|---|
| F1 | -1 | -1 | -1 | 1 | -1 | Tween60 | Ether injection Method |
| F2 | 0 | 0 | 0 | 0 | 0 | Tween60 | Ether injection Method |
| F3 | 0 | 0 | 0 | 0 | 0 | Tween80 | Hand Shaking Method |
| F4 | -1 | 1 | -1 | 1 | 1 | Tween80 | Hand Shaking Method |
| F5 | 1 | 1 | 1 | -1 | 1 | Tween80 | Hand Shaking Method |
| F6 | -1 | -1 | 1 | 1 | 0 | Tween80 | Hand Shaking Method |
| F7 | 1 | 1 | -1 | 1 | -1 | Tween80 | Ether injection Method |
| F8 | -1 | 1 | 0 | -1 | -1 | Tween60 | Hand Shaking Method |
| F9 | 1 | -1 | 0 | 1 | 1 | Tween80 | Ether injection Method |
| F10 | -1 | -1 | 1 | -1 | 1 | Tween60 | Hand Shaking Method |
| F11 | 1 | 0 | 1 | 1 | -1 | Tween60 | Hand Shaking Method |
| F12 | 1 | 1 | -1 | -1 | 0 | Tween60 | Ether injection Method |
| F13 | 0 | -1 | -1 | -1 | -1 | Tween80 | Hand Shaking Method |
| F14 | 1 | -1 | 1 | -1 | -1 | Tween60 | Ether injection Method |
| F15 | -1 | 1 | 1 | 0 | -1 | Tween80 | Ether injection Method |
| F16 | -1 | 0 | -1 | -1 | 1 | Tween80 | Ether injection Method |
| F17 | 1 | -1 | -1 | 0 | 1 | Tween60 | Hand Shaking Method |
| F18 | 0 | 1 | 1 | 1 | 1 | Tween60 | Ether injection Method |

Table 2: Composition of niosome by definitive screening design

***Characterization of Drug loaded Niosomal suspension:***

***Determination of Particle size:***

Particle size analysis was determined using digital microscope. The morphological characterization of niosomal vesicle such as shape and surface features were projected by using a digital microscope. Pixel Pro software was used for particle size analysis. Calibration of microscope was done using stage micrometer. After suitable dilution, a drop of niosomal dispersion was placed over the slide and viewed by Labomed microscope with a magnification of 10X[14].

***Determination of Entrapment Efficiency:***

Caffeine and Quercetin niosomal formulation were centrifuged at 13000rpm at $4^0$C for 30 min using cooling Centrifuge, so as to separate niosomes from non-entrapped drug. After centrifugation the concentration of free drug in the supernatant was determined by measuring absorbance at 273nm and 372nm with UV spectrophotometer (Schimadzu, UV 1700, Japan) the percent drug entrapment in niosomes was calculated by following formula[15].

Percent drug entrapment (%EE) = (Total drug – drug in supernatant / Total drug) x 100

***Determination of Drug Release****:*

The release of drug from niosomes is determined using the membrane diffusion technique i.e. by using Franz diffusion cell. Cellophane membrane was placed securely between donor and receptor compartment. Phosphate buffer solution of pH 6.4 was taken in the receptor chamber and the niosomal formulation was taken into the donor compartment. The quantity of niosomal formulation was taken approximately 1ml. The speed of magnetic stirrer was maintained at an optimal speed. Sampling was done at regular intervals, i.e. 5, 10, 15, 20, 25, 30, 35…….240min. The sink condition was maintained with phosphate buffer. And further measurement was carried out on the UV spectrophotometer at 273nm and 371nm [16].

***Differential Scanning Calorimetry (DSC) studies:***

DSC thermograms were obtained for pure drug and optimized formulation to investigate any drastic changes with thermal behavior of either drug or excipients. The interaction studies were carried out using differential scanning calorimetry, DSC curves (known as thermo grams) represented as heat flow v/s temperature or time. A typical DSC curve is characterized by the 'baseline' (part of the curve obtained during steady state conditions when no reaction or transition occurred), the peak caused from transitions or reactions, interpolated baseline, initial peak temperature (Ti), extrapolated peak onset temperature (Te), peak maximum temperature (Tp), extrapolated peak offset temperature (Tc), and final peak temperature (Tf). The thermograms of pure drug and optimized niosomal formulation were obtained. Sample was weighed (5.00-8.00+0.5 mg) and placed in sealed aluminum pans, before heating under nitrogen flow at a scanning rate of 10° C/min conducted over a temperature range below 200°C, respectively.

***FTIR Analysis***

FTIR was performed for Optimized Niosomal Suspension to determine for any Drug-Excipient In-compatibility. The graph obtained for this formulation was evaluated for the peaks of both pure drugs.

## III. Preparation of Niosomal In Situ Gel Formulation

PluronicF127 and Niosomal suspension were solubilized in distilled water containing polyethylene glycol400 (PEG400). The PluronicF127 vehicles used throughout this study were composed of 14% w/v of PluronicF127. The concentration of PluronicF127 was selected so as to obtain thermoreversible gel at minimum possible concentration. PluronicF127 vehicles with concentration varying from 16% w/v to 20% w/v were screened preliminarily to decide lowest possible concentration. PluronicF127, 14% w/v, was foundto be lowest concentration (when formulated in addition of niosomal suspension) that exhibited thermoreversible property below $34^0$C (temperature of the nasal cavity). Hence, 14% w/v of PluronicF127 was selected for further studies. The liquid was kept at $4^0$C until a clear solution was obtained. Thermoreversible niosomal gel was prepared using cold method. Bioadhesive anionic polymer Carbopol940 was slowly added to the solution with continuous agitation. Carbopol940 was added in concentration range given by design expert software to PluronicF127 solution [17]. The solution was made isotonic with sodium chloride (0.9%). Benzalkonium chloride was added as a preservative. The prepared gels were filled in glass vials and stored in refrigerator at a temperature of 4 to 8°C[18].

***Experimental Design for Niosomal Gel:***

Optimization studies for the given procedure was performed by using Central Composite design (Design Expert Software Trial version 12.0), 13 runs were generated.

| **Independent Variables** | **Level(-1)** | **Level (+1)** |
|---|---|---|
| Concentration of Carbopol | 0.1% | 0.6% |
| Concentration of Propylene glycol | 3% | 10% |
| **Dependent Variables** | | |
| Spreadability | Viscosity | % Drug Peameation |

Table 3: Independent and dependent variables selected for optimization studies of niosomal in-situ gel.

| **Run** | **A** | **B** |
|---|---|---|
| G1 | 4 | 0.5 |
| G2 | 6 | 0.0171573 |
| G3 | 8.82843 | 0.3 |
| G4 | 8 | 0.1 |
| G5 | 6 | 0.3 |
| G6 | 3.17157 | 0.3 |
| G7 | 6 | 0.582843 |
| G8 | 6 | 0.3 |
| G9 | 6 | 0.3 |
| G10 | 8 | 0.5 |
| G11 | 4 | 0.1 |
| G12 | 6 | 0.3 |
| G13 | 6 | 0.3 |

Table 4: Composition of niosomal gel by central composite design

***Characterization of Niosomal In-situ gel formulation:***

***Visual appearance and pH***

Visual appearance was observed for the presence of any particular matter. The pH of in-situ gels was measured using digital pH meter [19].

