**Research article**

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# OPTIMIZATION AND CHARACTERIZATION OF OXYMETAZOLINE BASED THERMOSENSITIVE IN SITU GEL FOR NASAL DRUG DELIVERY BY USING STAT-EASE DESIGN-EXPERT SOFTWARE

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## ABSTRACT

This work was focused on the optimization and characterization of temperature reverse *in situ* gels based on poloxamer 188, a thermosensitive polymer, and hydroxypropylmethylcellulose (HPMC), a bioadhesive polymer, planned for the nasal delivery of oxymetazoline hydrochloride. Seventeen runs of temperature reverse *in situ* gels s were composed of poloxamer 188 and HPMC. A 3-factor, 4-level Box-Behnken design was used to optimize the process parameters including Poloxamer 188 (A), HPMC (B) and Distilled water (C). Four dependent variables gel strength, mucoadhesive force, gelation time and gelation temperature was measured as responses. Gel strength of *in situ* gel was found to be in the range of 75-120 seconds. The mathematical model generated for mucoadhesive force was found to be significant with F-value of 362.28 ($p < 0.0001$) and $R^2$ value of 0.9979. The gelation time was found to be significant with F-value of 80.29 implies the model is significant. The interactive effect of A and B on the gelation temperature (GT) at fixed level of C. At low levels of A (P188), GT increased from 43.1 to 44.2 ºC. Similarly, at high levels of A, Gelation temperature increases from 54.1 to 56.3 ºC, but moderate level of A, the system gel at body temperature.

**Keywords:** Thermosensitive *in situ* gel, HPLC, Rabbit Model, Box-Behnken design

## INTRODUCTION

Oxymetazoline hydrochloride (6-tert-Butyl-3-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2,4-dimethylphenol hydrochloride) is an imidazoline derivative sympathomimetic amine. Oxymetazoline (OXY) is a vasoconstrictor that acts directly on nasal membranes and has been available as a over-the-counter intranasal drug in the United States for more than 40 years. It is approved for the relief of nasal congestion as a result of common colds and allergic rhinitis. Excessive doses of nonprescription oxymetazoline nasal products have been associated with cardiovascular and/or central nervous system adverse events, indicating systemic absorption from the nasal mucosa. [1-3].

Allergic rhinitis (AR), also known as hyper aesthetic rhinitis, is an allergic inflammatory disease that is caused by a variety of allergens. Allergic rhinitis is an allergic reaction that frequently appears in nasal passages during the spring, autumn and winter [4]. The clinical symptoms of allergic rhinitis are unlike in various people, but the foremost symptoms are nasal itching, sneezing and a runny, blocked nose. The intranasal administration of drugs has long been used for the treatment of rhinitis and nasal congestion. Intranasal administration can overcome the side effects that happen in the gastrointestinal tract and the hepatic first-pass effect. Furthermore, drugs are absorbed better, because of the abundant blood and lymphatic capillaries under the nasal mucosa. Since these properties, intranasal administration can effectively enhance the bioavailability of drugs. Intranasal administration has been stated to reach comparable blood concentrations as intravenous administration [5]. Most of the commercially available nasal preparations are now sprays. The scavenging effect of nasal cilia leads to a very short drug residence time on the human nasal mucosal surface (only 15–30 min) [6], which affects the clinical efficacy to some extent.

The *in situ* gel is made of polymeric materials that have a solution state that responds to external stimuli at the administration spot. These formulations also have conformations that can undergo reversible conversion to form a semisolid [7]. *In situ* gel types include thermo-sensitive, ion-activated and pH responsive. The most extensive and mature type of *in situ* gel currently used in research is the gel that reacts to changes in temperature, called a "temperature-sensitive *in situ* gel". A temperature-sensitive *in situ* gel is liquid or semisolid at room temperature and congeals into gel as the temperature increases from room temperature to body temperature, which results in good adhesion and slow release effects [8,9]. The polymers under thermo-sensitive include chitosan, pluronics, tetronics, xyloglucans, and

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hydroxypropylmethyl cellulose [10-12]. In most formulations, the nasal drops are frequent administration (2-3 drops, every 6 to 12 hours) with less nasal residence time. The objective of present studies a prolong release of drug at the site of action reducing the dosing frequency of drug [13-15].

**MATERIALS**

Oxymetazoline hydrochloride were gifted sample from Kotra Pharma (M) Sdn Bhd. Poloxamer 188 were purchased Merck (Germany). Hydroxypropylmethyl cellulose procured from Sigma Aldrich USA. All the chemicals were used as HPLC grade.

**PREPARATION OF OXYMETAZOLINE *IN SITU* GEL**

Oxymetazoline hydrochloride *in situ* gel was prepared by the cold method [16]. Specified amount of poloxamer 188 (P188) and hydroxypropylmethyl cellulose were stirred in the calculated amount of cold distilled water. The solution was cooled to 4°C by keeping it in a refrigerator for overnight. Equivalent to 0.05% w/v of oxymetazoline hydrochloride was added slowly in polymeric solution with continuous stirring. The polymeric solution was stored in amber bottle in a refrigerator for overnight to become clear solution. Finally the pH can be adjusted if desired.

**EVALUTION OF THERMOSENSITIVE IN SITU GEL**

*Gelation temperature:*

The gelation temperature was determined by heating the solution (2 ºC per minute) in a clear test tube with mild stirring until gel was formed. The temperature was recorded when there was no flow after container has overturned [17].

*Determination of mucoadhesive force:*

The mucoadhesive force has been derived from a previously published method [18,19]. A section of sheep nasal mucosa was cut from the slaughter house and instantly fixed with mucosal side out onto each glass vial using rubber band. The vial with nasal mucosa was connected to the balance in inverted position while first vial was placed on a height adjustable pan. The formulations were added onto the mucosa of first vial. Then the height of second vial was so adjusted that the mucosal surfaces of both vials come in intimate contact. Two minutes time of contact was given to the vials. Then, the switch of the infusion apparatus was opened to make the water drop into the glass vial with a constant flow rate of 5 mL/min. The weight of the water in the glass vial kept increasing until the gel and the mucosal tissue were detached. Mucoadhesive force, the detachment stress (dyne/$cm^2$), was determined from the minimal weights that detached the gel. The sheep nasal mucosa pieces were changed for each measurement.

*Determination of gel strength:*

Gel strength was measured by placing 50 g of formulation in a 100 ml graduated cylinder and gelled at 37°C using thermostat. A piston of weight 27 g was placed onto the gelled solution and allowed to penetrate 5 cm in the gel. Time taken by weight to sink 5 cm was measured [20].

*In vitro permeation studies:*

The in vitro permeation study was performed using nasal mucosa collected from slaughter house. The nasal conch was collected in phosphate buffer pH 6.4 and washed three times with phosphate buffer pH 6.4 and extraneous tissues were removed [21]. The prepared nasal mucosa was mounted on Franz diffusion cell to get a permeation area of 3.14 $cm^2$. Sixteen milliliters of phosphate buffer pH 6.4 was added to the acceptor chamber maintained at 37°C. Formulation equivalent to 0.05% w/v of oxymetazoline hydrochloride was placed in the donor chamber. At predetermined time points (0, 0.15,0.30, 1, 2, 3, 4, 5, 6, 8, 16 and 24 hours), 2-ml sample was withdrawn from the acceptor compartment and replaced with an equal volume of the phosphate buffer pH 6.4. The sample was then filtered and drug release was determined by RP HPLC method. RP HPLC chromatographic separation was performed on a Shimadzu liquid chromatographic system equipped with a LC- 20AD solvent delivery system (pump), SPD-20A photo diode array detector, and SIL-20ACHT injector with 50µL loop volume. The LC solution version 1.25 was used for data collecting and processing (Shimadzu, Japan). The HPLC was carried out at a flow rate of 1.0 ml/min using a mobile that is phase constituted of ACN: water (pH 4.5) (75:25, v/v), and detection was made at 270 nm. 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 most widely used method for formulation of the oxymetazoline hydrochloride *in situ* gel the by solvent evaporation method. In this study, we report the positive effect on the formulation of oxymetazoline hydrochloride *in situ* gel. Through preliminary experiments the Poloxamer 188 (A), HPMC (B) and Distilled water (C) were identified as the most significant variables influence the gel strength, mucoadhesive force, gelation time and gelation temperature. Seventeen runs were required for the

