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SCREENING AND SELECTION OF SURFACTANTS FOR SOLUBILIZATION OF TRAVOPROST USING DESIGN OF EXPERIMENTS

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ABSTRACT

Travoprost is a prostaglandin analogue widely used for reducing intraocular pressure (IOP) in patients affected with glaucoma and ocular hypertension. The current marketed formulation of Travoprost 0.004% in the US (Travatan Z) is a micellar solution with an ionically buffered preservative system *Sofzia*, recommended for treatment of open angle glaucoma. This formulation has several disadvantages and has the potential to form particulate matter during its shelf life due to interaction with the preservative system. The current marketed formulation also uses a patented primary packaging component to stabilize the formulation. Thus, an effort was made to select alternate surfactants for dissolving Travoprost in micellar solutions. Design of Experiments was used for screening a combination of four surfactants namely, Cremophor RH 40, Cremophor EL, Polysorbate 80 and Solutol HS 15 using *Optimal Custom Mixture design*. Particle size and polydispersity index (PDI) were measured as responses. A combination of two surfactants was chosen based on the lowest polydispersity index. Further optimization of surfactant concentrations was done using a *Response Surface Optimal design*. The results of particle size and PDI were evaluated statistically using regression analysis. All the experiments gave a particle size in the nano range of less than 20nm.

KEYWORDS: Travoprost, Micellar solution, Surfactants, Design of experiments, particle size, Polydispersity Index.

INTRODUCTION

Travoprost is a prostaglandin analogue widely used for reducing intraocular pressure (IOP) in patients affected with glaucoma and ocular hypertension.[1] It exerts its ocular hypotensive effect through the prostaglandin FP receptors, located in the ciliary muscle and the trabecular meshwork.[1] Travoprost is is an oily substance and insoluble in water. The primary objective of this work is to screen and select surfactant(s) for solubilization of Travoprost by forming micellar solutions having particle size in the nano range and low polydispersity index.

MATERIALS AND METHODS

Materials

Travoprost was sourced from Everlight Chemicals. Solutol HS 15 (Polyoxyl-15-Hydroxystearate), Cremophor RH 40 (Polyoxyl 40 Hydrogenated Castor Oil) and Cremophor EL (Polyoxyl 35 Castor Oil) were generous gifts from BASF. Polysorbate 80 was sourced from Croda International.

Screening of surfactants

Trials were conducted to select a surfactant or a mixture of surfactants to solubilize the drug substance. The aim was to select a surfactant or mixture of surfactants that gave nano particle size and low polydispersity index (less than 0.7).

From the literature, four non-ionic surfactants were selected for solubilising Travoprost by forming a micellar solution, namely, Cremophor RH 40, Solutol HS 15, Cremophor EL and Polysorbate 80.

Design of Experiments

Design of experiments (DOE) using a statistical software *Design expert 9* was employed for selecting the best combination of surfactant(s). *Optimal Custom Mixture* design[2,3] was chosen with two replicates and two center point experiments keeping the total concentration of surfactants to 5%. Nineteen experimental batches were manufactured by dissolving Travoprost in different combinations of surfactants in water. Particle size [d(90)] and polydispersity index (PDI) were chosen as the responses. The experimental runs are tabulated in Table 1.

Table 1: Experimental run for selection of surfactants by using *Optimal custom Mixture* design in *DesignExpert 9* software and responses.

Run	Component 1 A:Cremophor RH 40(%)	Component 2 B:Solutol HS 15(%)	Component 3 C:Cremophor EL(%)	Component 4 D:Polysorbate 80(%)	Response 1 Particle size in nm (D90)	Response 2 Polydispersibility Index (PDI)
1	1.250	1.250	1.250	1.250	12.78	0.67
2	0.000	0.000	5.000	0.000	12.44	3.66
3	0.000	0.000	2.500	2.500	12.44	3.82
4	0.625	0.625	3.125	0.625	14.42	2.03
5	1.875	0.625	1.875	0.625	13.83	4.89
6	0.000	0.000	0.000	5.000	11.11	0.22
7	1.250	1.250	1.250	1.250	15.27	1.13
8	0.000	5.000	0.000	0.000	13.74	0.31
9	0.000	2.500	2.500	0.000	13.45	0.7
10	0.000	2.500	0.000	2.500	13.71	0.21
11	3.125	0.625	0.625	0.625	14.07	0.34
12	2.500	0.000	2.500	0.000	13.78	1.47
13	0.000	0.000	2.500	2.500	12.74	4.45
14	5.000	0.000	0.000	0.000	15.56	4.16
15	0.625	0.625	0.625	3.125	11.58	4.13
16	2.500	0.000	0.000	2.500	12.07	19.8
17	2.500	2.500	0.000	0.000	14.34	0.62
18	0.000	2.500	0.000	2.500	11.88	0.23
19	0.625	3.125	0.625	0.625	17.6	3.44

