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# OPTIMIZATION AND IN-VITRO CHARACTERIZATION OF ERYTHROMYCIN NANOGELS BY USING SIMPLEX-LATTICE MIXTURE DESIGN

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**ABSTRACT**

Erythromycin nanogels (EM-NGs) are established as one of the most promising carriers for topical delivery system. Therefore, the aim of the present study was to develop a poloxamer based nanogels of erythromycin with a view to improve its high capacity to hold water, without dissolving into the aqueous medium. Nanogels of erythromycin were developed by probe sonicator and evaluated in vitro for, Fourier transforms infra-red (FTIR) spectroscopy, with good particle size, refractive index, gel strength, spreadability, mucoadhesive force and viscosity. In vitro characterizations of the erythromycin nanogels revealed that the mean globule sizes of the nanogels ranged from 192 nm to 247 nm, the refractive index ranged from 1.371 to 1.395, the gel strength values were in the range of 9 to 120 seconds, the spreadability were between 3.61 and 64.64 gm.cm/sec, the viscosity ranged from 15.3 to 809 cps and the mucoadhesive force was in the range of 15890.9 to 30193.9 dynes/cm.

**Keywords:** Erythromycin, Nanogels, Probe Sonicator, Simplex-Lattice Mixture Design

## INTRODUCTION

Polymeric nano systems which include micelles, liposomes, nanoparticles, and nanogels have been the most investigated drug delivery systems. Due to their prolonged circulation time, significant improved accumulation in the targeted site via the enhanced permeability and retention (EPR) effect, decreased adverse effects, and improved, drug tolerance [1–7]. Compared to other nanosystems, nanogels with internally cross-linked 3D structures are highly interesting for controlling drug delivery at the target site in fast response to external stimuli as well as for improving drug bioavailability [8–13]. Nanogels are 3D network hydrogel materials in the nanoscale size range formed by crosslinked swellable polymer networks with a high capacity to hold water, without dissolving into the aqueous medium. Their characteristics such as size, charge, porosity, amphiphilicity, softness, and degradability can be fine-tuned by varying the chemical composition of the nanogels. They are mostly spherical particles and allow fabrication of the nanogels of different shapes [14, 15]. Nanogels are mostly hydrophilic in nature and highly biocompatible with a high loading capacity for foreign molecules and their special physical properties provide them a lot of biomedical applications. They actively participate in the delivering process due to their characteristic properties like stimuli-responsive behavior, softness and swelling to help achieve a controlled, triggered response at the target site [16-22]. Erythromycin is in a class of medications called macrolide antibiotics. It works by stopping the growth of bacteria. The main advantage of erythromycin is to treat or prevent various types of infections caused by bacteria. For instance infections of the respiratory tract, including bronchitis, pneumonia, pertussis, pertussis (whooping cough; a serious infection that can cause severe coughing); diphtheria (a serious infection in the throat); sexually transmitted diseases (STD), including syphilis; and ear, intestine, gynecological, urinary tract, and skin infections. It is also used to prevent recurrent rheumatic fever. However, antibiotics such as erythromycin will not work for colds, flu, or other viral infections.

Poloxamers are nonionic polyoxyethylene-polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. The polyoxyethylene part is belong to hydrophilic while the polyoxypropylene part is hydrophobic. Poloxamers are white, waxy, free-flowing prilled granules, or as cast solids. They are odorless and tasteless. They are colorless liquid at room temperature. The pH of poloxamers is 5.0-

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7.4 while the melting point is 52-57degree celcius. Poloxamers are used as emulsifying agents in intravenous fat emulsions, and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups. Furthermore, it may also be used as wetting agents; in ointments, suppository bases, and gels; and as tablet binders and coatings. Poloxamer 188 has been used as an emulsifying agent for fluorocarbons and also used as artificial blood substitutes and in the preparation of solid-dispersion systems. Poloxamer 188 is incompatible with phenols and parabens depends on the relative concentration [23]. In this paper, Erythromycin nanogels with desired globule size, high gel strength and mucoadhesive force were obtained by using simplex-lattice mixture design (Stat-Ease Design-Expert software-DX9). The nanogel networks offer several advantages including prolonged drug release, excellent mucoadhesive properties, and spreadability.

**METHOD OF PREPARATION:** (Figure 1)

## MATERIALS

Erythromycin was gift sample from SM Pharmaceuticals Sdn Bhd, Malaysia .Tween 20 was purchased from R&M Chemical. Poloxamer 188 was purchased from Merck KGaA (Darmstadt, Germany). All water used in the formulation was of Milli-pore grade.