***Determination of viscosity:***

Viscosity determination was done by using Ostwald viscometer. The niosomal suspension was poured in to the apparatus through the left arm up to the mark A. The formulation was sucked in to the right arm slightly above the point B and the left arm was closed with the thumb to keep the liquid without dropping down. The apparatus was clamped vertically and the thumb was removed so as to allow the liquid to fall through the capillary under gravity then note down the time taken for the formulation to drop down from the point B to C. Then calculate the viscosity 14 by using poisulles equation as:

$$\eta l = \eta w \times tl \times dl / tw \times dw.$$ [20]

***Spreadability:***

The Spreadability of niosomal in-situ gel formulations was determined by using spreadability apparatus. 1gram of niosomal in-situ gel sample was placed on the lower slide and upper slide was placed on the top of the sample. The spreadability was determined by the formula

$S = (m \times l)/t$

Where $S$ is spreadability, $m$ is weight tied to upper slide, $l$ is length travel by upper slide, and $t$ is time [21].

***Drug Diffusion (Permeation):***

The horizontal diffusion chamber was used for the present study using cellophane membrane. Phosphate buffer solution of pH 7.4 was used in the receptor chamber. Before starting the study, the cellophane membrane was pre-incubated with phosphate buffer solution of pH 7.4 so as to saturate the cellophane membrane; so that there should not be any change in permeability. The niosomal nasal gel formulation was taken into the donor compartment. The quantity of the niosomal gel formulation was approximately about 1ml of gel. The speed of the magnet was adjusted at an optimum speed. Sampling was done at regular intervals, i.e. for 15, 30, 45, 60, 75, 90, 120, 150 min up to 6hrs. The sink condition was maintained with phosphate buffer solution. The samples were diluted with phosphate buffer and further measurements were carried out on the UV spectrophotometer at 273 nm and 372nm respectively[22].

***Stability study:***

Stability studies were assessed by keeping Niosomal suspension and Niosomal In-situ gel in sealed glass vials and storing them at $5\pm3^0$C for a period of 30 days. After 30days, Particle size, % drug release and % entrapment efficiency of vesicles were measured. The results were compared with the initial particle size, % drug release and % entrapment efficiency of optimized formulation for niosome. And in case of niosomal gel Spreadability, viscosity and % drug permeation compared with initial values of optimized batch[23].

## IV. Result and Discussion

***Effect On Particle Size*:**

ANOVA test for observed data of Particle size indicates that the quadratic model was significant and fitting for the data. The model was found to be significant (F- value99.56, p-value0.0100). The polynomial equation in terms of coded factors is given as follows:

Particle size =+0.3395+0.0670A-0.0542B+0.0313C+0.0036D+0.0050E+0.0130F-0.0217G+0.2098AB +0.1940AC+0.1403AD+0.4465AE+0.1703AF+AG-0.1753BC+0.2508BD

The vesicle size of all niosomal suspension batches with Tween 80 and Tween 60 ranged between 0.221±0.26µm to 0.502±0.16µm. From Response Surface Plot it was found that, increase in concentration of Cholesterol increases the particle size, at low concentration of cholesterol and surfactant it is believed to be closely packed, as the concentration of cholesterol increases, the hydrophobicity of bilayer membrane increases thereby increasing vesicle size this result occurred in case of both the surfactant[24]. Aswe increase in the concentration of surfactant there was decreasing vesicular diameter. Niosomal vesicles obtained from Tween 80 formulations with cholesterol were larger than those with Tween 60. This suggests that when the hydrophilicity of the surfactant increases, the particle size increases[25]. Increase in sonication time leads to decrease in particle size. In fig.1 we kept sonication time and rpm at middle level and the method of formulation and type of surfactant kept Tween60 and rotary evaporator. Ether injection method showed smaller vesicle as compare to thin film hydration method. A speed of 152 rpm yielded a uniformly thin lipid film resulting in spherical vesicles on hydration (data not shown).

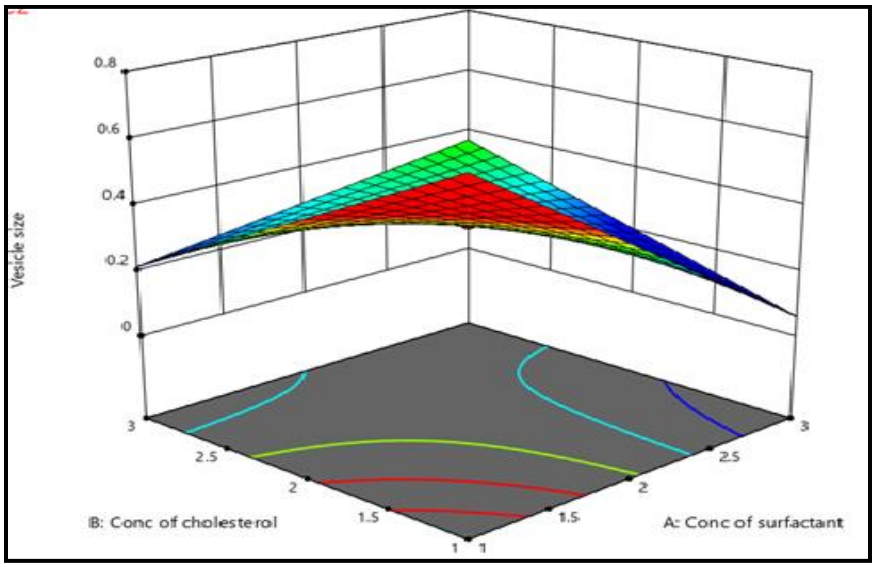


Figure 1: 3D Response Surface Plots for particle size

***Effect on Entrapment Efficiency:***

ANOVA test for observed data of an entrapment efficiency of both drugs indicates that the quadratic model was significant and fitting for the data. The model was found to be significant as the F-value 69.15, p-value 0.0143 for entrapment of Caffeine and the F-value73.10,p-value0.0136 for the entrapment of Quercetin. The polynomial equation in terms of coded factors is given as follows:

% Drug entrapment of caffeine =95.01-0.2479A+3.39B-1.05C-0.6793D+2.47E+2.02F+1.32G-59.83AB-54.13AC-48.80AD-149.09AE-50.61AF-2.99AG+74.27BC-79.89BD

% Drug entrapment of quercetin =98.99-0.04384A+0.7328B+0.5200C+0.2064D +0.5305E +0.3968F+0.2120G-4.13AB-3.40AC-3.17AD-9.01AE-3.33AF-0.9167AG+3.41BC-5.56BD

The % Entrapment Efficiency of all niosomal suspension batches ranged between 81.52±0.21% to 98.72±0.16% for Caffeine and 94.3±0.31 to 99.73±0.23 for Quercetin. With increase in cholesterol concentration there was increase in entrapment of Caffeine as well Quercetin. This could be due to the function of cholesterol which at high concentration, prevents the gel-state transformation in to liquid ordered phase which results in an increase in rigidity of the resulting bilayers, thus increasing the stability of niosomes and %Entrapment Efficiency[26]. Similarly, with increase in concentration of surfactant there was increase in entrapment of both drugs. In case of quercetin further increase in concentration of surfactant showed decrease in %EE. This could be due to formation of mixed micelles along with the niosomal vesicles with high concentration of surfactant, which may lead to lowering of entrapment efficiency[27]. Entrapment efficiency for niosomes prepared with Tween 60 was higher than that with Tween 80. This shows that the longer the alkyl chain of the surfactant, the fewer drugs will be entrapped. Tween 80 has a longer saturated alkyl chain than Tween 60 and lower entrapment efficiency. On studying the effect on drug entrapment it was found that thin film hydration method shows more entrapment than ether injection method. This may be attributed to the fact that vesicle size of the niosomes formed by thin film hydration more than ether injection method [28]. The % entrapment was also found to influences with RPM and sonication. The time for sonication does not had much effect on % entrapment but without sonication entrapment was increased for Caffeine being a soluble drug whereas with sonication entrapment increased for Quercetin an insoluble drug. The increase in entrapment efficiency was achieved by increasing drug concentration from 1:1 to 1:2; with further increase in drug ratio from 1:2 to 1:3 significant decrease in encapsulation efficiency was observed. The viscosity of solution at high drug loading was very high and was responsible for the formation of larger solvent droplets. It caused decreased rate of entrapment due to slower hardening of the larger particles, allowing time for drug diffusion out of the particle which tends to decrease in entrapment efficiency [29].

