Identification of Polymer Synergy with Help of DOE

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Abstract – Ophthalmic solutions undergo rapid clearance from eye due to instant tear drainage, lower volume of cul-de-sac and lesser contact time with eye. All these drawbacks of ophthalmic solutions results in less efficacious product or requires repeated dosing. Repeated dosing of formulation could lead to lesser patient compliance and possible adverse effects. There are numerous approved polymeric ingredients available for ophthalmic use; however less information is available on their synergies. Hence there was a need to identify synergies among various polymeric ingredients with increased adhesiveness for sustained release ophthalmic formulations. Formulation adhesiveness is the function of viscosity being directly proportional; it plays a major role to sustain the drug release by increasing the contact time in eye with help of muco-adhesive forces or by polymer Inter-Penetrated Network (IPN). Choices of selection of polymeric ingredient were based on their individual viscosities. Significant synergies were considered for those combinations which have higher viscosities compared to their individual viscosities at lower concentrations. The synergies were also studied for stress study with the impact of pH, Buffers and Temperature during steam sterilization process.

Index Terms – Ophthalmic, Polymer, Synergy, Viscosity.

1. INTRODUCTION

Generally it is assumed that drug applied topical in eye would be available completely for eliciting it's therapeutically action. However it is not the case due to rapid tear drainage, blinking of eye, lower residence time of ophthalmic formulation in eye and lower cul-de sac volume[1,2]. There are different routes of administration of drug in to eye are available however topical route remains the most preferred choice. One way to increase the ocular bioavailability is by prolonging drug residence time in eye. Residence time in eye can be increased by increasing the viscosity of formulation with use of various viscosity enhancing agents such as polymers. There are different kind of polymers such as natural, semisynthetic and synthetic polymers[3]. Various polymers are available which can be utilized for formulation of long acting ophthalmic formulation.

Rationale of work was to identify a suitable polymer based platform system which is derived from the polymer concentration approved in inactive ingredients guide approved by USFDA[4] or based on toxicity study data of polymers. This kind of polymer platform would serve as ready tool which can be incorporated in various ophthalmic formulations which requires frequent dosing. These systems would be resulting more viscous solution which remains in the eye for a longer period of time and thus enhances the sustained release of the medicament[5].

Systematic approach of quality by design was used to identify the synergistic polymer platform system. By use of quality by design interaction amongst various polymers can be studied in more effective way with less number of experiments.

As these polymer platform systems are to be used in ophthalmic formulation which needs to be sterile hence many times sterilization process, buffers or pH adversely or synergistically impact these polymers. Hence the impact of all sterilization process & pH or buffers data becomes useful for further formulation development activity.

2. EXPERIMENT METHODOLOGY

2.1 Selection of polymer:

Polymers generally used in ophthalmic formulations were selected to identify the synergy. Polymers were studied at concentration 0.5 %.

Table No.1: Viscosity of single polymer (0.5%)

Polymer	Viscosity (cps)
Sodium carboxy methyl cellulose (Sodium CMC)	4.78
Hydroxy propyl methyl cellulose (Hypromellose 2910)	6.79
Gellan gum (Kelcogel CG-LA)	616
Carbomer homopolymer Type B (Carbopol 974 PNF)	31.8
Methyl Cellulose	1.92
Guar gum	123
Xanthan gum (Xantural-75)	2753

2.2 Preparation of polymer solution

0.5 % aqueous solution were prepared by dissolving polymer in purified water under stirring. Viscosity of these aqueous solution was measured by using appropriate spindle and rpm.

2.3 Screening of polymers

Polymer shortlisting was done based on viscosity studies. Polymer of more than 100 cps viscosity was selected for further interaction study.

Gellan gum, Guar gum, Xantural 75 were selected. Carbopol 974 P NF was additional selected as after neutralization of Carbopol 974, it would result higher viscosity.