response surface methodology based on the Box-Behnken design. Based on the experimental design, the factor combinations produced different responses as presented in Table 1. These outcomes obviously specify that all the dependent variables are strongly dependent on the selected independent variables as they show a wide variation among the 17 batches as shown in Table 2. Data were analyzed using Stat-Ease Design-Expert software (DX9) to obtain analysis of variance (ANOVA), regression coefficients and regression equation as presented in Table 3. These equations represent the quantitative effect of Poloxamer 188 (A), HPMC (B) and distilled water (C) and their interaction on gel strength, mucoadhesive force, gelation time and gelation temperature. The values of the coefficient A, B and C are related to the effect of these variables on the responses R1, R2, R3 and R4. 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.

Gel strength of oxymetazoline *in situ* gel was found to be in the range of 75-120 seconds as shown in Table 2.The factorial equation for gel strength exhibited a good correlation coefficient (1.000) and the Model F value of 29.86 which implies the model is significant. Values of "Prob> F" less than 0.0500 indicate model terms are significant. In this case A, C and $A^2$ are significant model terms as shown in Table 4. Results of the equation indicate that the effect of A (P188) is more significant than B and C. All the three variables having the positive effect on the gel strength, which means these factors, are directly proportional to the response. The influence of the main and interactive effects of independent variables on the gel strength was further elucidated using the perturbation and 3D response surface plots. The individual main effects of A, B and C on gel strength are as shown in Figure 1. It is found that all the variables are having interactive effects for the response R1. The 2D contour plots and the 3D response surfaces of the response R1 are shown in Figure 2 & 3 to depict the interactive effects of independent variables on response R1, one variable was kept constant while the other two variables varied in a certain range. The shapes of response surfaces and contour plots reveal the nature and extent of the interaction between different factors. The interaction between A and B on gel strength at a fixed level of C. At low levels of A, R1 range from 70 to 80 seconds. Similarly at high levels of A, R1 increased from 110 to 120 seconds. The "Lack of Fit F-value" of 135.42 implies the Lack of Fit is significant. There is only a 0.02% chance that a "Lack of Fit F-value" this large could occur due to noise.

**Table-1: List of Independent variable and Dependent variables in Box-Behnken design**

| **Independent variable** | | | **Levels** | | |
|---|---|---|---|---|---|
| **Variable** | Name | Units | Low | Middle | High |
| A | Poloxamer 188 | gm | 15 | 17.5 | 20 |
| B | HPMC | gm | 0.01 | 0.03 | 0.05 |
| C | Distilled water | mL | 10 | 30 | 50 |
| **Dependent variable** | | | | **Goal** | |
| R1 | Gel Strength | Seconds | | Maximum | |
| R2 | Mucoadhesive Force | Dynes/$cm^2$ | | Maximum | |
| R3 | Gelation Time | Seconds | | Rapid | |
| R4 | Gelation Temperature | Celsius | | Near 37ºC. | |

**Table-2: Factorial design of oxymetazoline hydrochloride *in situ* gel**

| Std | Run | Factor 1 A:P188 gm | Factor 2 B:HPMC gm | Factor 3 C:Distilled w... mL | Response 1 Gel strength Seconds | Response 2 Mucoadhesi... dynes/cm2 | Response 3 Gelation time Seconds | Response 4 Gelation tem... Celsius |
|---|---|---|---|---|---|---|---|---|
| 3 | 1 | 15 | 0.05 | 30 | 75 | 27.45 | 90 | 43.5 |
| 16 | 2 | 17.5 | 0.03 | 30 | 85 | 30.14 | 26 | 34.1 |
| 5 | 3 | 15 | 0.03 | 10 | 80 | 26.33 | 88 | 43.1 |
| 1 | 4 | 15 | 0.01 | 30 | 72 | 24.13 | 91 | 45.3 |
| 8 | 5 | 20 | 0.03 | 50 | 101 | 33.41 | 110 | 54.1 |
| 7 | 6 | 15 | 0.03 | 50 | 70 | 25.11 | 98 | 44.2 |
| 14 | 7 | 17.5 | 0.03 | 30 | 86 | 30.16 | 24 | 33.7 |
| 13 | 8 | 17.5 | 0.03 | 30 | 85 | 30.15 | 25 | 33.1 |
| 4 | 9 | 20 | 0.05 | 30 | 112 | 33.87 | 120 | 56.2 |
| 2 | 10 | 20 | 0.01 | 30 | 110 | 33.1 | 115 | 55.2 |
| 9 | 11 | 17.5 | 0.01 | 10 | 83 | 27.01 | 28 | 33.5 |
| 12 | 12 | 17.5 | 0.05 | 50 | 82 | 29.41 | 48 | 35.8 |
| 10 | 13 | 17.5 | 0.05 | 10 | 84 | 31.12 | 27 | 32.2 |
| 15 | 14 | 17.5 | 0.03 | 30 | 85 | 30.16 | 25 | 33.1 |
| 6 | 15 | 20 | 0.03 | 10 | 120 | 33.22 | 126 | 56.3 |
| 11 | 16 | 17.5 | 0.01 | 50 | 81 | 29.17 | 45 | 36.1 |
| 17 | 17 | 17.5 | 0.03 | 30 | 85 | 30.15 | 25 | 33.3 |

**Table-3: Regression equation for the response**

| Response | Regression equation |
|---|---|
| Gel strength | = +85.20+18.25* A+0.88* B-4.13* C-0.25* AB-2.25* AC+6.280* BC+8.65* $A^2$-1.60* $B^2$-1.10* $C^2$ |
| Mucoadhesive Force | = +30.15+3.82* A+1.06* B-0.073* C-0.64* AB+0.35* AC-0.97* BC-0.087* $A^2$-0.43* $B^2$-0.55* $C^2$ |
| Gelation time | = +25.00+13.00* A+0.75* B+4.00* C+1.50* AB-6.50* AC+1.00* BC+73.75* $A^2$+5.25+6.75* $C^2$ |
| Gelation temperature | = +33.46+5.71* A-0.30* B+0.64* C+0.70* AB-0.82* AC+0.25* BC+15.81* $A^2$+0.78* $B^2$+0.16* $C^2$ |

**Table-4: ANOVA results of the quadratic model for the response gel strength (R1)**

| Source variations | Sum of Squares | DF | Mean Square | F Value | p-value Prob> F | $R^2$ |
|---|---|---|---|---|---|---|
| Model | 3149.95 | 9 | 349.99 | 29.86 | < 0.0001 | 0.9746 |
| A-P188 | 2664.50 | 1 | 2664.50 | 227.32 | < 0.0001 | |
| B-HPMC | 6.13 | 1 | 6.13 | 0.52 | 0.4932 | |
| C-Distilled water | 136.13 | 1 | 136.13 | 11.61 | 0.0113 | |
| AB | 0.25 | 1 | 0.25 | 0.021 | 0.8880 | |
| AC | 20.25 | 1 | 20.25 | 1.73 | 0.2301 | |
| BC | 0.000 | 1 | 0.000 | 0.000 | 1.0000 | |
| $A^2$ | 315.04 | 1 | 315.04 | 26.88 | 0.0013 | |
| $B^2$ | 10.78 | 1 | 10.78 | 0.92 | 0.3695 | |
| $C^2$ | 5.09 | 1 | 5.09 | 0.43 | 0.5308 | |
| Residual | 82.05 | 7 | 11.72 | | | |
| Lack of Fit | 81.25 | 3 | 27.08 | 135.42 | 0.0002 | |