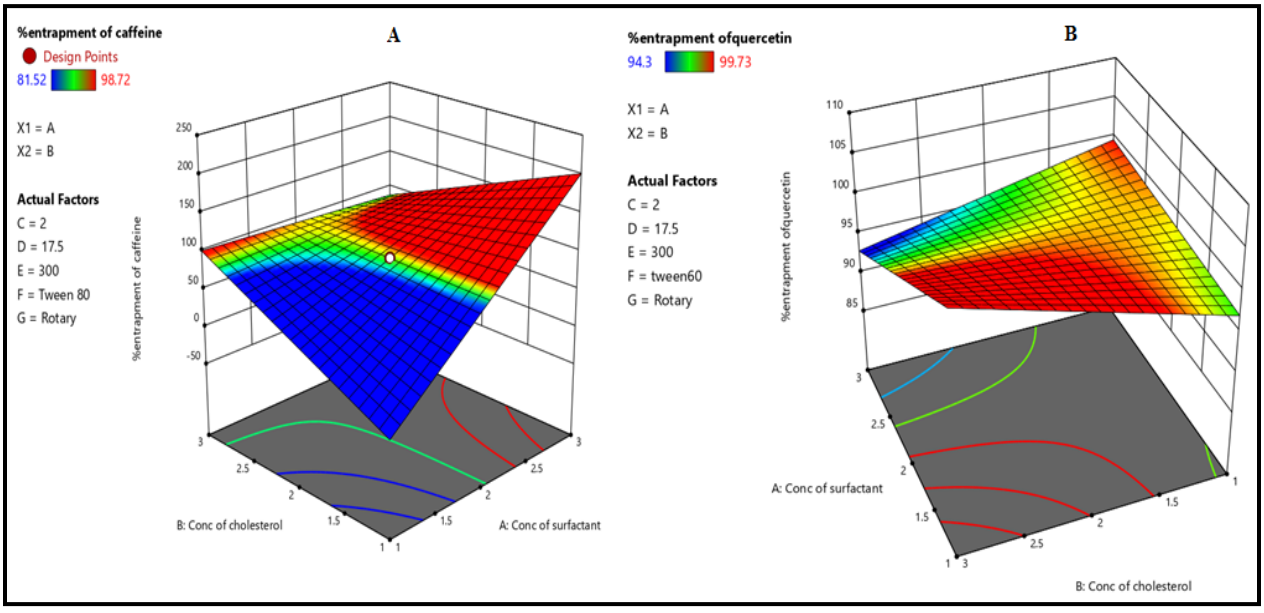


Figure 2: 3D Response Surface Plots for A: Entrapment of Caffeine, B: Entrapment of Quercetin

***Effect On Drug Release:***

ANOVA test for observed data of the entrapment efficiency of both drugs indicates that the quadratic model was significant and fitting for the data. The model was found to be significant as the F-value 626.97, p-value 0.0016 for drug release of Caffeine and the F-value 186.02, p-value 0.0054 for the drug release of Quercetin. The polynomial equation in terms of coded factors is given as follows:

% Drug Release of caffeine =102.25-2.53A+3.43B-1.68C-1.17D-1.78E+2.31F-1.19G-52.00AB-50.06AC-45.07AD-126.80AE-47.20AF-10.08AG+57.15BC-65.11BD

% Drug Release of quercetin =12.85+2.72A+4.63B-4.99C+2.40D+0.8764E+2.06F-1.11G+106.91AB+96.06AC+80.69AD+238.69AE+90.70AF+9.48AG-108.49BC+129.09BD

The % Drug Release of all niosomal suspension batches ranged between 72.09±0.18% to 103.3±0.26% for Caffeineand 10.9±0.31 to 37.06±0.15 for Quercetin at 4hrs.With increase in concentration of cholesterol and surfactant there was decrease in % drug release. That might be due to the fact that increased surfactant acts as depot, whereas with increase in cholesterol ratio there is increase in rigidity of vesicle, which decrease permeability of entrapped drug [30]. In case of caffeine maximum drug release was found might be due to release of absorb drug from surface of lipophilic region and bilayer portion of niosomes and also caffeine had high solubility and permeability due to this it show maximum release. While quercetin release was sustain release as it entrapped in lipophilic portion of niosomes. The fatty acid chain length of polyoxyethylene sorbitan type surfactant influences drug release. Increase in drug release occurred in case of Tween 80 as compared with Tween 60 this might be due to greater the chain length, the slower the release rate. In case of Tween 80, the unsaturation might be responsible for higher release rate as unsaturation in chain increase chain fluidity and permeability [31]. The % was also found to influences with RPM and sonication. As sonication time and RPM increases % drug release also increases, this might be due to smaller vesicle size larger surface area. Niosomes prepared with thin film hydration showed good drug release as compared with ether injection [32].