Measurement of particle size and polydispersity index

Particle size measurement was carried out using *Sympatec Nanophox* instrument which employs the principle of Dynamic Light Scattering[4] (DLS) to measure nano particles in the range of 1 nm to 10,000 nm. Particle size d(90) and polydispersity index (PDI) were measured on undiluted samples of Travoprost solutions. The results are tabulated in table 1.

Statistical analysis (ANOVA)

The results of particle size and PDI were evaluated statistically using regression analysis.[2,3] The following polynomial equations were generated in which the model *F* ratios were statistically significant at $\alpha < 0.05$, with a statistically non-significant lack of fit at $\alpha > 0.05$. The model for statistical analysis of particle size is *linear mixture* model and that for PDI is *reduced cubic mixture* model.

$$\text{Particle size[d(90)]} = 15.01*A+15.04*B+13.34*C+11.03*D \quad (1)$$

$$\text{PDI} = 4.71*A+0.32*B+3.67*C+0.23*D-6.43*AB-9.73*AC+70.47*AD-5.11*BC-0.18*BD+8.78*CD-4.71*ABC-196.80*ABD+363.87*ACD-466.36*BCD-200.17*AB(A-B) \quad (2)$$

Where A= Concentration of Cremophor RH 40; B=Concntration of Solutol HS 15 15; C= Concentration of Cremophor EL and D= Concentration of Polysorbate 80.

The results of statistical analysis for particle size and PDI is tabulated in Table 2.

Table 2: Results of statistical analysis (ANOVA) of the experimental design.

Responses	Model p value	Adequate Precision	Lack of fit test; p value
Particle size	0.0176	7.083	0.6038
PDI	< 0.0001	77.364	0.6848

Model p value indicates that there is only a 1.76% chance that that the model F value of the model equation (1) for particle size, occurred due to noise. For PDI, the model p value indicates that there is only less than 0.01% chance that model F value of the model equation (2) occurred due to noise. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. For both responses, ratios were higher than 4, indicating adequate signal. The p values of the lack of fit test of both models were not significant, indicating that these model equations fitted the data well and can be used for prediction.