**Table-5: ANOVA results of the quadratic model for the response mucoadhesive force (R2)**

| Source variations | Sum of Squares | DF | Mean Square | F Value | p-value Prob> F | $R^2$ |
|---|---|---|---|---|---|---|
| Model | 133.93 | 9 | 14.88 | 362.28 | < 0.0001 | 0.9979 |
| A-P188 | 116.89 | 1 | 116.89 | 2845.77 | < 0.0001 | |
| B-HPMC | 8.90 | 1 | 8.90 | 216.78 | < 0.0001 | |
| C-Distilled water | 0.042 | 1 | 0.042 | 1.02 | 0.3453 | |
| AB | 1.63 | 1 | 1.63 | 39.58 | 0.0004 | |
| AC | 0.50 | 1 | 0.50 | 12.10 | 0.0103 | |
| BC | 3.74 | 1 | 3.74 | 91.15 | < 0.0001 | |
| $A^2$ | 0.032 | 1 | 0.032 | 0.78 | 0.4064 | |
| $B^2$ | 0.77 | 1 | 0.77 | 18.71 | 0.0035 | |
| $C^2$ | 1.26 | 1 | 1.26 | 30.70 | 0.0009 | |
| Residual | 0.29 | 7 | 0.041 | | | |
| Lack of Fit | 0.29 | 3 | 0.096 | 1367.86 | < 0.0001 | |

**Table-6: ANOVA results of the quadratic model for the response gelation time (R3)**

| Source variations | Sum of Squares | DF | Mean Square | F Value | p-value Prob> F | $R^2$ |
|---|---|---|---|---|---|---|
| Model | 25445.38 | 9 | 2827.26 | 80.29 | < 0.0001 | 0.9904 |
| A-P188 | 1352.00 | 1 | 1352.00 | 38.39 | 0.0004 | |
| B-HPMC | 4.50 | 1 | 4.50 | 0.13 | 0.7313 | |
| C-Distilled water | 128.00 | 1 | 128.00 | 3.63 | 0.0983 | |
| AB | 9.00 | 1 | 9.00 | 0.26 | 0.6287 | |
| AC | 169.00 | 1 | 169.00 | 4.80 | 0.0646 | |
| BC | 4.00 | 1 | 4.00 | 0.11 | 0.7460 | |
| $A^2$ | 22901.32 | 1 | 22901.32 | 650.34 | < 0.0001 | |
| $B^2$ | 116.05 | 1 | 116.05 | 3.30 | 0.1123 | |
| $C^2$ | 191.84 | 1 | 191.84 | 5.45 | 0.0523 | |
| Residual | 246.50 | 7 | 35.21 | | | |
| Lack of Fit | 244.50 | 3 | 81.50 | 163.00 | 0.0001 | |

**Table-7: ANOVA results of the quadratic model for the response gelation temperature (R4)**

| Source variations | Sum of Squares | DF | Mean Square | F Value | p-value Prob> F | $R^2$ |
|---|---|---|---|---|---|---|
| Model | 1338.02 | 9 | 148.67 | 138.31 | < 0.0001 | 0.9944 |
| A-P188 | 261.06 | 1 | 261.06 | 242.86 | < 0.0001 | |
| B-HPMC | 0.72 | 1 | 0.72 | 0.67 | 0.4401 | |
| C-Distilled water | 3.25 | 1 | 3.25 | 3.02 | 0.1256 | |
| AB | 1.96 | 1 | 1.96 | 1.82 | 0.2189 | |
| AC | 2.72 | 1 | 2.72 | 2.53 | 0.1555 | |
| BC | 0.25 | 1 | 0.25 | 0.23 | 0.6443 | |
| $A^2$ | 1052.11 | 1 | 1052.11 | 978.78 | < 0.0001 | |
| $B^2$ | 2.58 | 1 | 2.58 | 2.40 | 0.1654 | |
| $C^2$ | 0.10 | 1 | 0.10 | 0.097 | 0.7643 | |
| Residual | 7.52 | 7 | 1.07 | | | |
| Lack of Fit | 6.77 | 3 | 2.26 | 12.01 | 0.0181 | |

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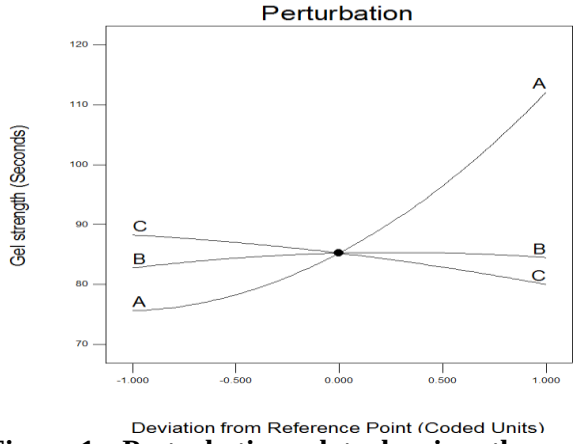


**Figure-1: Perturbation plot showing the main effect of P188 (A), HPMC (B) and Distilled water (C) on gel strength (R1)**

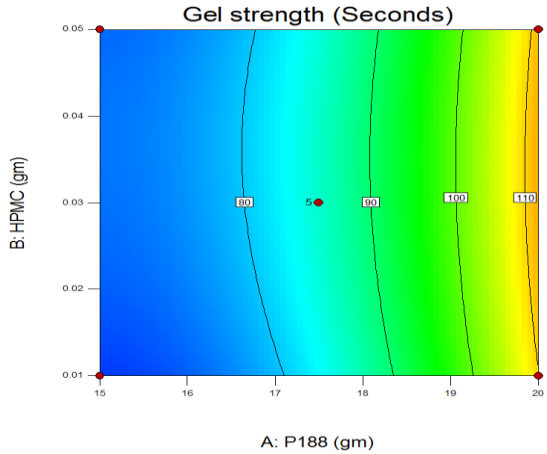


**Figure-2: Contour plot presenting the interaction between the P188 and HPMC affecting the gel strength at constant level of C.**

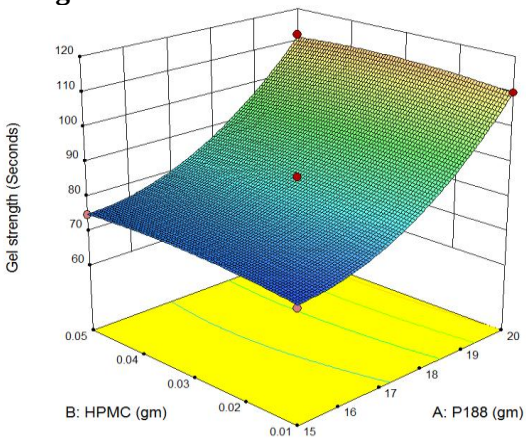


**Figure-3: 3D surface plot presenting the interaction between the P188 and HPMC affecting the gel strength at constant level of C.**

The mathematical model generated for mucoadhesive force (R2) was found to be significant with F-value of 362.28 ($p < 0.0001$) and $R^2$ value of 0.9979. The independent variables A, B, C, AB, AC, BC, $B^2$ and $C^2$ are significant model terms. The P-values less than 0.0500 represent the significant model terms as shown in Table 5. Results of the equation indicate that the effect of A and B is more significant than C. The influence of the main and interactive effects of independent variables on the mucoadhesive force was further elucidated using the perturbation and 3D response surface plots. The perturbation plot (Figure 4) showing the main effects of A, B and C on the mucoadhesive force (R2) of *in situ* gel. This figure clearly shows that A and B has the main and the major effect on R2 followed by C which has a moderate effect on R2. The relationship between the dependent and independent variables was further elucidated using 3D response surface plots & 2D contour plots. Figure 5&6 shows the interactive effect of A and B on the mucoadhesive force (R2) at fixed level of C. At low levels of B (HPMC), R2 decreases from 29.17 to 24.13 dynes/cm$^2$. Inversely, at high levels of B, R2 increases from 27.45 to 33.87 dynes/cm$^2$.