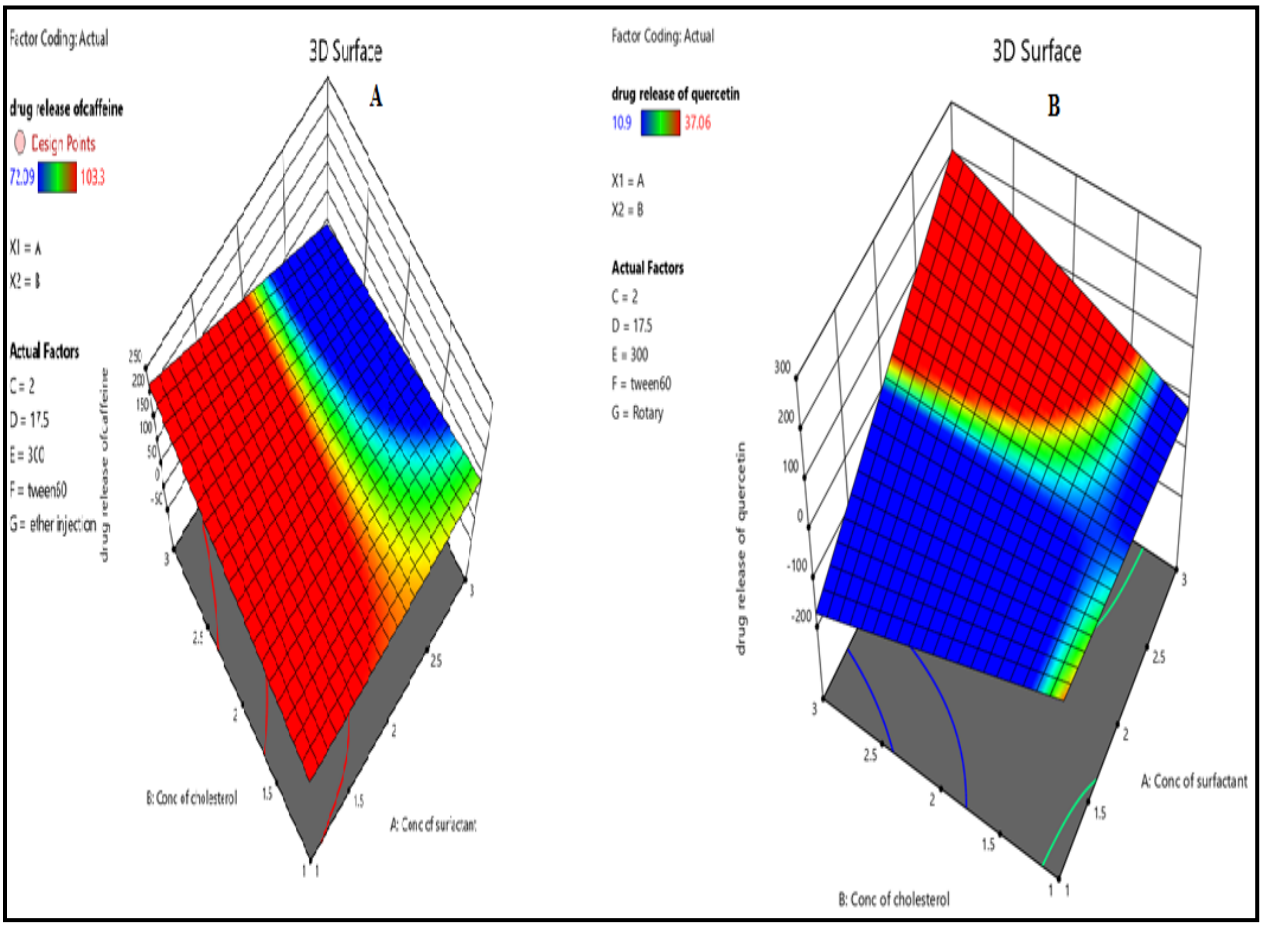


Figure 3:3D Response Surface Plots for A: Drug Release of Caffeine, B: Drug Release of Quercetin

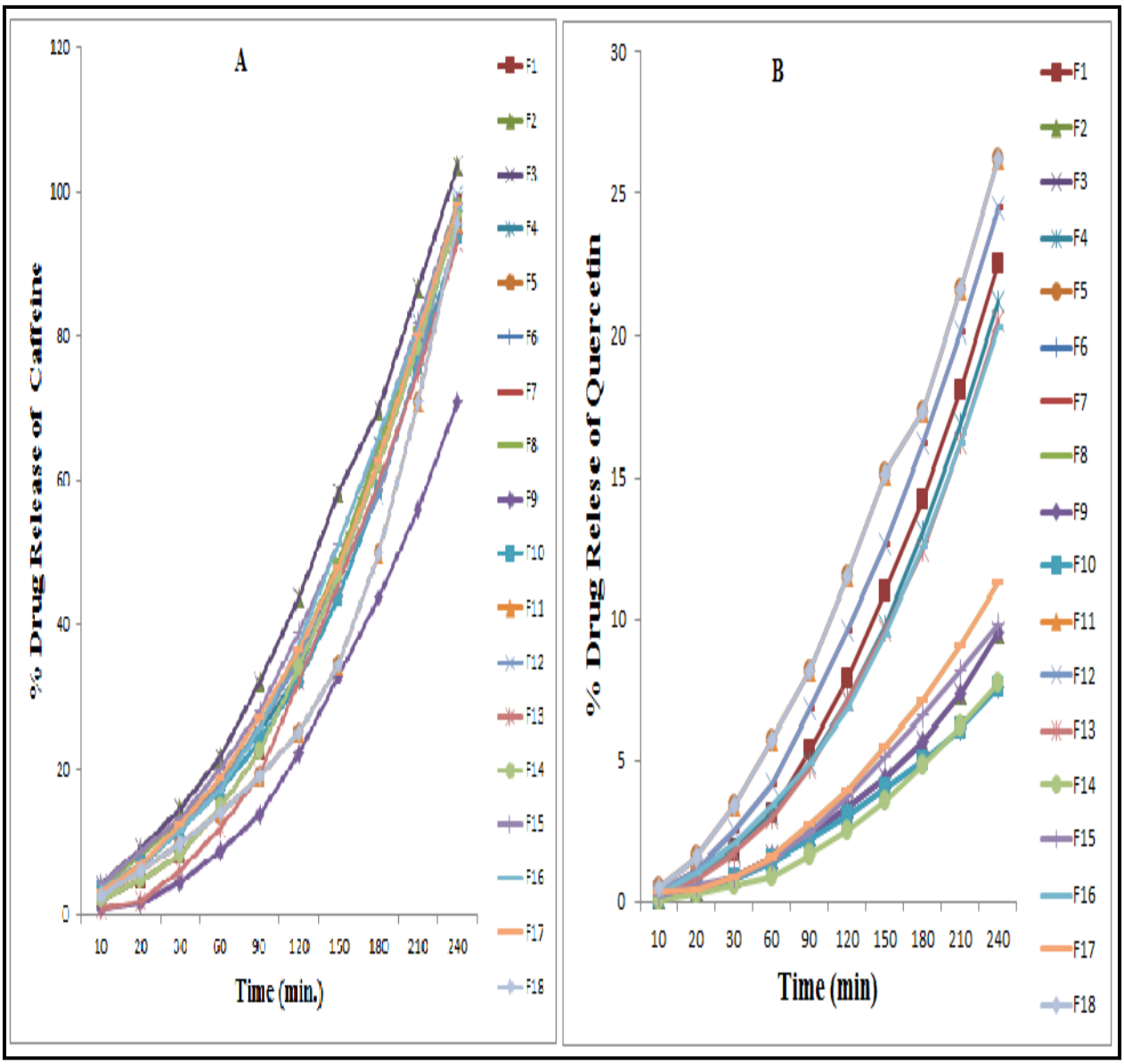


Figure 4: In Vitro drug release of A: Caffeine, B: Quercetin

***Desirability And Optimized Batch:***

Design Expert Software criterion being one having the maximum desirability value. The optimization process was performed by setting $Y_1$ at minimum, $Y_2$, $Y_3$, $Y_4$ and $Y_5$ at maximum while all independent variables within range obtained. The optimized formulation was achieved at A=2.85, B=2.26, C=1.45, D=20.55, E=152.01, F=Tween60, G=Thin film hydration method with corresponding desirability value of 0.908 (fig. 5). This factor level combination predicted the responses $Y_1$=0.273µm, $Y_2$=88.32%, $Y_3$=98.26%, $Y_4$=103.54% and $Y_5$=37.071% where, observed responses was $Y_1$=0.281µm, $Y_2$=87.5%, $Y_3$=97.9%, $Y_4$=102.1% and $Y_5$=38.67%.