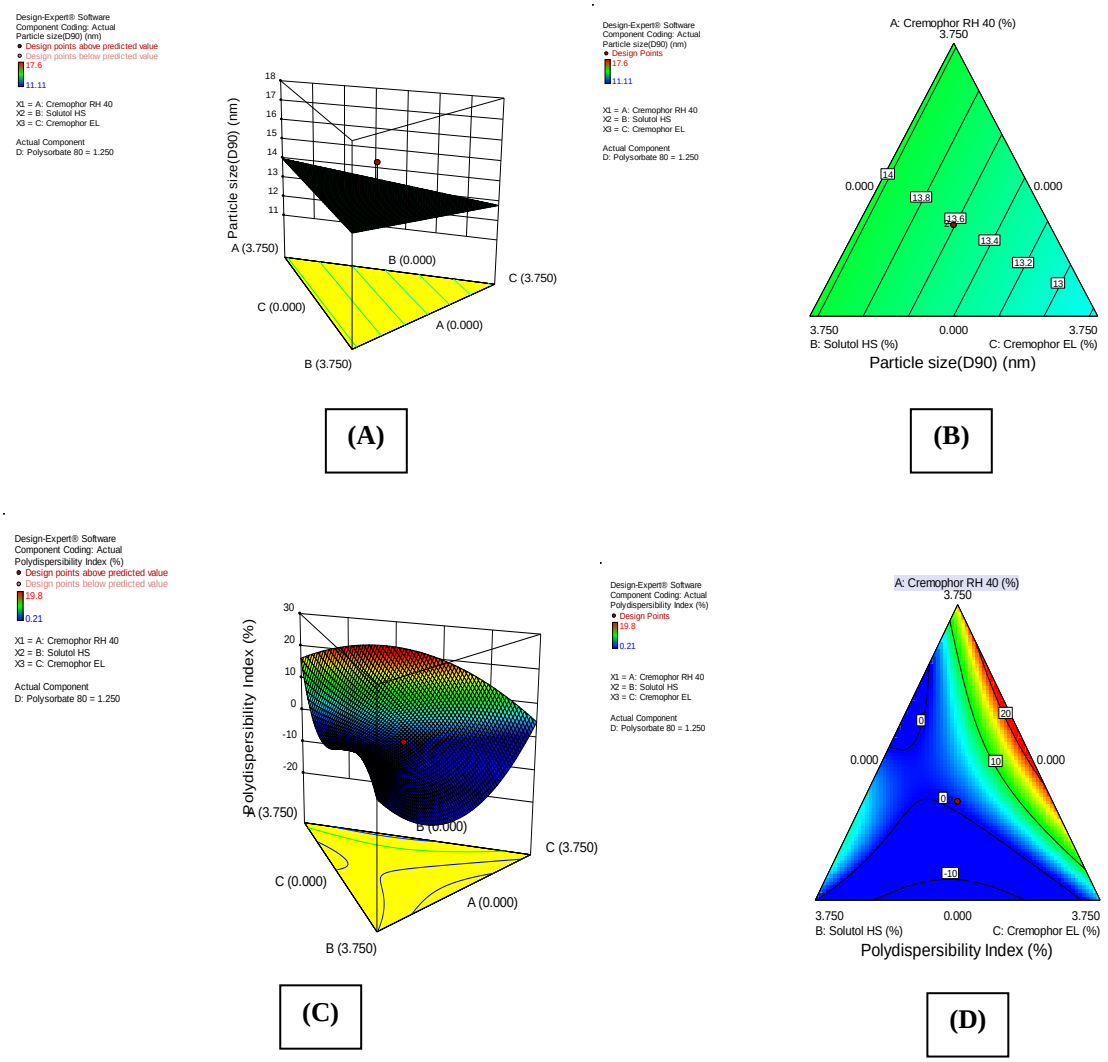


Fig 1: (A) – Response surface plot of effect of surfactant concentrations on particle size d(90); (B) – Contour plot showing effect of Solutol HS 15 and Polysorbate 80 on particle size d(90); (C) - Response surface plot of effect of surfactant concentrations on polydispersity index; (D) – Contour plots showing effect of surfactant concentration on polydispersity index.

Interpretation of results

All the trials yielded nano particle sizes for the micelles in the range of 11-17 nm for d(90). From the graphs, no clear correlation of particle size was seen w.r.t concentration of surfactants. However, polydispersity index vary widely for these experiments. PDI also shows a curvature in the 3D plot indicating that there is a significant interaction between the factors. Polydispersity index (PDI) is an indication of width of distribution and values ≤ 0.7 are considered acceptable for nano systems. The lowest value of PDI was found to be 0.21 in experiment 10. Experiment 18 is a replicate of experiment 10 also showed low PDI of 0.23. Both these experiments had a combination of Solutol HS 15 and Polysorbate 80 at 2.5% concentration each. Thus the combination of Solutol HS 15 and Polysorbate 80 was chosen for further optimization of concentrations.

Optimization of surfactant concentration

To optimize the concentration of Solutol HS 15 and Polysorbate 80 in combination at various concentrations, another DOE was used. Here *Mixture design* was not selected as the concentration would have to be fixed at 5% for the surfactant. But since the formulation is for ophthalmic use, aim is to arrive at the minimum concentration of surfactant, in order to avoid damage to the corneal surface. Hence a *Response surface design, optimal design*[3] was chosen for this study so that different combinations of both surfactants are achieved without limiting the total to 5%. A range of 0.1 to 2.5% for both surfactants were chosen. 2 replicates were included and a total of twenty experimental runs were identified by the software. All twenty experiments were performed by dissolving Travoprost in the surfactant combination at different concentrations as shown in Table 3. The particle size was measured and d(10), d(50) and d(90) were recorded. The experimental runs and their responses are tabulated below in Table 3.

Table 3: Experimental run for optimizing concentration of two surfactants by using *Response Surface design, Optimal design* in *DesignExpert 9* software.