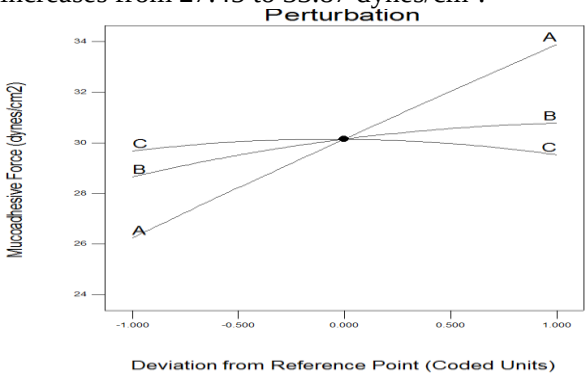


**Figure-4: Perturbation plot showing the main effect of P188 (A), HPMC (B) and Distilled water (C) on mucoadhesive force (R2)**

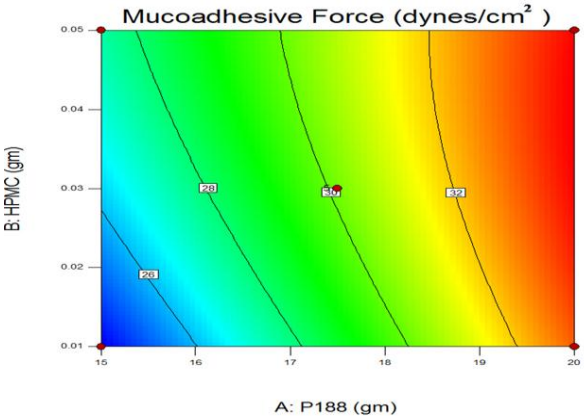


**Figure-5: Contour plot presenting the interaction between the P188 and HPMC affecting the mucoadhesive force at constant level of C.**

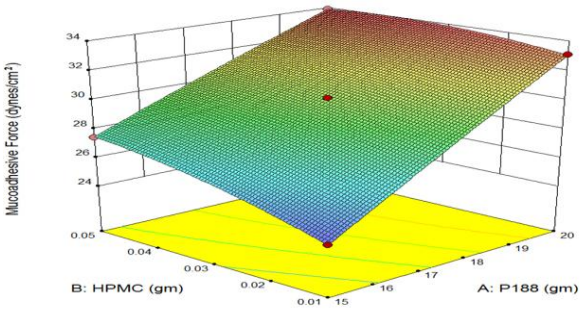


**Figure-6: 3D surface plot presenting the interaction between the P188 and HPMC affecting the mucoadhesive force at constant level of C.**

The gelation time (R3) was found to be significant with F-value of 80.29 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, $A^2$ are significant model terms shown in Table 6. The influence of the main and interactive effects of independent variables on the gelation time was further elucidated using the perturbation and 3D response surface plots. The perturbation plot (Figure 7) showing the main effects of A, B and C on the gelation time (R3) of formulations. The relationship between the dependent and independent variables was further elucidated using response surface plots. Figure 8 & 9 shows the interactive effect of A and B on the gelation time (R3) at fixed level of C. At high levels of A (P188), R3 increases from 120 to 126 seconds. Inversely, at low levels of A, R3 range from 90 to 98 seconds and moderate level of A, all the formulations gel at lesser time (24-28 seconds).

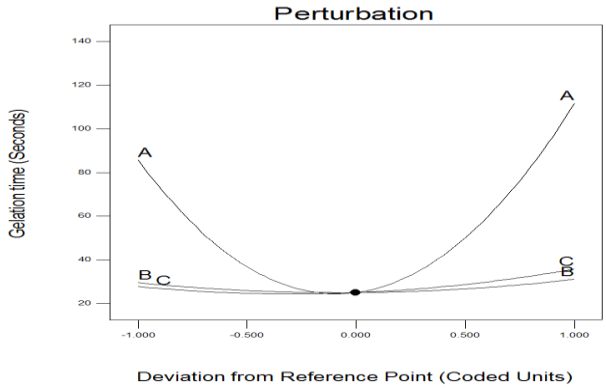


**Figure-7: Perturbation plot showing the main effect of P188 (A), HPMC (B) and Distilled water (C) on gelation time (R3)**

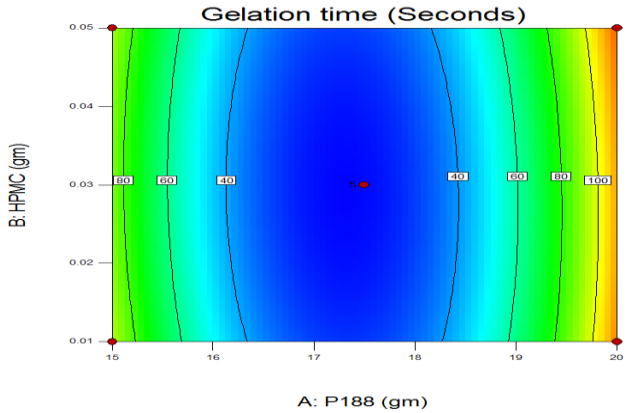


**Figure-8: Contour plot presenting the interaction between the P188 and HPMC affecting the gelation time at constant level of C.**

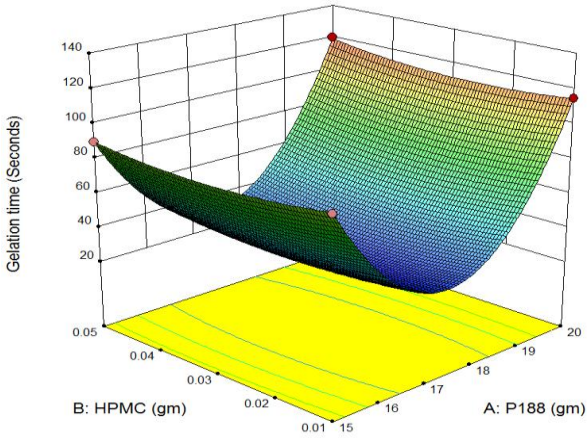


**Figure-9: 3D surface plot presenting the interaction between the P188 and HPMC affecting the gelation time at constant level of C.**

The mathematical model generated for gelation temperature (R4) was found to be significant with F-value of 138.31 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. In this case A, A2 are significant model terms. The P-values less than 0.0500 represent the significant model terms as shown in Table 7. The influence of the main and interactive effects of independent variables on the gelation temperature was further elucidated using the perturbation and 3D response surface plots. The perturbation plot (Figure 10) showing the main effects of A, B and C on the gelation temperature (R4) of *in situ* gel. This figure clearly shows that A has the main and the major effect on R4 followed by B which has a moderate effect on R4. The relationship between the dependent and independent variables was further elucidated using response surface plots. Figure 11 & 12 shows the interactive

effect of A and B on the gelation temperature (R4) at fixed level of C. At low levels of A (P188), R4 range from 43.1 to 44.2 ºC. Inversely, at high levels of A, R4 increases from 54.1 to 56.3 ºC and moderate level of A, all the formulations gel at near to body temperature as shown in figure 12a.