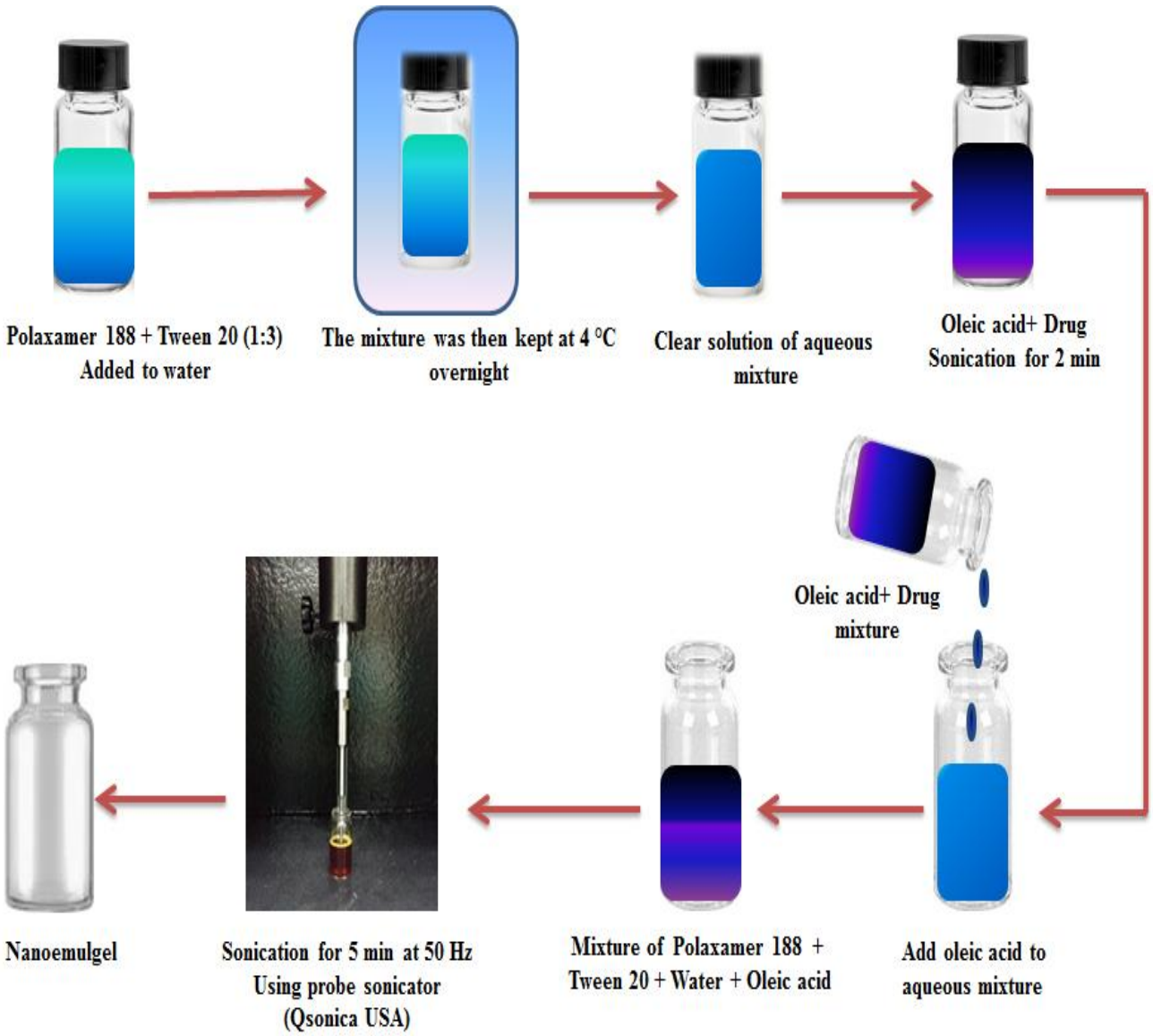


**Figure 1: Preparation of erythromycin nanogels**

**IN-VITRO EVALUATION**

*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 [24].

*Measurement of Spreadability:* A small portion of the nanogels was applied on a glass slide and was compressed to uniform thickness by placing 50 g for 5 minutes [25]. The time in which the upper glass slide move over the lower slide was taken as measurement of spreadablility (S)

S= ML/T where,

M= weight tide to upper slide (g)

L= length moved on the glass tide (cm)

T= time taken (sec)

*Measurement of gel strength:*

A sample of 50gm of nanogels was placed in a 100 ml graduated. The apparatus for measuring gel strength (weighing 27 gm) was allowed to penetrate in 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 [26].

*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. Nanogels 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 [25].

*Particle size analysis of nanogels:*

Particle size of nanoparticles was determined using malvern particle size analyzer (Zetasizer 4000S, Japan).

*Determination of refractive index:*

The refractive index of nanogels was determined by using Lan Optics. A small portion of samples were placed on the glass slide of the equipment. The refractive index will be shown by the equipment. Measurement was considered after reaching equilibrium.

*In-vitro permeation studies of nanogel formulations:*

In-vitro studies of the gel were carried out across the egg membrane extracted by using the concentrated hydrochloric acid. The receptor compartments were filled with phosphate buffered saline (PBS) pH 6.8, Study was carried out using excised egg membrane. The entire setup was placed on a thermostatic magnetic stirrer and the temperature was maintained at 37◦C throughout the study.

Permeability studies were carried out over a period of 8 hrs at regular intervals. Samples were withdrawn and analyzed spectrophotometrically at 264 nm.

**RESULT AND DISCUSSION**

*Optimization of process variables for the erythromycin nanogels:*

The effects of the three factors (Water, Oleic Acid and Tween 20: Poloxamer 188) on the refractive index, globule size, gel strength, spreadability, mucoadhesive force and viscosity were tested. Through preliminary screening the water, oleic acid and surfactant/Co-surfactant ratio were identified as the most significant variables within the range of 5-7g, 1-3g and 1-3g, respectively. On the basis of the preliminary trials a simplex-lattice mixture design was employed to study the effect of each independent variable on dependent variables (refractive index, globule size, gel strength, spreadability, mucoadhesive force and viscosity). 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. The independent factors and the dependent variables used in the design are listed in Table 1**.** The experiments were conducted as for the design of experiments and the responses for the dependent variables were entered in Table 2. The response surfaces of the variables inside the experimental domain were analyzed using Stat-Ease Design-Expert software (DX9). Subsequently, three additional confirmation experiments were conducted to verify the validity of the statistical experimental strategies.

**Table-1: List of Independent variable and Dependent variables in simplex-lattice mixture design**

| **Independent variable** | **Levels** | | | | |
|---|---|---|---|---|---|
| **Variable** | Name | Units | Low | Middle | High |
| A | Surfactant | % | 5 | | 7 |
| B | Speed | rpm | 5000 | 6500 | 8000 |
| C | Time | time | 60 | 120 | 180 |
| **Dependent variable** | **Goal** | | | | |
| Y1 | Refractive index | Optimized value | | | |
| Y2 | Globule size | | | | |
| Y3 | Gel strength | | | | |
| Y4 | Spreadability | | | | |
| Y5 | Mucoadhesive force | | | | |
| Y6 | Viscosity | | | | |

**Table 2: Factorial design of erythromycin nanogels**

| Run | A:water w/w | B:oil w/w | Tween 20:P188 (1:3) w/w | Refrac -tive index | Size nm | Gel strength sec | Spread-ability g.cm/sec | Muco-adhesive force dynes/cm | Viscosity cps |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 2 | 2 | 1.387 | 192 | 90 | 3.61 | 30193.9 | 449.9 |
| 2 | 6.3 | 1.3 | 1.3 | 1.371 | 195 | 9 | 10.21 | 15890.9 | 15.3 |
| 3 | 5.6 | 1.6 | 1.6 | 1.377 | 198 | 12 | 16.67 | 15940.5 | 18.1 |
| 4 | 5 | 3 | 1 | 1.394 | 214 | 22 | 18.39 | 15880.5 | 60.4 |
| 5 | 6 | 1 | 2 | 1.38 | 229 | 18 | 64.64 | 15891.1 | 55.3 |
| 6 | 5 | 1 | 3 | 1.393 | 190 | 90 | 13.62 | 28691.9 | 403.1 |
| 7 | 5.3 | 1.3 | 2.3 | 1.384 | 195 | 120 | 12.13 | 28469.6 | 809.3 |
| 8 | 5.3 | 2.3 | 1.3 | 1.383 | 193 | 21 | 31.31 | 15890.3 | 60.2 |
| 9 | 6 | 2 | 1 | 1.373 | 232 | 19 | 60.82 | 15885.7 | 55.4 |
| 10 | 5 | 1 | 3 | 1.393 | 189 | 90 | 13.55 | 28692.5 | 402.8 |
| 11 | 7 | 1 | 1 | 1.364 | 246 | 31 | 11.86 | 20679.2 | 100.2 |
| 12 | 5 | 3 | 1 | 1.395 | 213 | 23 | 18.21 | 15879.5 | 77.1 |
| 13 | 7 | 1 | 1 | 1.363 | 247 | 32 | 11.63 | 20678.2 | 105.6 |
| 14 | 6 | 2 | 1 | 1.374 | 231 | 18 | 59.15 | 15895.1 | 58.6 |