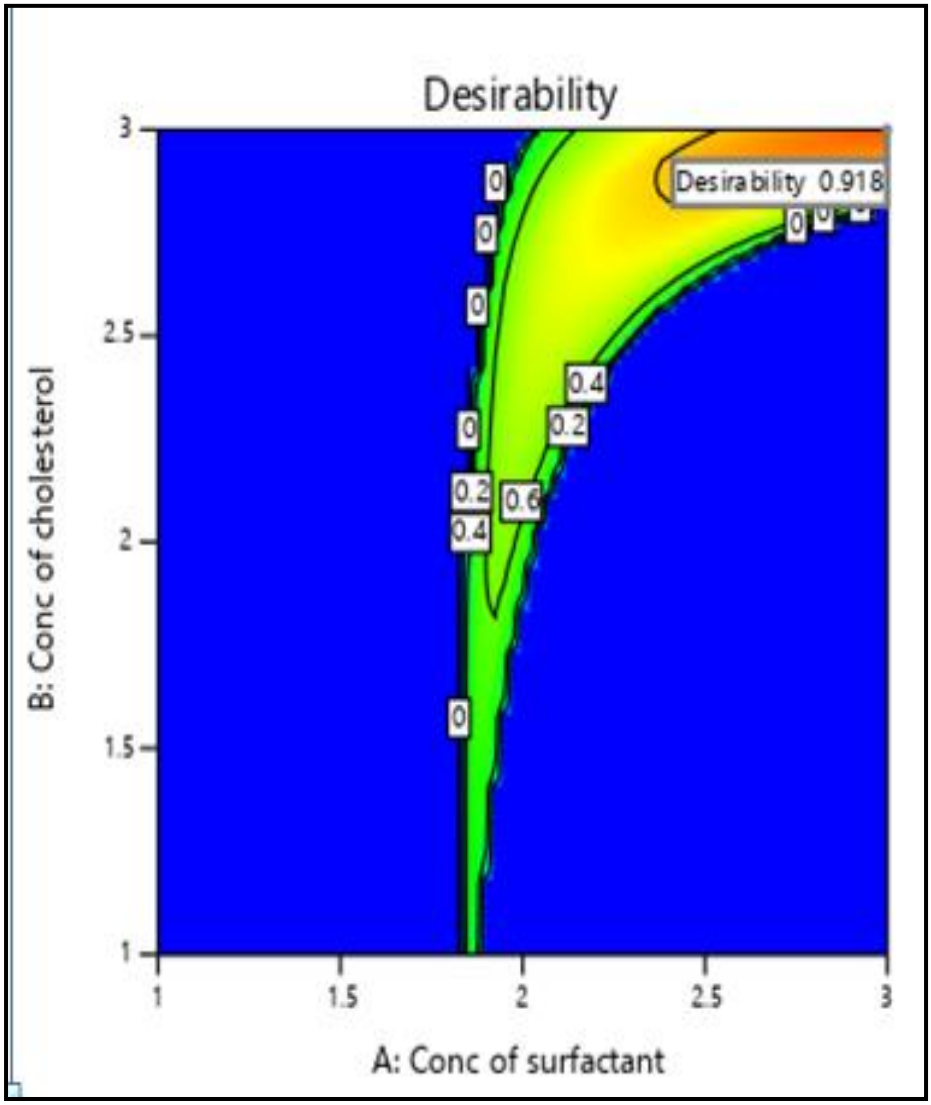


Figure 5: Desirability plot of Niosome

***DSC Studies:***

DSC studies were performed to observe the thermal properties and intermolecular reaction between Quercetin, Caffeine and excipients used in the formulation of niosomal dispersion. Pure Quercetin showed endothermic peak at 138.94ºC and pure Caffeine showed endothermic peak at 238.97ºC which corresponds to its melting point. The presence of sharp endothermic peak primarily indicates the crystalline nature of drugs. In the DSC thermogram of optimized formulation of Quercetin and Caffeine loaded niosomal suspension the endothermic peak shifted at 93.49ºC. This might be attributed to the presence of both the drugs in the amorphous state in the niosomal suspension (fig.6).

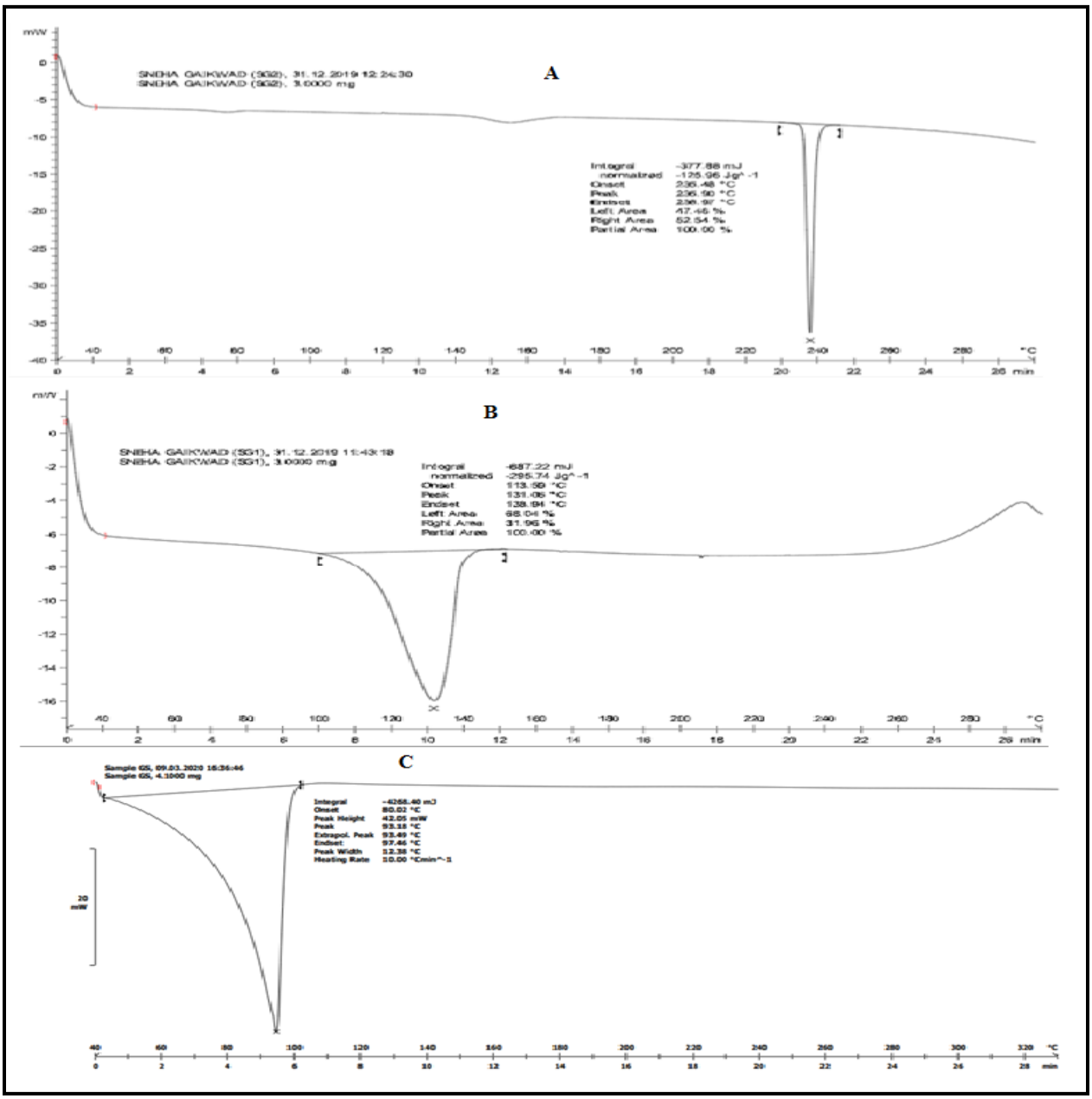


Figure 6: DSC thermogram of A: Caffeine, B: Quercetin and C: Optimized batch of Niosomal in-situ gel