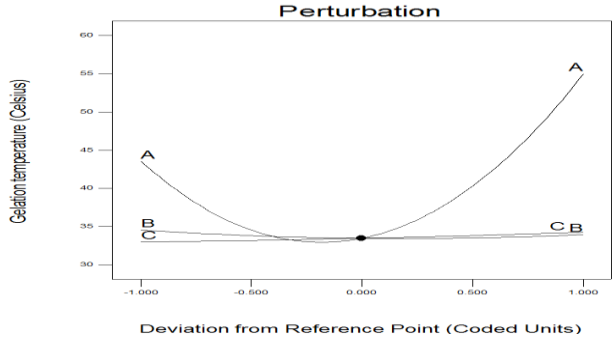


**Figure-10: Perturbation plot showing the main effect of P188 (A), HPMC (B) and Distilled water (C) on gelation temperature (R4)**

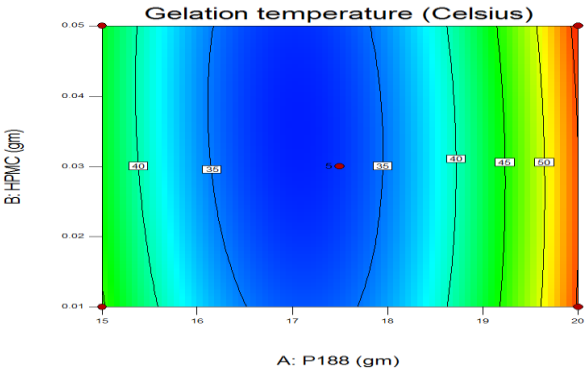


**Figure-11: Contour plot presenting the interaction between the P188 and HPMC affecting the gelation temperature at constant level of C.**

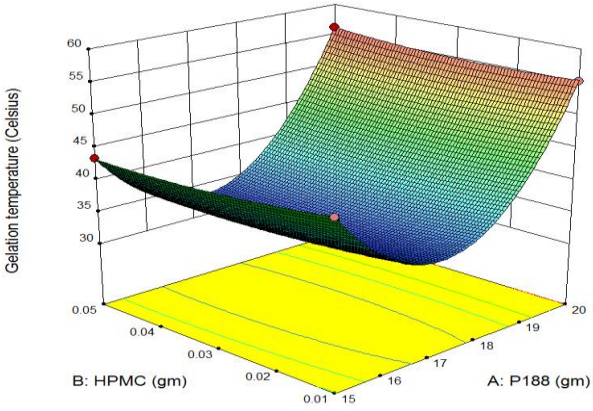


**Figure-12: 3D surface plot presenting the interaction between the P188 and HPMC affecting the gelation temperature at constant level of C.**

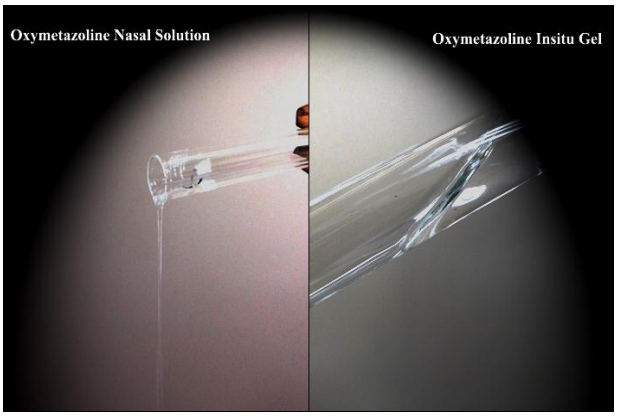


**Figure-12a: Photography shows solution to gel state**

The normality of the data could be proved through the normal % probability plot of the externally studentized residuals. If the points on the plot lie on a straight line, the residuals are normally distributed as confirmed in Figure 13 to 16.

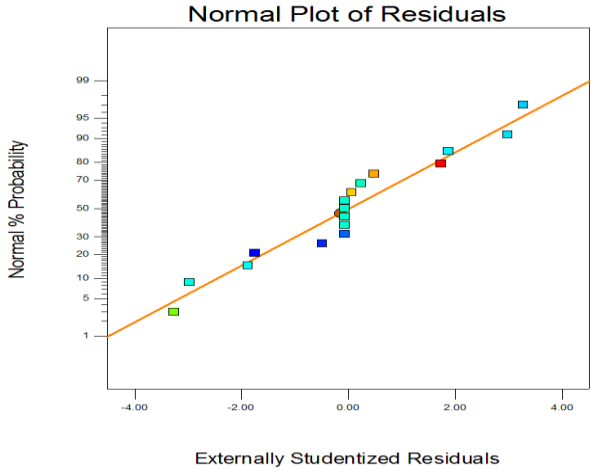


**Figure-13: Normal % probability plot of the externally studentized residuals (R1)**

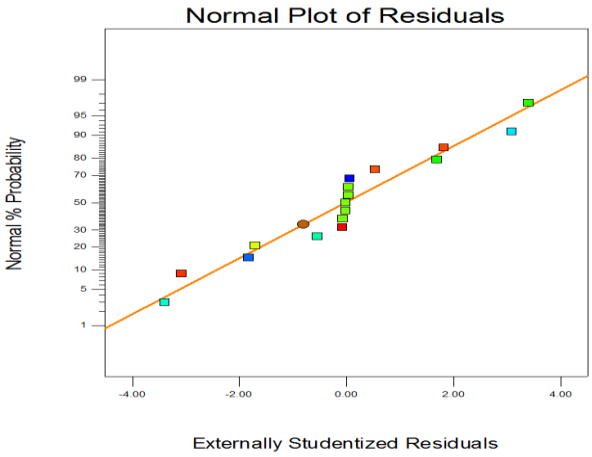


**Figure-14: Normal % probability plot of the externally studentized residuals (R2)**

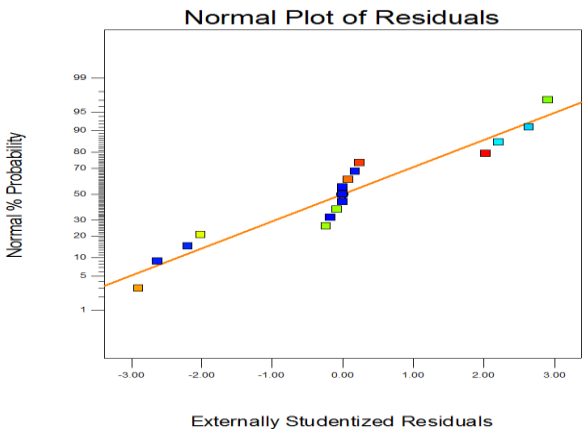


**Figure-15: Normal % probability plot of the externally studentized residuals (R3)**

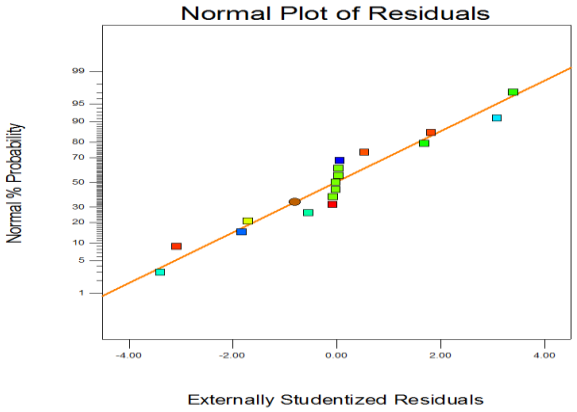


**Figure-16: Normal % probability plot of the externally studentized residuals (R4)**

The assumption of constant variance was tested by plotting externally studentized residual versus predicted values as illustrated in above figures. The studentized residuals are located by dividing the residuals by their standard deviations. According to evident from this Figure 17 to 20, the points are scattered randomly between the outlier detection limits +4.25 to -4.25.