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

**Table 3: Multiple linear regression analysis**

| | |
|---|---|
| **Y1** | +1.36 A+1.39 B+1.39 C-0.022 AB+7.085 AC-0.027 BC-0.050 ABC |
| **Y2** | +245.91A+212.91 B+188.91 C+3.62AB+36.86 AC-45.14 BC-2698.81 $A^2BC$ -394.81$AB^2C$+818.01 $ABC^2$ |
| **Y3** | +30.06 A+19.78 B+98.65 C-61.42 AB-165.91 AC+132.38 BC |
| **Y4** | +11.43 A+17.98 B+13.27 C+178.57 AB+204.06 AC-53.17 BC-3222.27$A^2BC$+1017.63 $AB^2C$-1028.63 $ABC^2$ |
| **Y5** | +20750.64 A+15951.93 B+28764.12 C-9268.19 AB-34314.40 AC+32494.26 BC-1.598 $A^2BC$-5.020 $AB^2C$+3.90$ABC^2$ |
| **Y6** | +111.50 A+77.35 B+411.55 C-80.92 AB-687.35 AC+959.35 BC-18663.79 $A^2BC$ -26790.79 $AB^2C$+41523.11 $ABC^2$ |

The quartic mixture model represent the quantitative effect of Water (A), Oil (B) and ratio of surfactant: Co-surfactant (C) and their interaction on Refractive index (Y1), Globule size (Y2), Gel strength (Y3), Spreadability (Y4), Mucoadhesive force (Y5) and Viscosity (Y6). The values of the coefficient A, B and C are related to the effect of these variables on the responses Y1 to Y6. 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 mixture 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 (DX9).

Refractive index of erythromycin nanogels was found to be in the range of 1.371– 1.395 as shown in Table 2. The factorial equation for refractive index exhibited a good correlation coefficient (1.000) and the Model F-value of 771.58 implies the model is significant. There is only a 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 are significant model terms. All the three variables having the positive effect on the refractive index, which means these factors, are directly proportional to the response. The influence of the main and interactive effects of independent variables on the refractive index was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on particle size are as shown in Figure 2. It is found that all the variables are having interactive effects for the response Y1. The trace graph (piepel), 2D contour, 2D real contour and 3D response surface plots of the response Y1 are shown in Figure 2, 3, 4 and 5 to depict the interactive effects of independent variables on response Y1.