***FTIR* Analysis:**

Infrared spectral analysis of Quercetin and Caffeine was performed to characterize both the drugs. The FTIR spectra showed the prominent peaks of various groups present in the Quercetin and caffeine chemical structure. The infrared spectra of Quercetin exhibited transmittance peaks at 3415 $cm^{-1}$,1667 $cm^{-1}$, 1611 $cm^{-1}$ and 1522 $cm^{-1}$ owing to presence of -OH stretching, C=O stretching, C-C stretching, and C=C stretching for aromatic group respectively. Peaks at 3336.25 $cm^{-1}$, 2956 $cm^{-1}$, and 1697 $cm^{-1}$ in infrared spectra of Caffeine are attributed to N-H stretching, C-H stretching, and –C=N ring stretching group respectively. These characteristic peaks of both the drugs are well presented in optimized batch of niosomal in-situ gel indicating absence of any interactions between both drugs and drugs with excipients. Therefore, both drugs can be administered together in a combination. (Fig. 7)

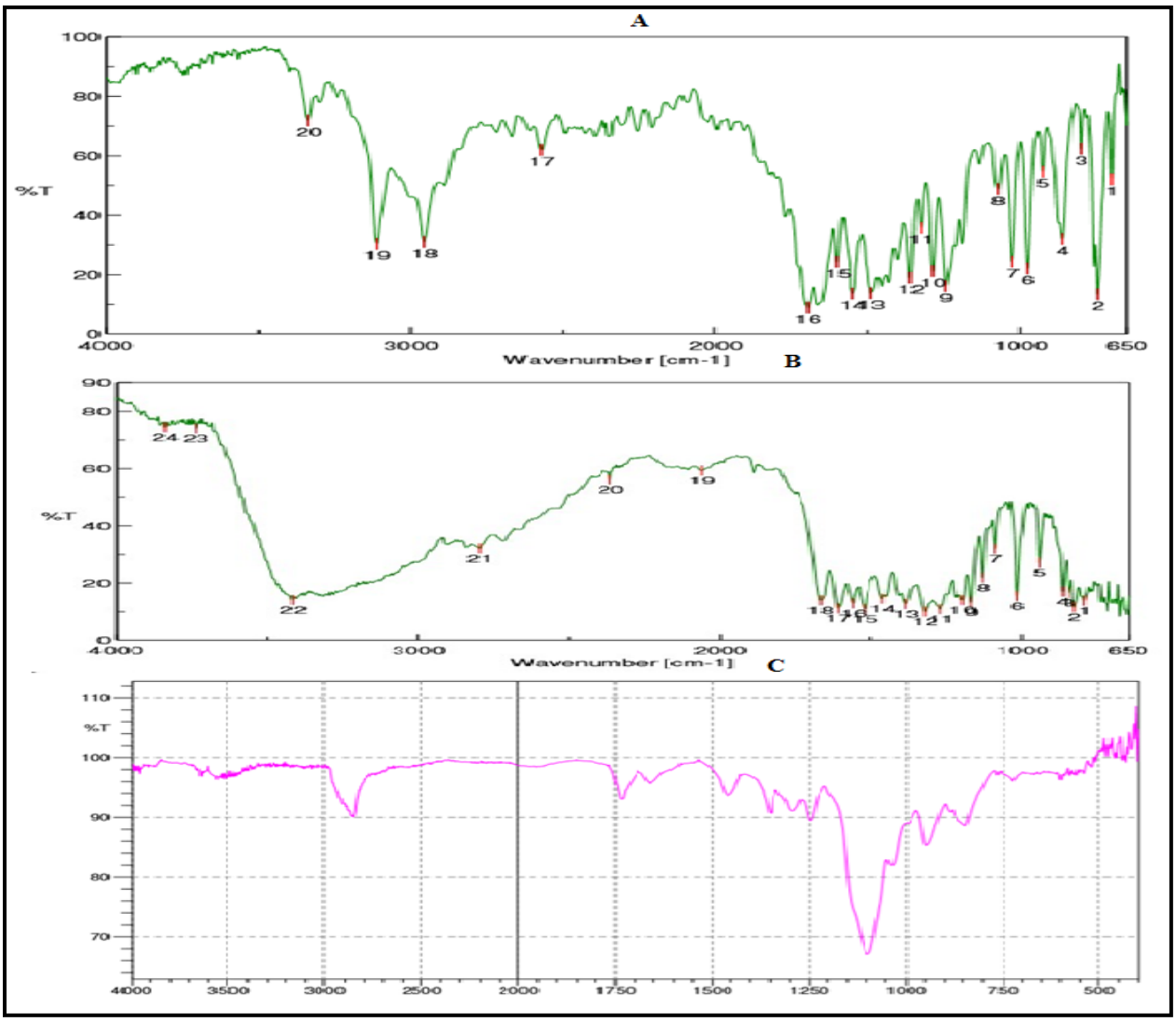


Figure 7: FTIR spectra of A: Caffeine, B: Quercetin, C: Optimized batch of Niosomal in-situ gel.

***Evaluation Of Niosomal In-Situ Gel:***

***Effect on Spreadability:***

ANOVA test for observed data of Spreadability indicates that the quadratic model was significant and fitting for the data. The model was found to be significant (F- value48.79, p-value < 0.0001). The polynomial equation in terms of coded factors is given as follows:

Spreadability= +6.42+0.8825A+0.2320B

The Spreadability of all niosomal in-situ gel batches ranged between 5.1±0.26cm to 7.9±0.16cm. When concentration of both Carbopol940 and polyethylene glycol400 increases results in increasingSpreadability. It might be due to Caropol940 is a well-known compound for its excellent gel property such as sparkling clarity and Spreadability etc. [33]