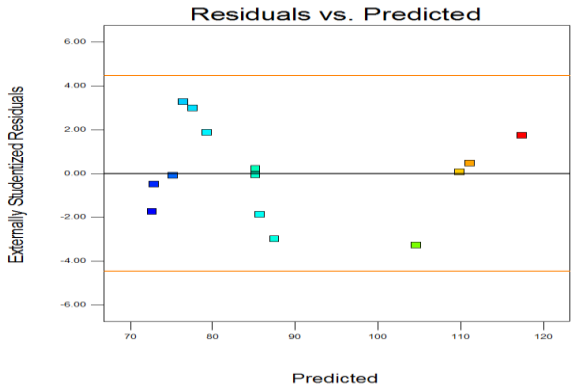


**Figure-17: Residuals vs. Predicted (R1)**

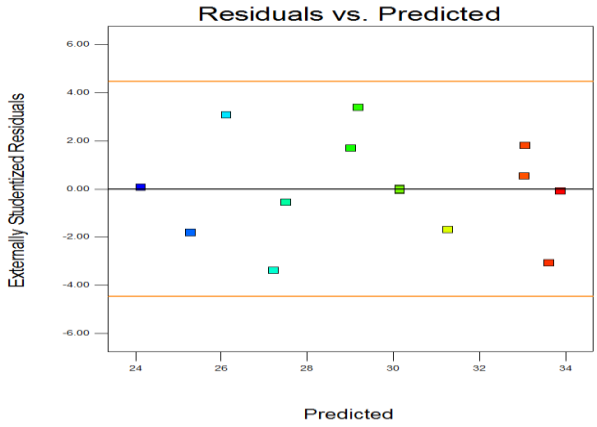


**Figure-18: Residuals vs. Predicted (R2)**

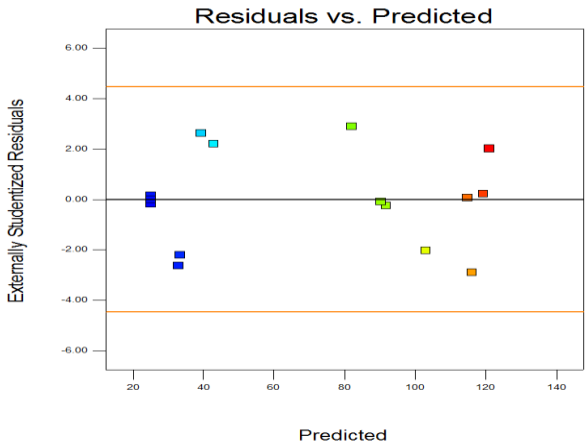


**Figure-19: Residuals vs. Predicted (R3)**

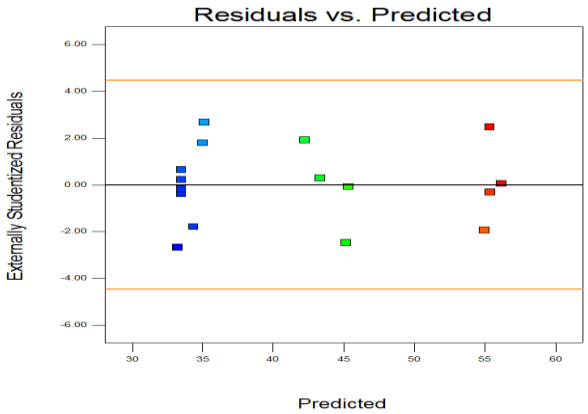


**Figure-20: Residuals vs. Predicted (R4)**

The Residuals vs. Predicted and Residuals vs. Run were scattered randomly as shown in Figure 21 to 28. From the results it can therefore be seen that the model is suitable for use and can be used to identify the optimal parameters.R1, R2, R3 and R4 results are quite satisfactory. Also, a high correlation between observed and predicted data indicates their low discrepancies