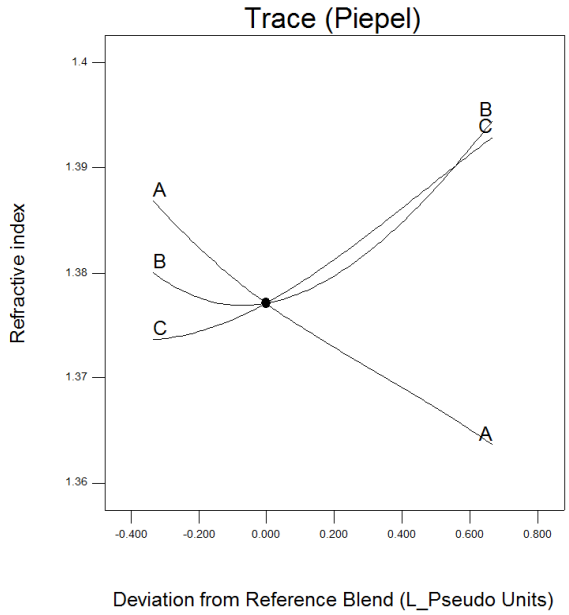


**Figure 2: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on refractive index.**

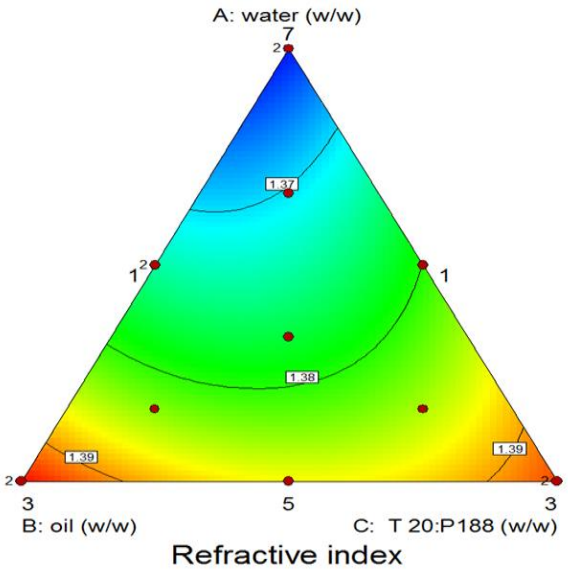


**Figure 3: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

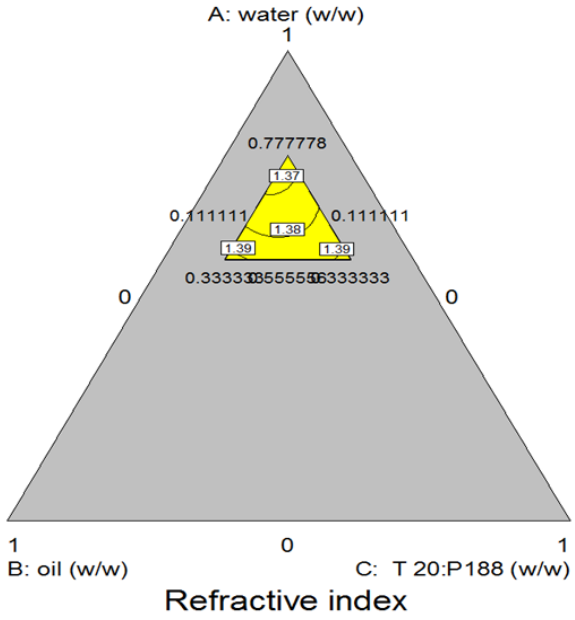


**Figure 4: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

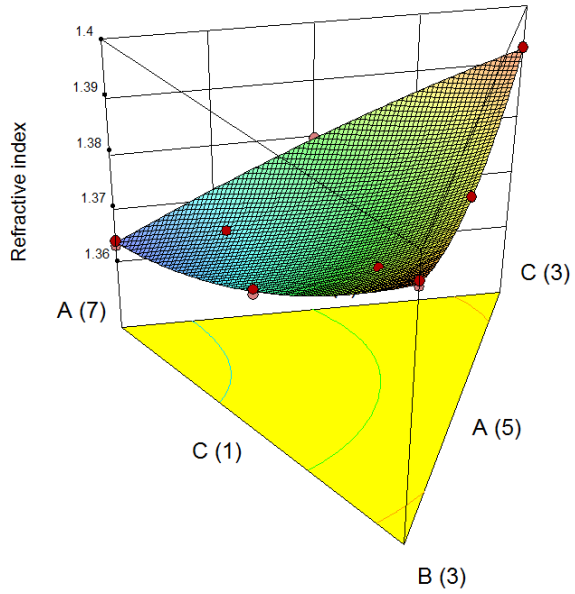


**Figure 5: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

The independent variables A, B, C and the quadratic term of A, B and C have significant effects on the globule size, since the Model F-value of 12.46 implies the model is significant. There is only a 0.65% 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, $A^2BC$ are significant model terms. It was found that all the variables are having interactive effects for the response Y2. The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response Y2 were shown in figure 6, 7, 8 and 9 to depict the interactive effects of independent variables on globule size. During erythromycin nanogels preparation, we sonicated the emulsion for 5 min using a probe sonicator set at 50 Amplitude power. As a result, we achieved a size under 250 nm globule sizes shown in figure 10.

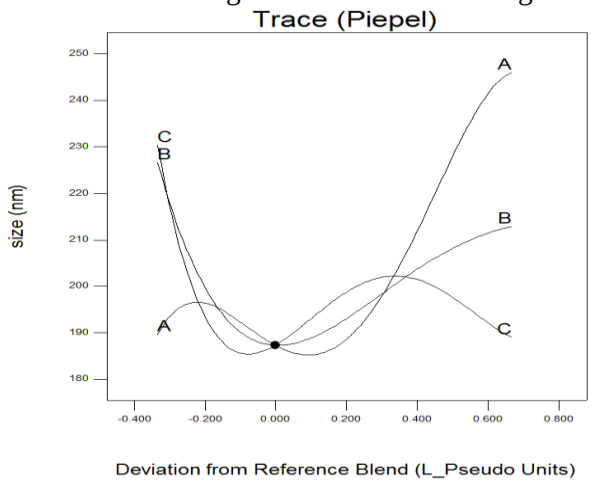


**Figure 6: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on refractive index.**

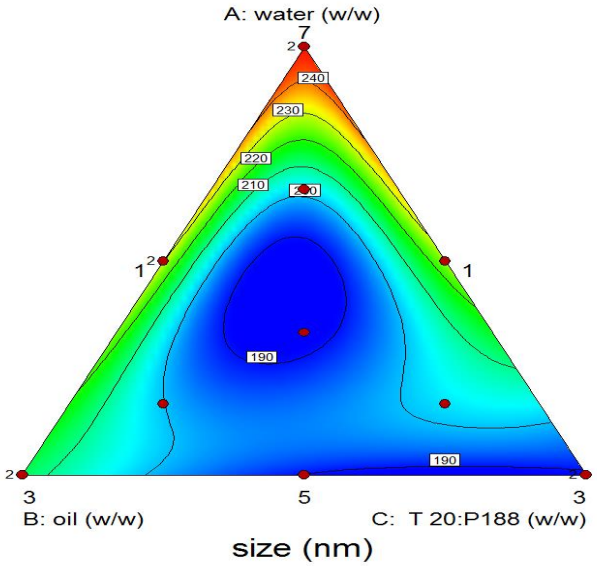


**Figure 7: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

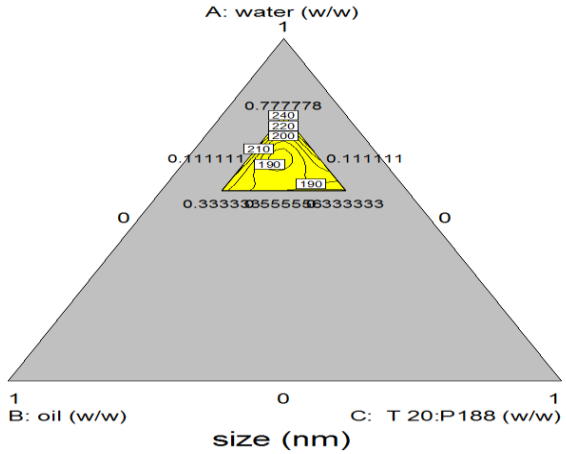


**Figure 8: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

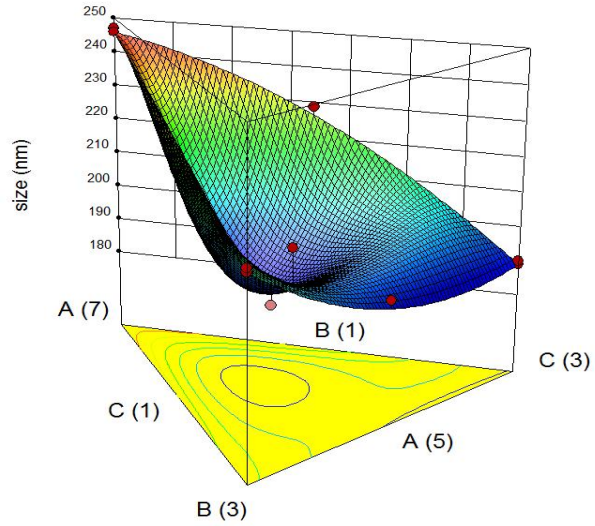


**Figure 9: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the refractive index.**

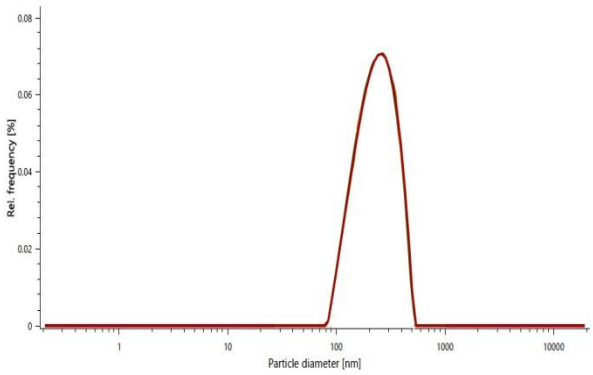


**Figure 10: Particle size analyzer**

Gel strength of erythromycin nanogels was found to be in the range of 9-120 seconds as shown in Table 2. The factorial equation for response Y3 exhibited a correlation coefficient (1.000) and the Model F-value of 5.47 implies the model is significant. There is only a 1.76% chance that an F-value .Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case C is a significant model term. The two variables were having the positive effect on the gel strength, which means these factors, were 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 trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response Y3 were shown in figure 11, 12, 13 and 14 to depict the interactive effects of independent variables on response Y3.