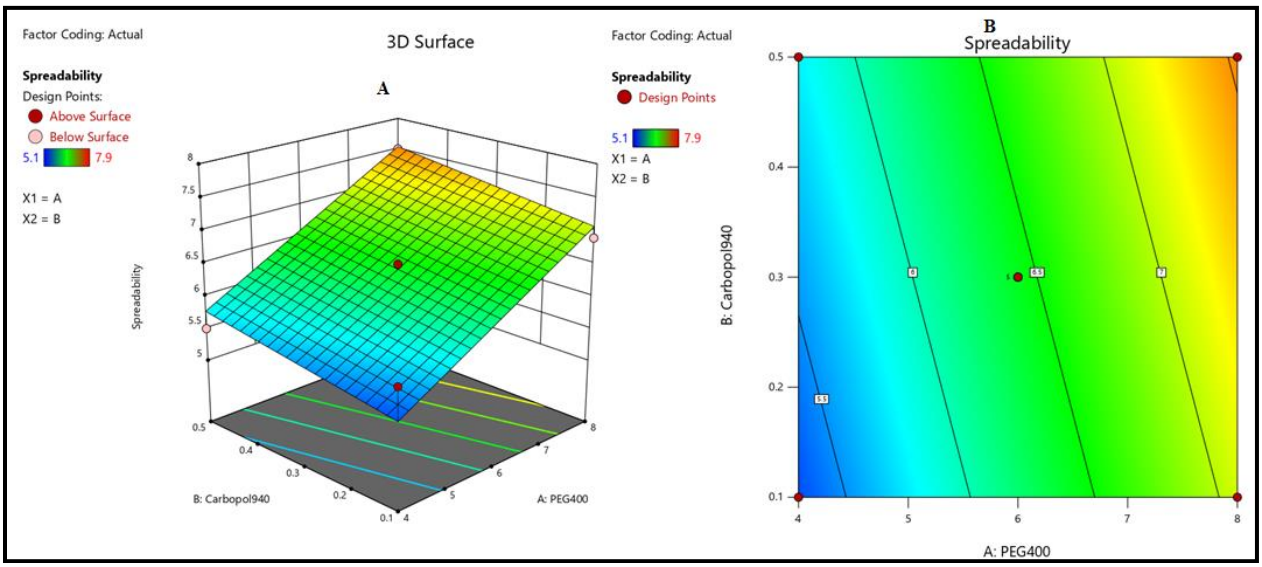


Figure 8: A: 3D Response Surface Plots B: Contour Plot for Spredability

***Effect on Viscosity:***

ANOVA test for observed data of Spreadability indicates that the quadratic model was significant and fitting for the data. The model was found to be significant (F- value 11.79, p-value 0.0027). The polynomial equation in terms of coded factors is given as follows:

Viscosity =+4460.00+548.48A+748.04B+392.50AB-1065.00$A^2$-507.50$B^2$.

The Viscosity after gelation of all niosomal in-situ gel batches ranged between 1800±0.11cps to 4780±0.26cps. When concentration of carbopol940 increases results in increasing Viscosity. Carbopol940 is weakly acidic compound and when dispersed in water and neutralized carbomer uncoil its chain and results in the gelling that, in turn, increases viscosity of solution [33]. Similarly, as concentration of PEG400 increases leads to increasing viscosity.

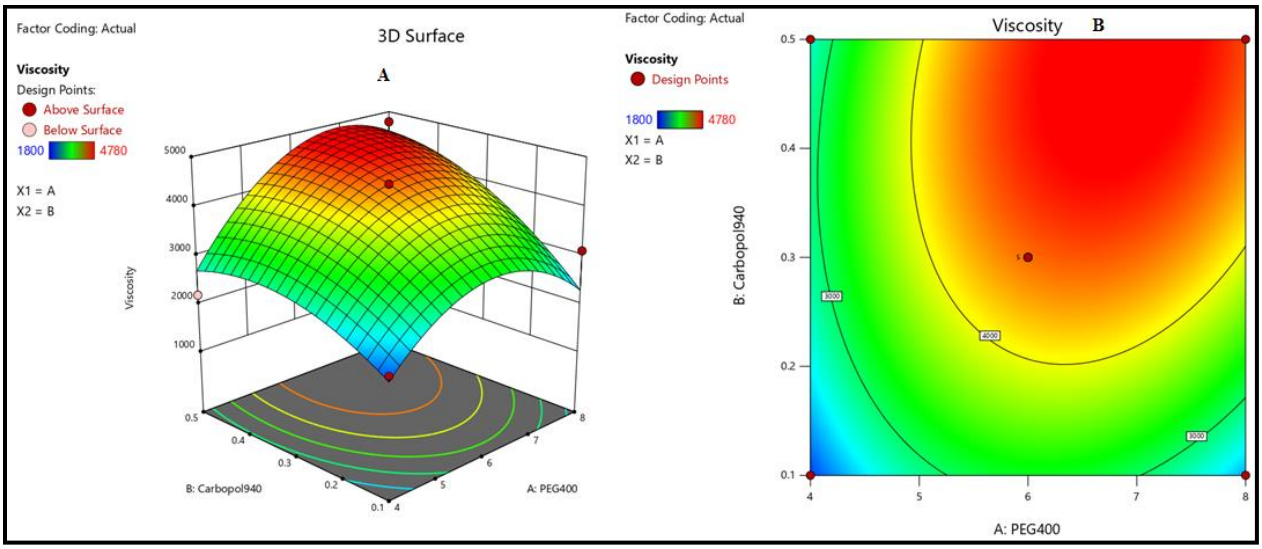


Figure 9: A: 3D Response Surface Plots B: Contour Plot for Viscosity

***Effect on Drug Permeation:***

ANOVA test for observed data of the % Drug Permeation of both drugs indicates that the quadratic model was significant and fitting for the data. The model was found to be significant as the F-value 32.92, p-value 0.0001 for drug permeation of Caffeine and the F-value 15.55, p-value 0.0011 for the drug permeation of Quercetin. The polynomial equation in terms of coded factors is given as follows:

Drug Permeation of Caffeine =+97.18+3.44A-1.73B-1.34AB-2.52$A^2$-2.47$B^2$.

Drug Permeation of Quercetin =+29.82+3.00A-1.01B+0.2975AB-0.7356$A^2$-1.62$B^2$.

The % Drug Permeation after gelation of all niosomal in-situ gel batches ranged between 85.86±0.015% to 98.61±0.024% for Caffeine and 22.65±0.19% to 33.23±0.34% for Quercetin at 6hrs. As we increase in carbopol940 content was associated with a corresponding decrease in the drug-permeation rate of both the drugs. This could be due to extensive swelling of the polymer which created a thick gel barrier for drug diffusion[34]. The drug permeation was increased with the increasing concentration of PEG400. This might be due to the fact that dissolution of aqueous soluble fraction of the polymer matrix leads to the formation of gelaneous pores. The formation of these kind of pores leads to decrease the mean diffusion path length of drug molecules to release into the diffusion medium and hence, to cause higher release rate [35].

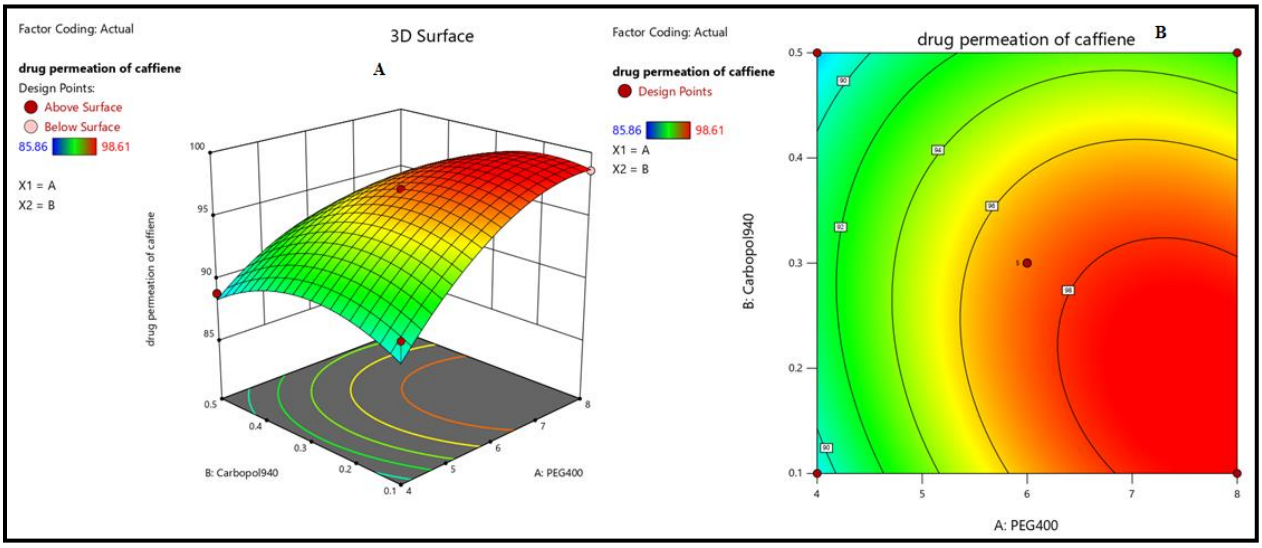


Figure 10: A: 3D Response Surface Plots B: Contour Plot for Drug Permeation of Caffeine