2.4 Experimental design

2.4.1 For identification of synergistic polymer combination

Table No. 2: Mixture design for 4 polymer mixture

Run	Gellan gum (Kelcogel CG-LA) (%)	Carbopol 974 PNF (%)	Guar gum (%)	Xanthan gum (Xantural -75) (%)	Viscosity (cps)
1	0.000	0.000	0.250	0.250	2816
2	0.250	0.250	0.000	0.000	14.7
3	0.000	0.000	0.000	0.500	2753
4	0.000	0.250	0.250	0.000	105.4
5	0.125	0.125	0.125	0.125	1575
6	0.063	0.063	0.063	0.313	7828
7	0.063	0.313	0.063	0.063	166.8
8	0.000	0.500	0.000	0.000	21.1
9	0.500	0.000	0.000	0.000	235
10	0.250	0.000	0.000	0.250	1773
11	0.125	0.125	0.125	0.125	1356
12	0.063	0.063	0.313	0.063	2615
13	0.167	0.000	0.167	0.167	342.5
14	0.250	0.000	0.250	0.000	82
15	0.000	0.250	0.250	0.000	192.8
16	0.000	0.250	0.000	0.250	6284
17	0.313	0.063	0.063	0.063	91.6
18	0.000	0.000	0.500	0.000	4.26
19	0.000	0.250	0.000	0.250	7453

The design of experiment was employed systematically to identify the synergistic polymeric combination.

Mixture design was selected as this design is used when the response changes as function of the relative proportion of the component.

Optimal mixture design with cubic mixture model was used to study interaction. Total 19 experimental runs were conducted with 2 center point and 2 replicate. Total concentration for mixture was kept 0.5%.

Aqueous solution were prepared by dissolving polymer in purified water under stirring. Viscosity of these aqueous solution was measured by using appropriate spindle and rpm.

2.4.2. Response surface and contour plot

The regression analysis of the data obtained from the experimental runs generated the following polynomial equations in which the model F ratio was statistically significant at $\alpha < 0.05$ with Adj – R^2 value in the range close to 1 with a statistically non-significant lack of fit at $\alpha > 0.05$.

$$+ 2.38 \times A + 1.33 \times B + 0.64 \times C + 3.45 \times D - 2.88 \times AB + 1.68 \times AC + 1.40 \times AD + 4.21 \times BC + 5.81 \times BD + 5.68 \times CD + 66.99 \times ABC - 120.27 \times ABD - 15.95 \times ACD + 86.54 \times BCD - 11.55\ BD(B-D)$$

Where A, B, C and D are concentration of Gellan gum, Carbopol 974, Guar gum and Xantural 75 respectively.

Table No. 3: Statistical analysis of experimental design

Response	Model p value	Adj – R^2	Lack of fit test p value
Viscosity (cps)	< 0.0001	0.9949	0.1063

Table no 3 suggest that less than 0.01 % chance that the model F value of polynomial equation occurred due to noise. The p value for lack of fit was not significant, indicating that this model equations fitted the data well. Therefore reduced cubic mixture model could adequately describe adequately the data and could be employed to arrive at synergy.

A positive sign represents a synergistic effect, while a negative sign indicates antagonistic effect. The negative coefficient of A and B in the model refers to decrease in viscosity due to for gellan gum and guar gum combination. Highest positive coefficient of 5.81 between B and D showed the highest viscosity which in turn means maximum synergy.

3-D surface and contour plots in which the responses were represented by curvature surface as a function of independent variables. The relationship between the response and independent variables can be directly visualized from the response surface plots. The Contour plots and 3 D graphs are presented in fig 1 and 2.

From the viscosity study data it can be concluded that four polymer combination system and Carbopol 974 PNF & Xantural 75 (0.250:0.250) gave the best synergy. The viscosity of synergistic combination even at lower concentration (0.250:0.250) was higher than the viscosity of individual polymer at higher concentration (0.500). Further to identify the best ratio of binary polymer combination Carbopol PNF & Xanthan gum again mixture design was applied.