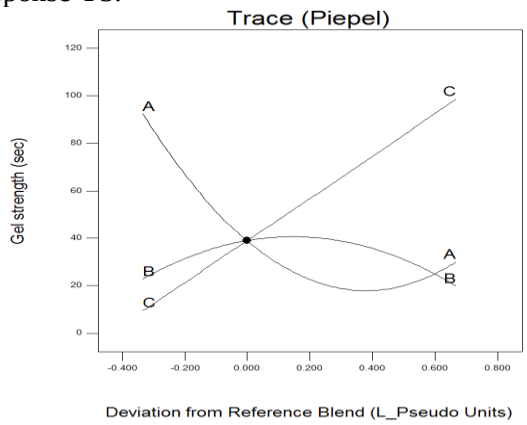


**Figure 11: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on gel strength.**

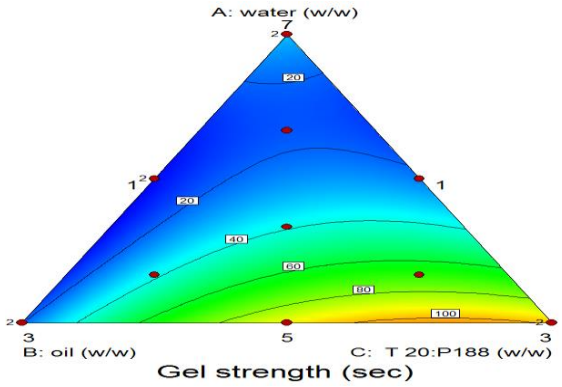


**Figure 12: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the gel strength.**

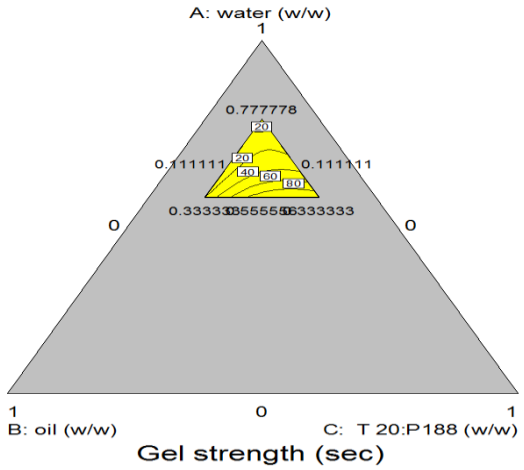


**Figure 13: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the gel strength.**

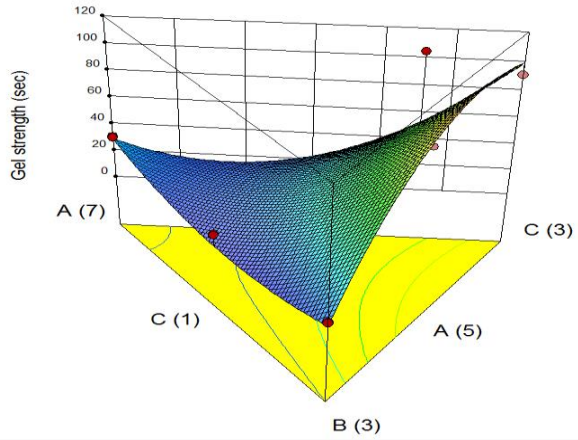


**Figure 14: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the gel strength**.

The response of Y4 was found to be in the range of 3.61- 64.64 gm.cm/sec as shown in Table 2. Results of the equation indicate that the effect of A, B and C is more significant. The Model F-value of 41.86 implies the model is significant. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AB, AC, BC, $A^2BC$ are significant model terms. The influence of the main and interactive effects of independent variables on the spreadability was further elucidated using the perturbation and 3D response surface plots. The trace graph (piepel), 2D contour, 2D real contour and 3D response surface plot showing the main effects of A, B and C on the response Y4 in figure 15, 16, 17 and 18.