Run	Factor 1	Factor 2	Particle size in nm		
	A:Solutol HS 15(%)	B:Polysorbate 80(%)	d(10)	d(50)	d(90)
1	1.28	1.47	6.17	7.16	8.31
2	1.00	1.95	5.73	6.65	7.73
3	2.22	2.01	5.7	6.81	8.16
4	2.50	2.50	5.7	6.8	8.13
5	1.68	1.88	5.34	6.3	7.42
6	1.00	1.00	7.06	8.49	10.21
7	2.13	1.00	5.78	6.72	7.81
8	1.79	2.50	5.36	6.28	7.32
9	2.50	1.53	5.73	6.79	8.03
10	1.57	1.00	7.17	8.32	9.65
11	1.00	2.50	5.66	6.83	8.25
12	2.50	1.53	6.06	7.08	8.28
13	1.68	1.88	6.5	7.1	8.5
14	0.25	0.25	7.15	8.1	8.5
15	0.25	0.5	6.59	7.21	8.4
16	0.25	0.75	7.2	7.8	8.8
17	0.25	1	7.5	8.4	9.6
18	0.25	0.1	7.4	8.7	9.2
19	0.1	0.25	7.8	8.9	9.8
20	0.1	0.1	7.9	9.1	10.5

Statistical analysis (ANOVA)

The results of particle size [d(90)] were evaluated statistically using regression analysis. The following polynomial equation was generated in which the model *F* ratios were statistically significant at $\alpha < 0.05$, with a statistically non-significant lack of fit at $\alpha > 0.05$. The model for statistical analysis of particle size is *Response surface Linear* model.

Particle size d(90) = +8.90906-0.28813*A - 0.48084*Polysorbate 80 (3)

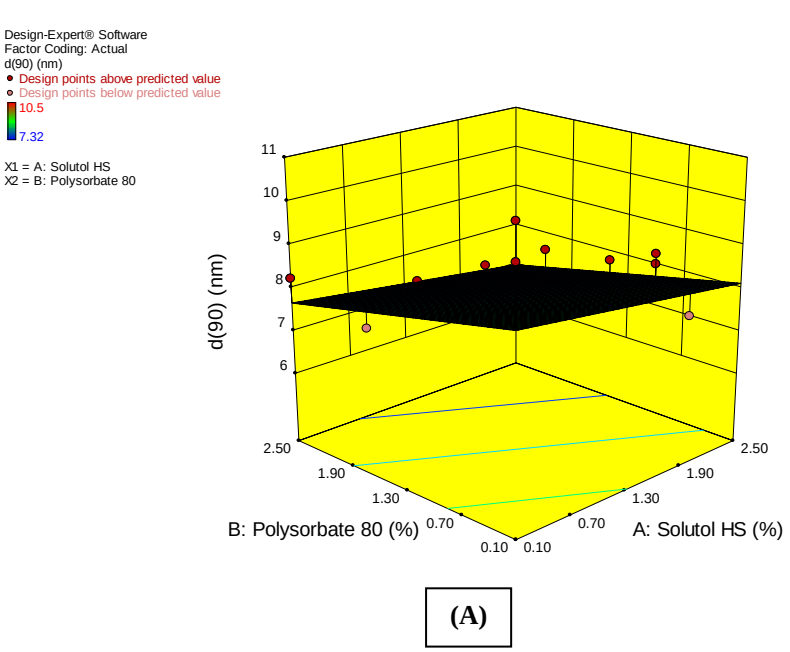
Where A= concentration of Solutol HS 15 and B= concentration of Polysorbate 80.

The results of statistical analysis for particle size is tabulated in Table 4.