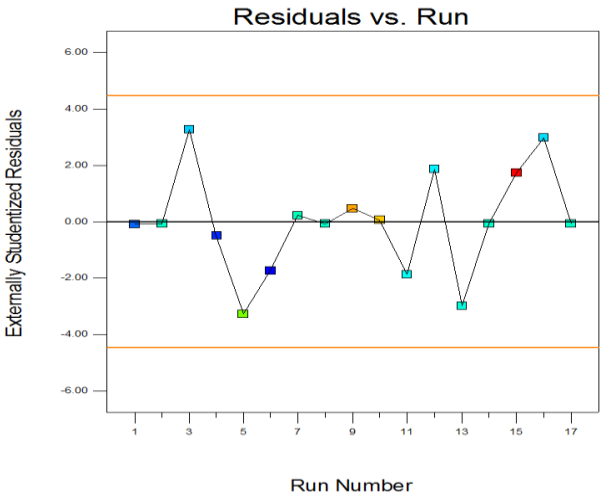


**Figure -21: Residuals vs. Run (R1)**

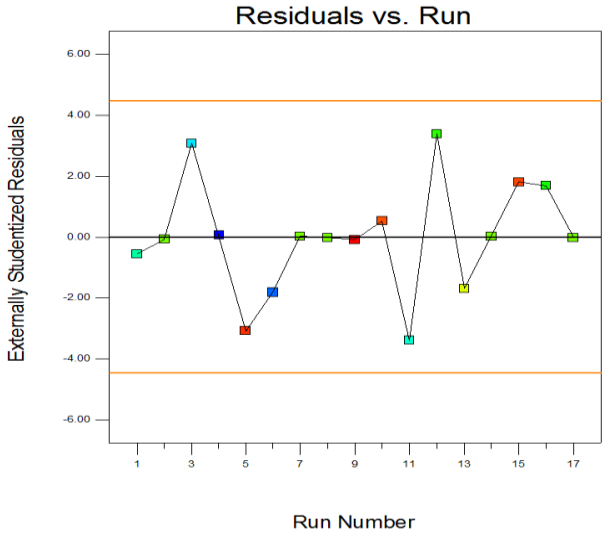


**Figure -22: Residuals vs. Run (R2)**

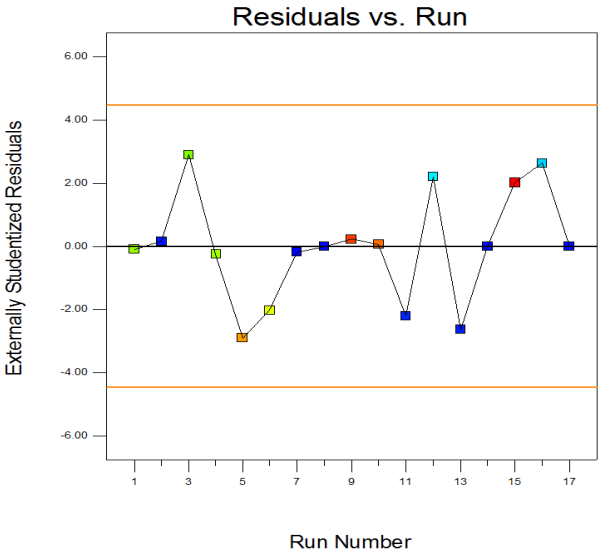


**Figure -23: Residuals vs. Run (R3)**

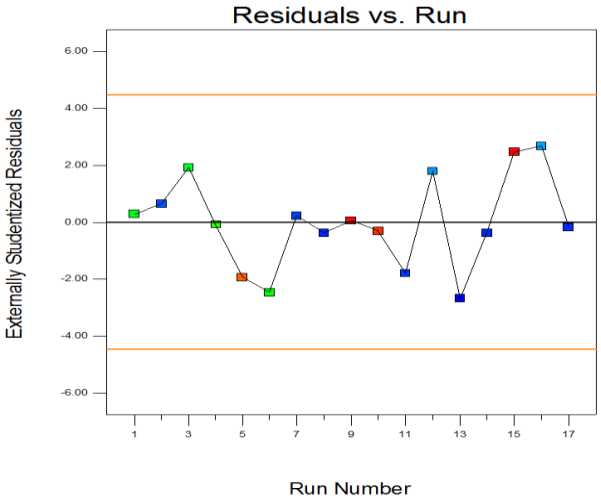


**Figure -24: Residuals vs. Run (R4)**

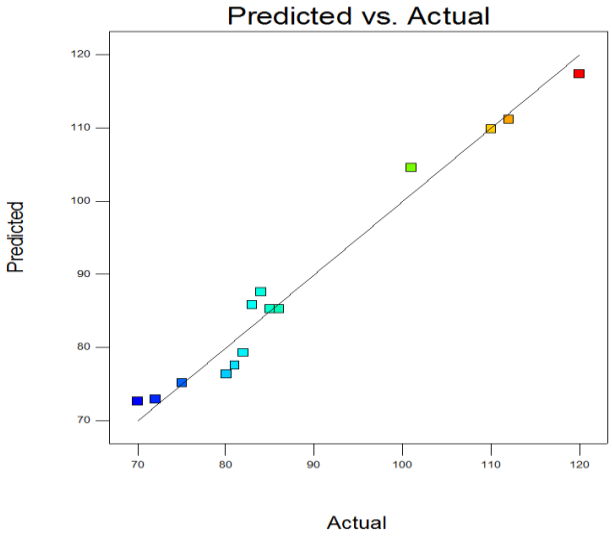


**Figure -25: Actual Response vs. Predicted (R1)**

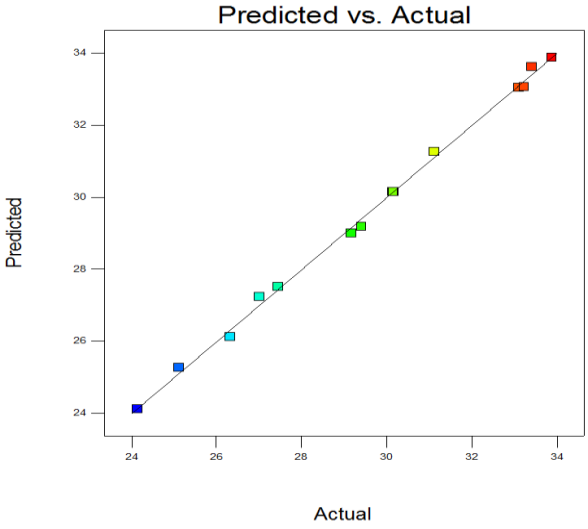


**Figure -26: Actual Response vs. Predicted (R2)**

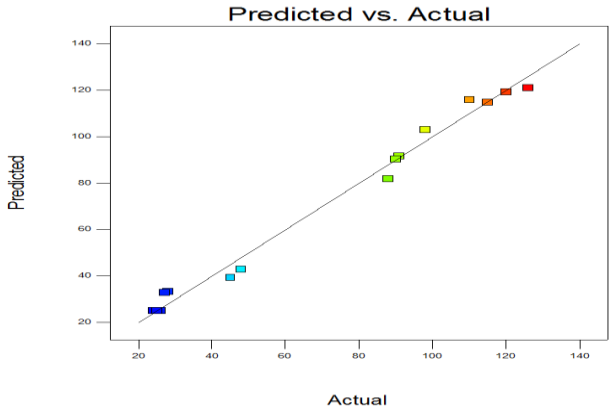


**Figure -27: Actual Response vs. Predicted (R3)**

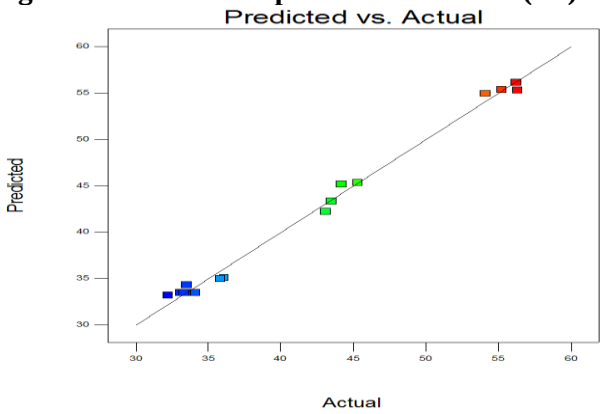


**Figure -28: Actual Response vs. Predicted (R4)**

The transformation parameter, λ, is chosen such that it maximizes the log-likelihood function. The maximum likelihood estimate of λ agrees to the value for which the squared sum of errors from the fitted model is a minimum. This value of λ is determined by fitting a numerous values of λ and choosing the value corresponding to the minimum squared sum of errors. t can also be chosen graphically from the Box-Cox normality plot. Value of λ = 1.00 indicates that no transformation needed and produces results identical to original data [22] shown in Figure 29 to 32.