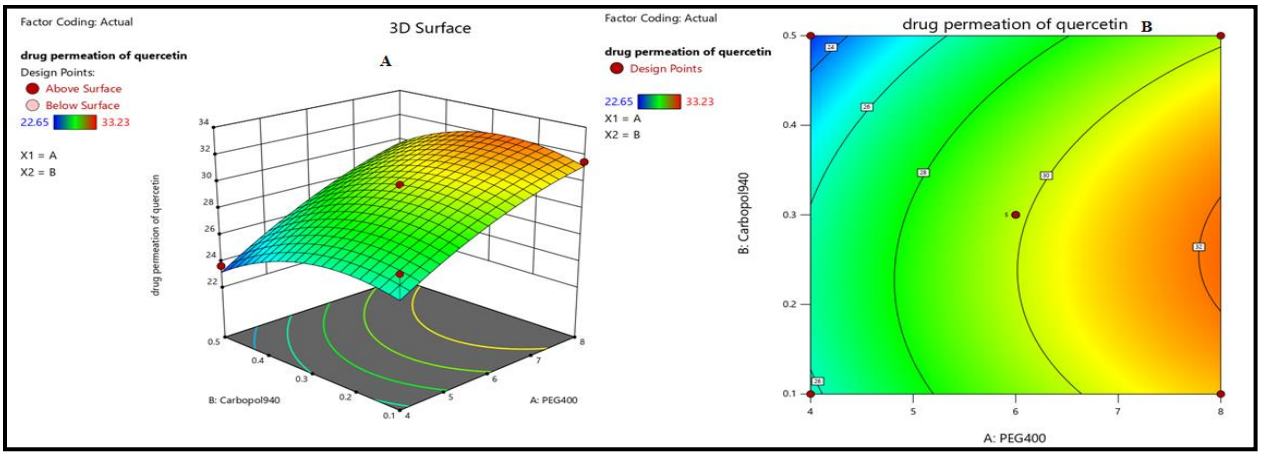


Figure 11: A: 3D Response Surface Plots B: Contour Plot for Drug Permeation of Quercetin

***Desirability and Optimized Batch:***

Design Expert Software criterion is the one having the maximum desirability value. The optimization process was performed by setting all dependent variable at maximum while all independent variables within range obtained. The optimized formulation was achieved at A=4.2 and B=0.3 with corresponding desirability value of 0.846 (fig. 12). This factor level combination predicted the responses spredability=5.6cm, viscosity=3191cps, drug permeation of caffeine=92.32%, drug permeation of quercetin=26.67%where, observed responses was spredability=5.7cm, viscosity=3201cps, drug permeation of caffeine=92.68%, drug permeation of quercetin=29.15%.

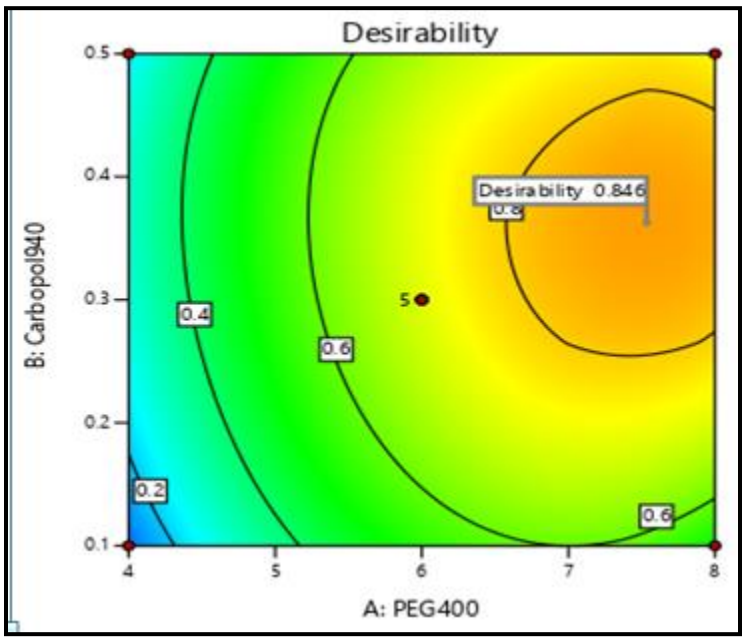


Figure 12: Desirability plot of Gel

***Visual Appearance, Clarity and pH*:**

All gels were found to be yellowish in colour and free from presence of particle. The pH range of the gel formulations was in the range of 6.3 to 6.9.

***Stability Studies:***

Stability studies performed for optimized formulation of both niosomal suspension and niosomal insitu gel. In which both formulation stored at 5±3$^0$C for a period of 30 days. Results were showed in Table 3 and 4.

Table 5: Stability studies of Niosomal Suspesion

| Sr. No. | Parameters | Initial days | After 30 days |
|---|---|---|---|
| 1 | Particle size | 0.281µm | 0.280µm |
| 2 | % Entrapment Efficiency of Caffeine | 87.32% | 87.32% |
| 3 | % Entrapment Efficiency of Quercetin | 97.9% | 97.8% |
| 4 | % Drug Release of Caffeine | 102.1% | 102.09% |
| 5 | % Drug Release of Quercetin | 38.67% | 38.61% |

Table 6: Stability studies of Niosomal In-situ Gel

| Sr. No. | Parameters | Initial days | After 30 days |
|---|---|---|---|
| 1 | Spreadability | 5.7cm | 5.7cm |
| 2 | Viscosity | 3201cps | 3200cps |
| 3 | % Drug Permeation of Caffeine | 92.68% | 92.66% |
| 4 | % Drug Permeation of Quercetin | 29.15% | 29.13% |
| 5 | pH | 6.4 | 6.4 |

Optimized formulations were stored at $5\pm3^0$C for a period of one month and later evaluated for various parameters. Both the formulations were found to be more stable at refrigerated temperature of $5\pm3^0$C.

## V. Conclusion

These results indicated that niosomal In-situ gel can be used to overcome the shortcomings of the pure drug and maintaining therapeutically effective drug concentration for a prolonged period indirectly minimizing drug administration frequency and enhancing the bioavailability of drug by directly delivering the drug to the brain by avoiding first pass effect. Thus, it can be concluded that niosomal In-situ gel represents a promising drug delivery system.

## VI. Acknowelgement

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