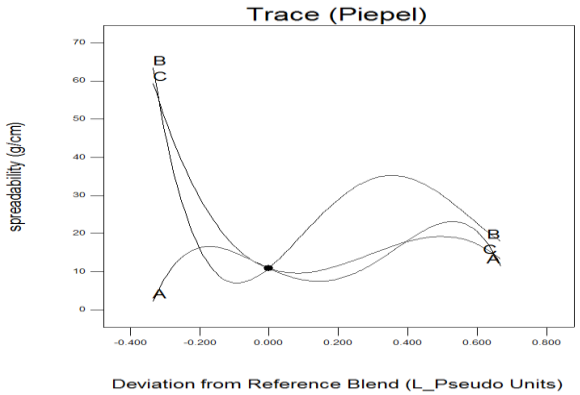


**Figure 15: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on spreadability.**

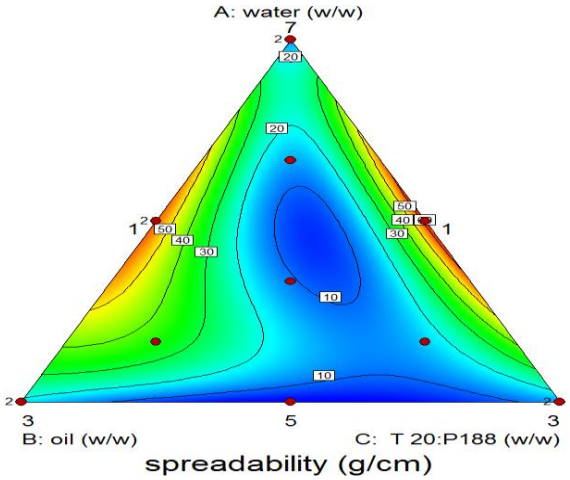


**Figure 16: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the spreadability.**

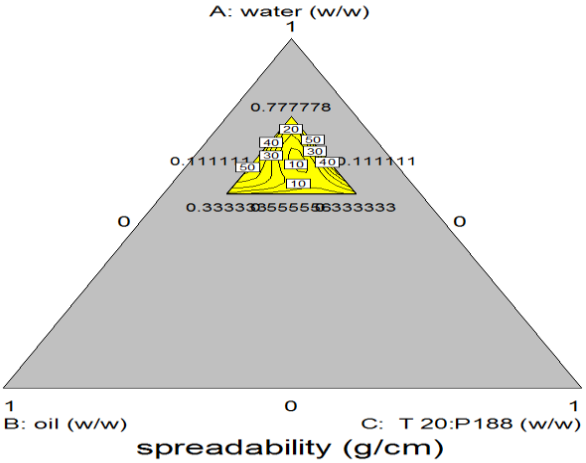


**Figure 17: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the spreadability.**

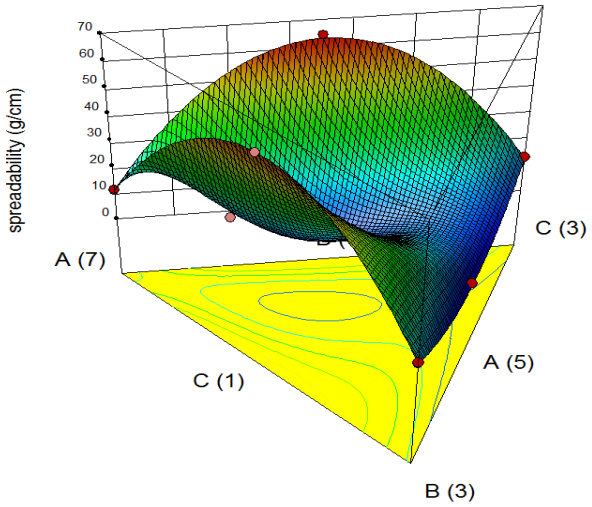


**Figure 18: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the spreadability.**

The mathematical model generated for response Y5 was found to be significant with F-value of 68.84 ($p < 0.0001$). In this case A, B, C, AB, BC, $A^2BC$, $AB^2C$, $ABC^2$ were significant model terms. The P values less than 0.0500 represented the significant model terms as shown in Table 2. It was found that all the variables were having interactive effects for the response Y5. The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response (viscosity) were shown in figure 19, 20, 21 and 22 to depict the interactive effects of independent variables on mucoadhesive force.

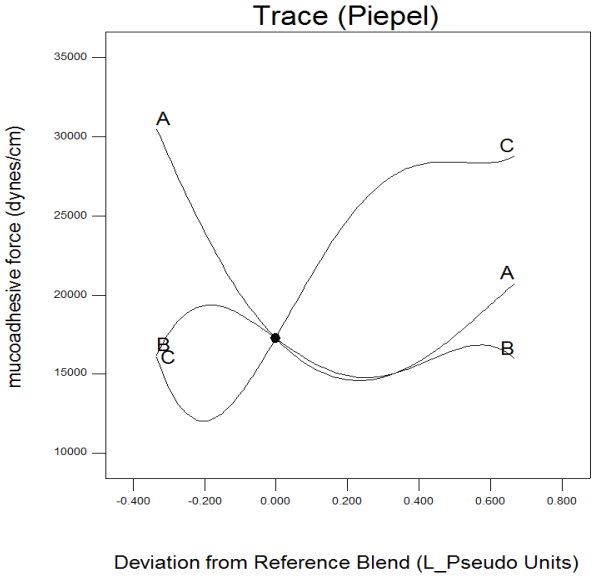


**Figure 19: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on mucoadhesive force.**

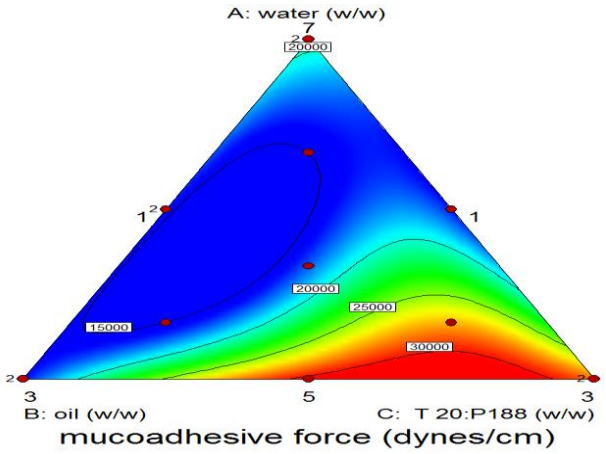


**Figure 20: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the mucoadhesive force.**

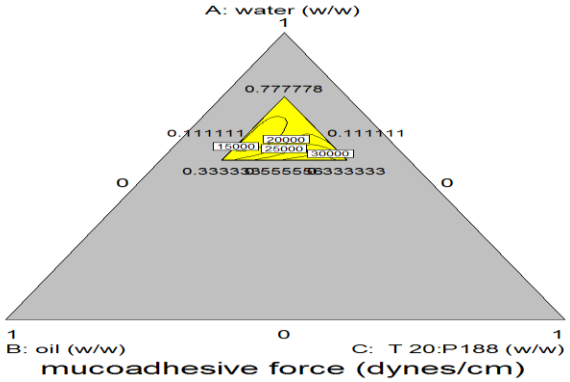


**Figure 21: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the mucoadhesive force.**

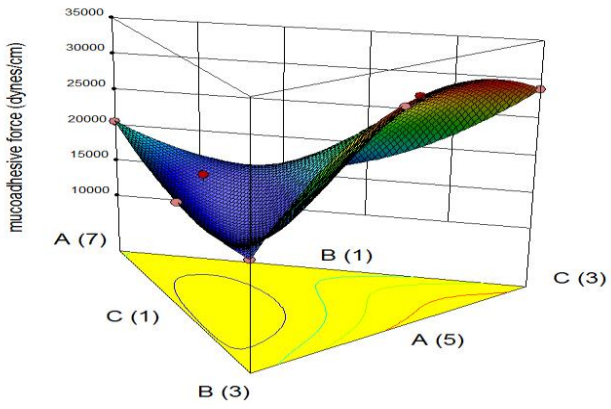


**Figure 22: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the mucoadhesive force.**

Viscosity of nanogels was found to be in the range

of 15.3-809cps as shown in Table 2.The factorial equation for viscosity exhibited a good correlation coefficient (1.000) and the Model F value of 6.92 which implied the model were significant. Values of "Prob> F" less than 0.0500 indicate model terms were significant. In this case C, $ABC^2$ are significant model terms. The trace graph (piepel), 2D contour, 2D real contour and 3D response surface of the response (viscosity) were shown in figure 19, 20, 21 and 22 to depict the interactive effects of independent variables on mucoadhesive force.