Figure 1: Contour Plot for 4 polymer mixture

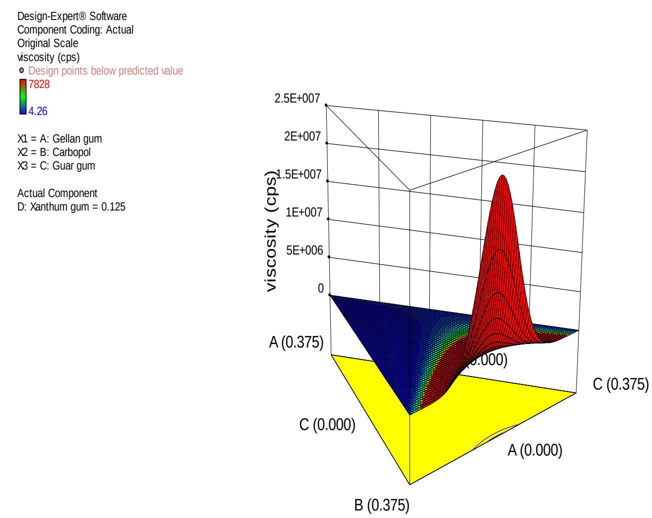
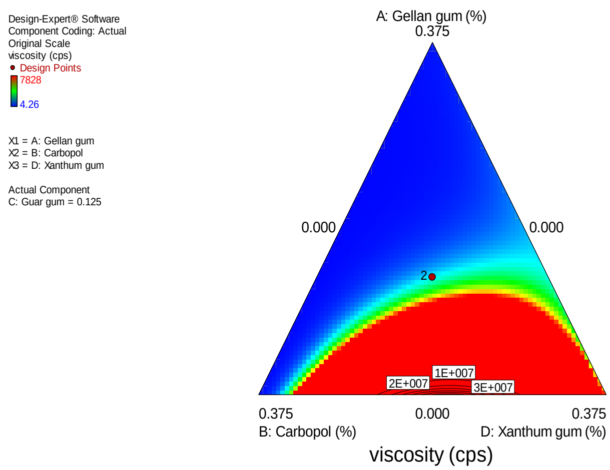


Figure 2: 3 D Graph for 4 polymer mixture design



2.4.3 Identification for optimal synergistic ratio:

Mixture I-optimal design, quartic model was used for this study. 10 experimental runs were conducted. Total concentration for mixture was kept 0.5%.

Table No. 4: Experimental run along with viscosity data

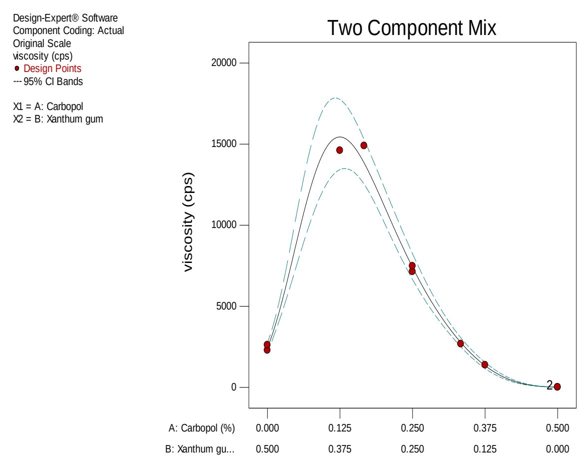
Run	Carbopol 974 PNF	Xantural 75	Viscosity (cps)
1	0.000	0.500	2615
2	0.500	0.000	13.8
3	0.500	0.000	13.9
4	0.375	0.125	1380
5	0.250	0.250	7483
6	0.125	0.375	14607
7	0.250	0.250	7138
8	0.167	0.333	14297
9	0.333	0.167	2681
10	0.000	0.500	2289

The regression analysis of the data obtained from the experimental runs generated the following polynomial equations in which the model F ratio was statistically significant at $\alpha < 0.05$ with Adj – R^2 value in the range close to 1

+ 1.14 x A + 3.39 x B + 6.42 x AB + 0.33 x AB(A –B) + 4.05 x AB (A-B) ^ 2

Where A and B are concentration of Carbopol 974 and Xantural 75 respectively.

Figure 3: Graph of two component mixture



It can be concluded that Carbopol 974 PNF & Xantural 75 in ratio of 0.125: 0.375 showed the best synergy. This ratio was further selected to understand the impact of pH, temperature, buffers.

2.4 Impact of pH:

Carbopol 974 PNF & Xantural 75 was dissolved in purified water in ratio of 0.125:0.375 under stirring. pH of as such solution was observed to be 4.30. pH impact study was studied from pH 4.0 to 8.0. pH was adjusted with either 0.1N hydrochloric acid or 1 N Sodium hydroxide.