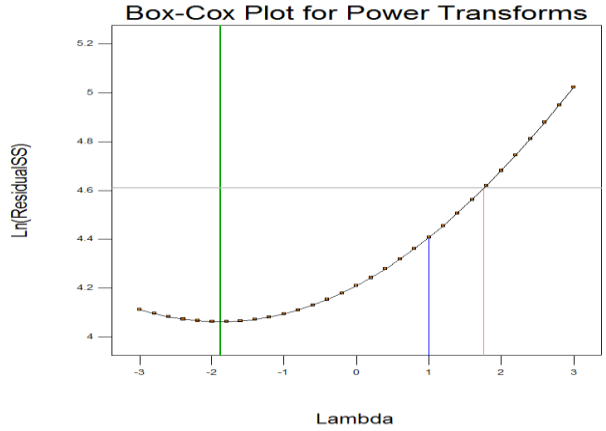


**Figure -29: Box-Cox Plot (R1)**

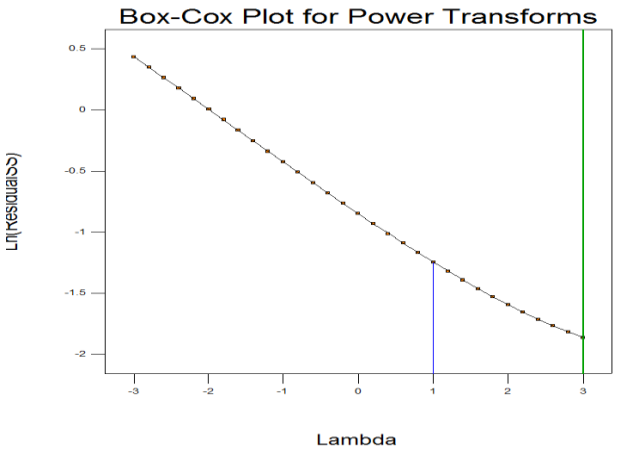


**Figure -30: Box-Cox Plot (R2)**

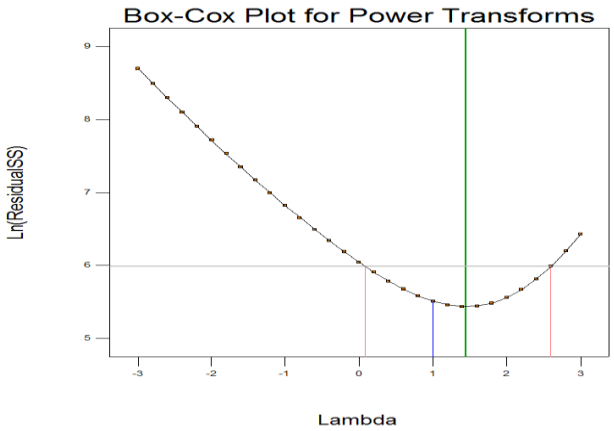


**Figure -31: Box-Cox Plot (R3)**

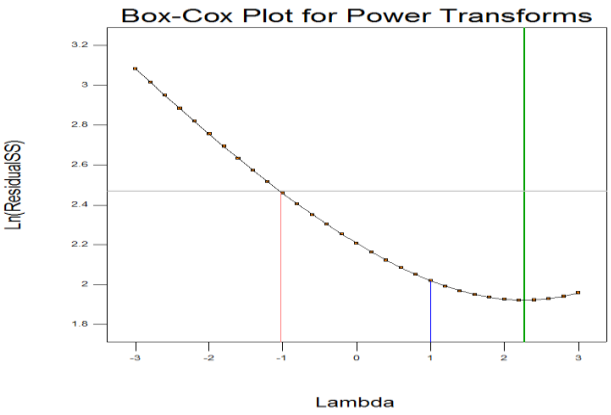


**Figure -32: Box-Cox Plot (R3)**

The Run 17 was prepared again according to these optimized levels. Observed responses were in close agreement with the predicted values of the optimized process, thereby demonstrating the feasibility. The percentage of drug release from optimized formulations over the period of 540 min for formulations R8, R14 and R17 was found to be

99.3%, 99.1% and 97.9% respectively as shown in Figure 34& 35.

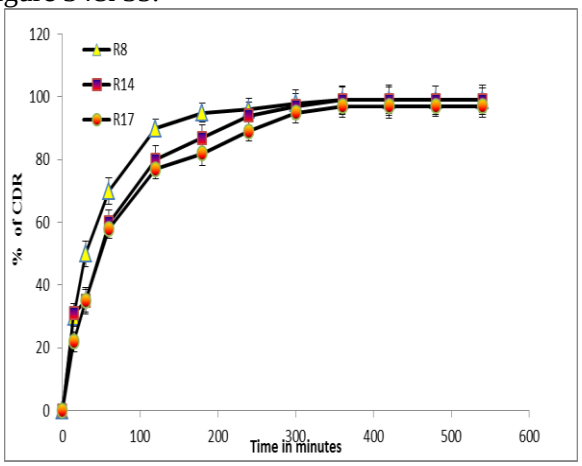


**Figure-34: Shows drug release from *in situ* gel**

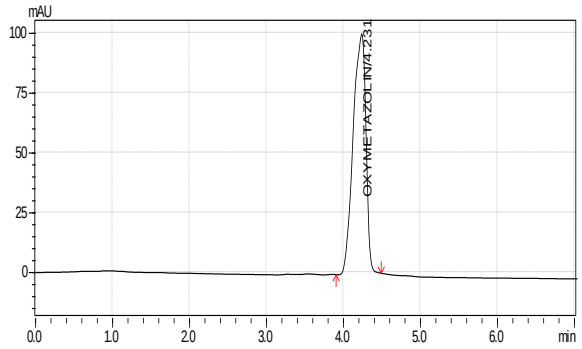


**Figure-35: Shows sample HPLC chromatogram.**

The FTIR spectra of oxymetazoline hydrochloride has its original peaks retains even after the polymerization. There is interaction between drug and polymer. Figure 17 shows the relative spectra of physical mixture.

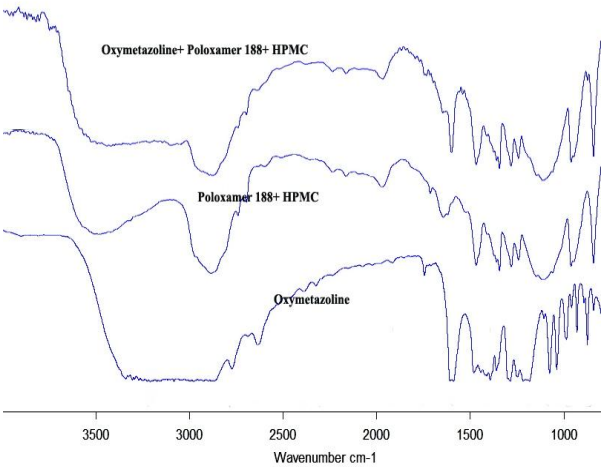


**Figure-33: Shows FTIR spectra**

The DSC thermogram of oxymetazoline hydrochloride showed a sharp endothermic peak at 186.80°C which is related to pure drug. The DSC curve of the poloxamer 188 and HPMC showed a broad endothermic peak at 59.25°C which is related to the moisture adsorbed by polymers. Physical mixture of polymer and drug exhibited an endothermic peak at 183.85°C. From this study it was concluded that there were no any significant changes between drug and polymer.

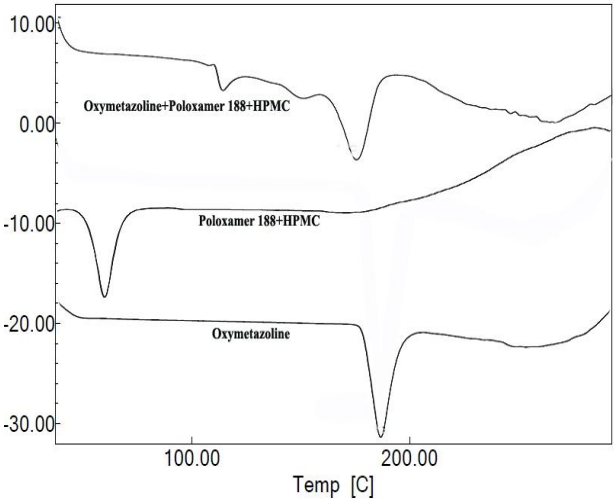


**Figure-33: Shows DSC spectra**

Transparent thermosensitive *in situ* gel was prepared by the cold method. Fig. 34 shows SEM images of the composite of oxymetazoline *in situ* gel.

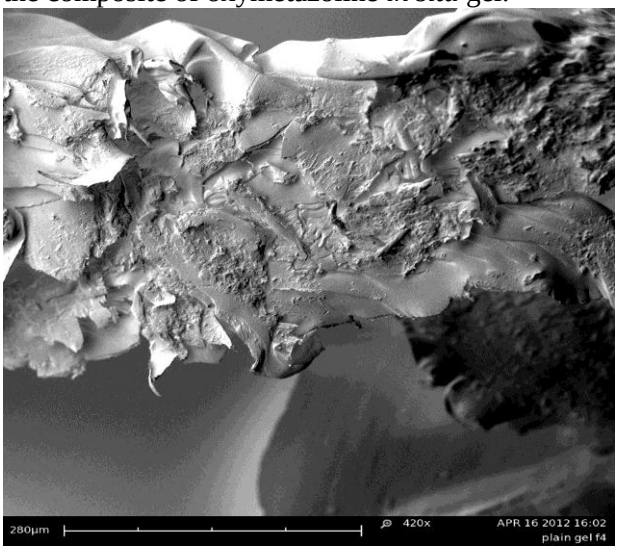


**Figure-34: Shows SEM image of oxymetazoline *in situ* gel**

## CONCLUSION

The *in situ* system has been proven to be safe, effective, efficient, acceptable, nontoxic, and easy to be administered via the nasal route. It was explored that as the concentration of P188 and water increased, the gelation temperature of thermosensitive gel system decreased. Similarly increasing concentration of P188: HPMC tends to

increase the mucoadhesiveforce of *in situ* gel. Thus from the above results it can be concluded that a thermosensitive *in situ* gel of oxymetazoline hydrochloride can be formulated using optimum quantity of polaxmer 188 and HPMC ratio. The thermosensitive gels have a increase in nasal residence time and patient comfort.

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acetaminophen liquid suppository. *Int J Pharm*, 165:33–44 (1998).

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