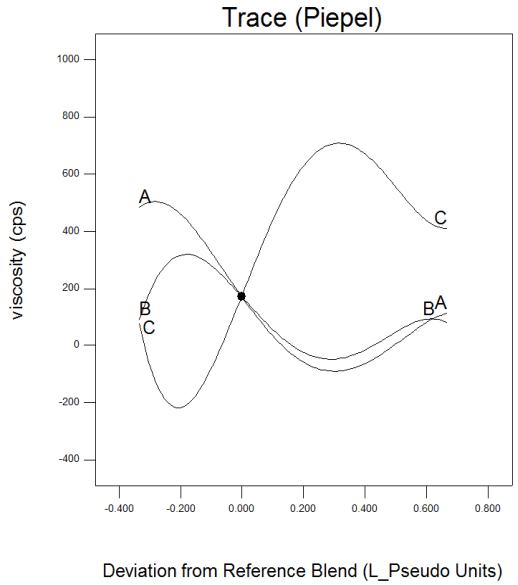


**Figure 19: Trace graph (piepel) shows the main effect of water (A), oil (B) and surfactant: Co-surfactant ratio (C) on viscosity.**

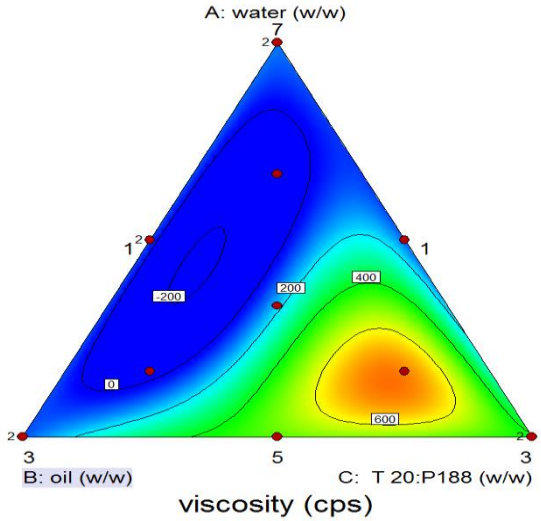


**Figure 20: Response 2D contour plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the viscosity.**

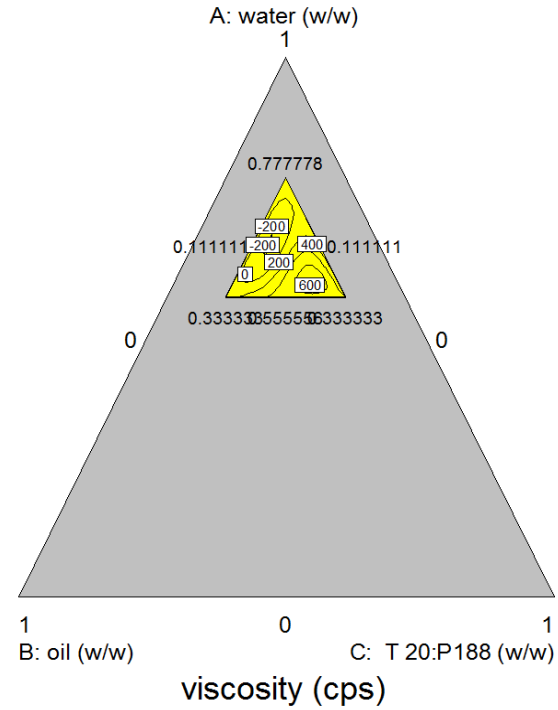


**Figure 21: Real contour graph represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the viscosity.**

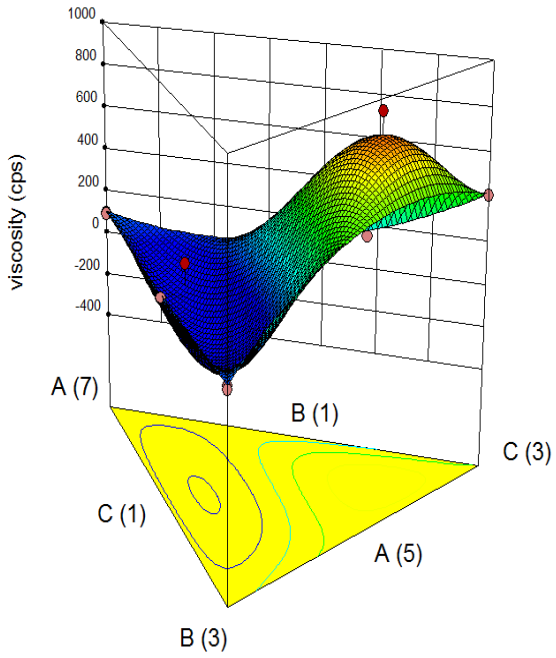


**Figure 22: 3D surface plot represents the interaction between the water, oil and surfactant: Co-surfactant ratio affecting the viscosity.**

Rapports De Pharmacie Vol.3 (2), 2017, 371-383
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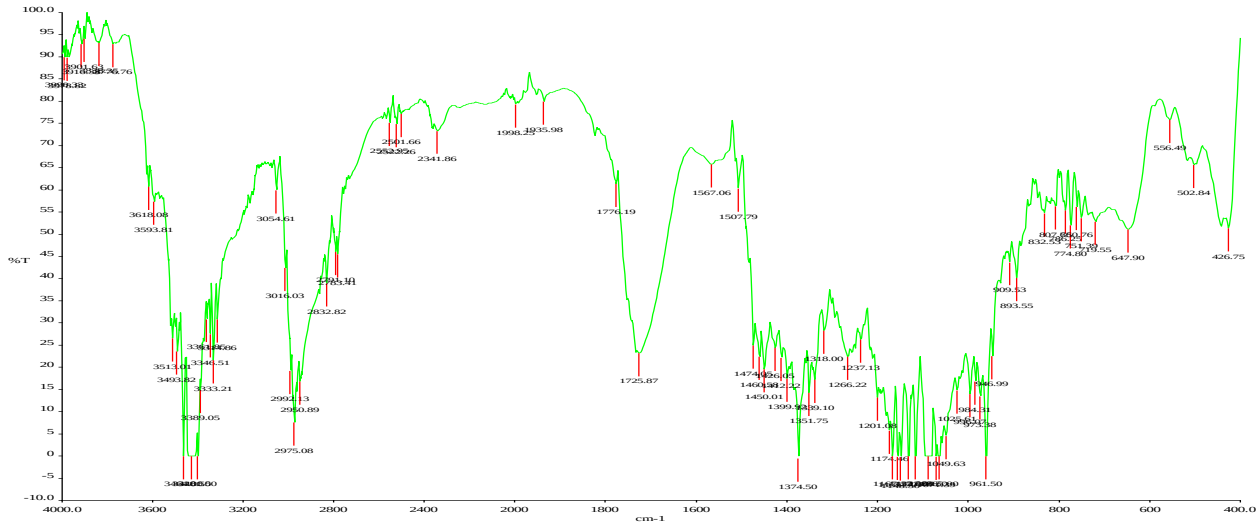