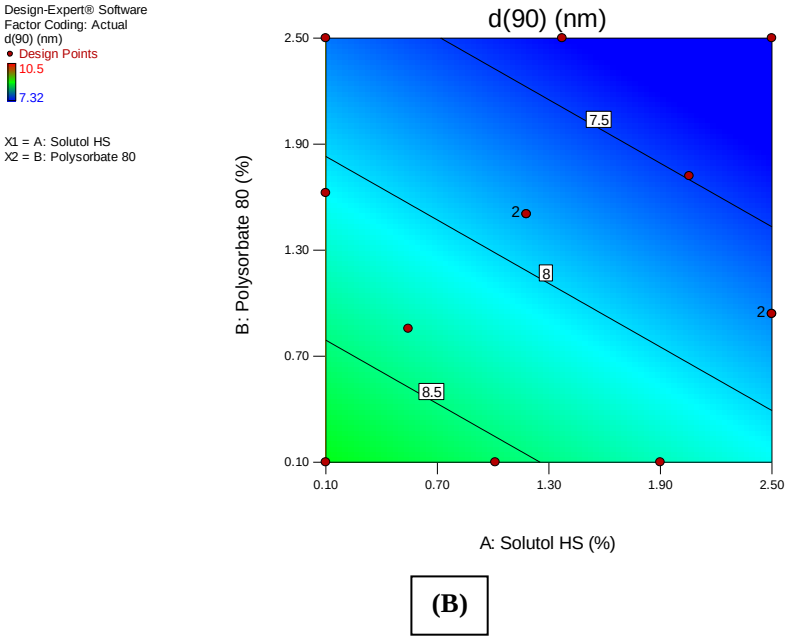
Table 4: Results of statistical analysis (ANOVA) of the experimental design.

Responses	Sources		
	Model p value	Adequate Precision	Lack of fit test; p value
Particle size d(90)	0.0453	7.009	0.4391

Model p value indicates that there is only a 4.53% chance that that the model F value of the model equation (3) occurred due to noise. Adequate precision measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 7.009 indicates adequate signal. The p values of the lack of fit test of the model were not significant, indicating that these model equations fitted the data well and can be used for prediction.



(A)



(B)

Fig 2: (A) – Response surface plot of effect of Solutol HS and Polysorbate 80 concentrations on particle size d(90); (B) – Contour plots of effect of Solutol HS 15 and Polysorbate 80 concentrations on particle size d(90).

Interpretation of results

All the trials were satisfactory and particle size of d(90) between 7.32 to 10.5 nm was achieved. From the contour plots, it is evident that as the concentration of surfactant increases, the particle size decreases. However, since all the particle size is in the narrow range, this observation is not significant.

Analysis of Travoprost in the micellar solutions

Experiments with low concentration of Solutol HS (0.25% and less) in combination with different concentrations of Polysorbate 80 (Experiments 14 to 20 in Table 3) were tested for assay of active substance using an HPLC method in order to verify that the drug is solubilised completely within the micellar structure. If free drug remains in the solution, it tends to adsorb onto the LDPE vials.[5] Hence assay of solution was done for both glass vials and plastic LDPE vials to check if there is any difference in the assay. The assay values for all seven experiments were satisfactory (between 90-110%).

Table 5: Different combinations of Solutol HS 15 with Polysorbate 80 to dissolve Travoprost and their assay values.

Solutol HS 15(%)	0.25	0.25	0.25	0.25	0.25	0.1	0.1
Polysorbate 80(%)	0.25	0.5	0.75	1.0	0.1	0.25	0.1
% Assay values in glass vials	107.9	101.4	102.3	99.7	107.9	106.2	99.2
% Assay values in LDPE vials	107.7	100.8	101.5	99.2	107.7	106.2	99.4

RESULTS AND DISCUSSION

All the surfactants chosen in the DOE studies gave particle size in the nano range of less than 20nm. There was no clear correlation of particle size seen w.r.t concentration of surfactants in the screening DOE. However, based on results of polydispersity index, a combination of Polysorbate 80 and Solutol HS 15 were chosen in combination to dissolve Travoprost. Further optimization of surfactant concentrations was done in order to arrive at the lowest possible surfactant concentration, being an ophthalmic drug. The lowest combination of 0.1% each of Solutol HS 15 and Polysorbate 80 also gave satisfactory assay in both glass and LDPE vials indicating that micellazation is complete and no free drug is available for adsorption onto the plastic vials. Complete formulation development of Travoprost ophthalmic solution at 0.004% would be undertaken based on the finalized surfactant concentrations.

CONCLUSION

Design of experiments is a systematic statistical tool to perform screening experiments and arrive at the optimum concentrations using suitable designs. Software *Design expert 9* was used to screen a combination of 4 surfactants at different concentrations to solubilize Travoprost, an insoluble oily substance. Based on the results of particle size and polydispersity index, a combination of Polysorbate 80 and Solutol HS 15 was further optimized, using another design. Both the statistical models were analysed using ANOVA and yieled model *F* ratios that were statistically significant at $\alpha < 0.05$, with a statistically non-significant lack of fit at $\alpha > 0.05$, indicating that the model equations fitted the data well and could be employed to navigate the design space.

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