2.5 Impact of steam sterilization (autoclave):

Being ophthalmic product it needs to be sterilized. In ophthalmic formulation sterilization process plays important role. Carbopol 974 NF & Xantural 75 was dissolved in Purified water in ratio of 0.125:0.375 under stirring. Solution was autoclaved at 121 degree centigrade for 15 minutes & viscosity was checked again.

2.6 Impact of buffers:

Various buffers are used in ophthalmic formulation. These buffers again could modify the viscosity.

In purified water respective buffers were added one after, to dissolve each ingredient. Carbopol 974 PNF was added under stirring to buffer solution to get clear solution followed by Xantural 75 addition. Viscosity of these aqueous solution was measured by using appropriate spindle and rpm.

Table No. 5: Various buffer studied

Citrate buffer	% w/v	Borate buffer	% w/v	Acetate buffer	% w/v	Phosphate buffer	% w/v
Carbopol 974 PNF	0.125	Carbopol 974 PNF	0.125	Carbopol 974 PNF	0.125	Carbopol 974 PNF	0.125
Xantural 75	0.375	Xantural 75	0.375	Xantural 75	0.375	Xantural 75	0.375
Citric acid monohydrate	0.20	Boric acid	1.8	Acetic acid	0.2	Monobasic sodium phosphate monohydrate	1.3
Trisodium citrate dihydrate	0.45	Borax	1.1	Sodium acetate trihydrate	1.28	Dibasic sodium phosphate dihydrate	1.2
Purified water	q.s	Purified water	q.s	Purified water	q.s	Purified water	q.s

3. RESULT AND DISCUSSION

3.1 Impact of pH on viscosity:

With increase in pH from 4.0 to 6.0 there was increase in viscosity observed. There was not much change in viscosity observed from pH 6.0 to 8.0.

Table No. 6: Impact of pH on viscosity

pH	Viscosity (cps)
pH 4.0	875.8
pH 6.0	2408
pH 8.0	2525

3.2 Impact of autoclaving on viscosity:

Viscosity drops significantly in both scenario’s when the neutralization done prior to autoclaving or neutralization done post autoclaving. This could be due to nature of Xanthan gum. Xanthan gum exits in helix nature. Temperature breakdown this helical nature, which results in drop in viscosity. Hence for sterilization of xanthan gum other sterilizing techniques can be used such as radiation or gaseous sterilization.

Table No. 7: Impact of Autoclaving

Study	**Viscosity (cps)**
Viscosity before autoclaving (pH 4.3)	875.8
Viscosity after autoclaving (pH 4.3)	114
Viscosity of autoclaved sample (neutralization post autoclaving)	604.4
Viscosity before autoclaving (pH 7.4)	2729
Viscosity after autoclaving (pH 7.4)	231

3.3 Impact of autoclaving on viscosity:

In comparison with un- buffered system there was not much change in viscosity observed with various buffers.

Table No. 8: Viscosity with various buffers

Sample	**Viscosity (cps)**
Unbuffered system	875.8
Citrate buffer	713
Borate buffer	863.8
Acetate buffer	653
Phosphate buffer	764

4. CONCLUSION

Design of experiment tool was used to identify polymeric synergy. Shortlisting of polymers were done based on individual polymer viscosity data. Four polymers viz. Gellan gum, Carbopol 974 PNF, Guar gum and Xanthan gum were shortlisted. Design of experimental study data showed Carbopol 974 PNF in combination with Xanthan gum (0.250:0.250) gave best synergy. Optimal ratio for Carbopol 974 PNF and Xanthan gum was identified (0.125:0.375) with help of mixture design.

With increase in pH from 4.0 to 6.0 there was increase in viscosity further there no significant change was observed from pH 6.0 to 8.0.

Significant drop in viscosity was observed after steam sterilization which could be due to breakdown of helical structure of xanthan gum hence steam sterilization is not a choice which can be used for this kind of platform system. Buffers did not show any significant impact.

From all the studies conducted it can be concluded that a synergistic polymer platform system was identified by use of systematic approach with help of design of experiment. This platform system can be used for formulation of various ophthalmic formulation which would give sustain or modified release action.

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