**Figure 23: Shows FTIR spectra of erythromycin**

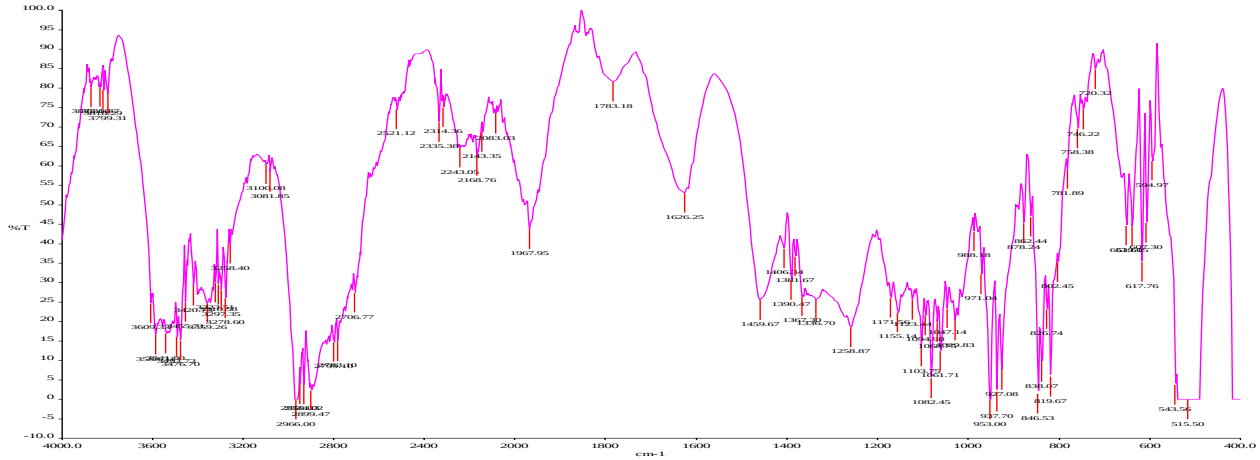


**Figure 24: Shows FTIR spectra of poloxamer 188**

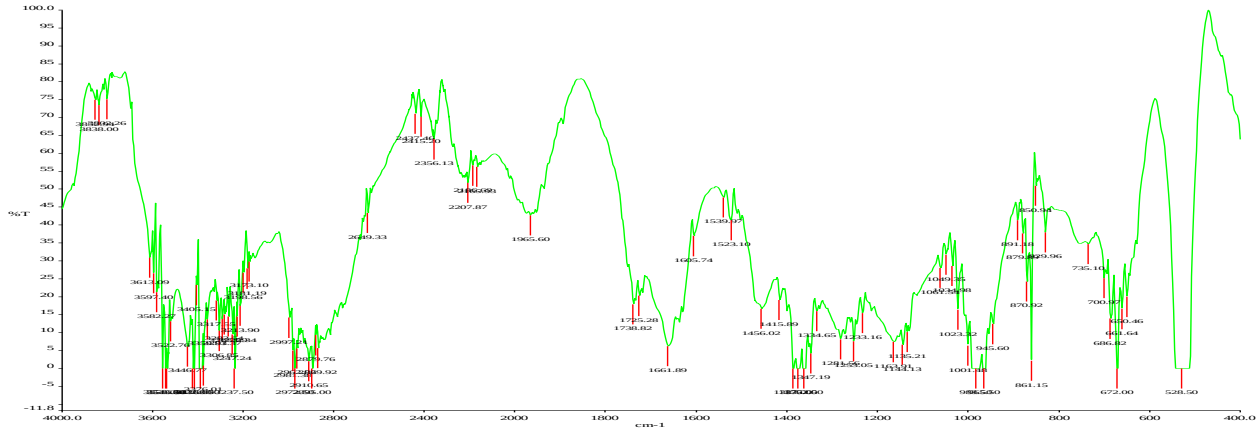


**Figure 25: Shows FTIR spectra of erythromycin and poloxamer 188**

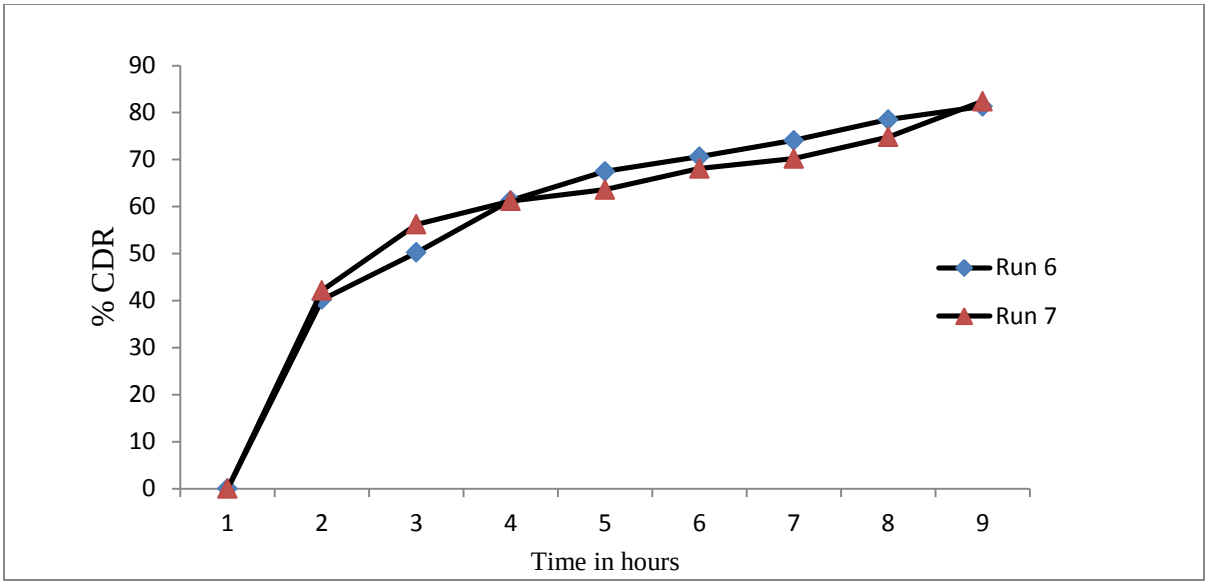


**Figure-26: Showing the percentage of drug release**

As depicted in Figure 23, 24 and 25 the FT-IR spectra of erythromycin revealed high intensity broad bands at approximately 2975.08, 2341.86, 1998.25 and 1725.87 cm−1. These peaks have also been observed in a physical mixture of drug and poloxamer 188 peaks at 2972.02, 2356.13, 1965.60 and 1725.28 cm−1.

Run 6 and Run 7 were selected according to spreadability values. The in vitro release profile of nanogels in PBS (pH = 6.8) is shown in Fig. 26. Drug release at the end of 8 hours (approximately 81.3 and 82.4 %)

**CONCLUSION**

Erythromycin nanogels were prepared by using probe sonicator. The application of factorial design gave a statistically systematic approach for the formulation of nanogels with ideal particle size. Oil phase, water phase and surfactant: Cosurfactant ratios were found to influence the globule size, refractive index, gel strength, spreadability, mucoadhesive force and viscosity of erythromycin loaded nanogels. In vitro drug release study of selected factorial formulations (run 6 and run 7) showed 81.3 % and 82.4% release respectively at the end of 8 hours. The overall results suggest that erythromycin loaded nanogels could be a potential option for topical delivery.

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