DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(4), 405-424 (1993)

RESPONSE SURFACE METHODOLOGY AS AN APPROACH TO OPTIMIZATION OF AN ORAL SOLUTION

Edith Senderak, Marion Merrell Dow Inc., Cincinnati, Ohio
Holly Bonsignore, Marion Merrell Dow Inc., Indianapolis, Indiana
Deborah Mungan, Marion Merrell Dow Inc., Cincinnati, Ohio

ABSTRACT

Response surface methodology was used to optimize the cloud point and turbidity of a nonionic surfactant solution that had been used to solubilize a very slightly water soluble drug. A formulation suitable for oral administration with turbidity less than four parts per million and cloud point greater than 60°C was desired. A first-order experiment, specifically, a 2^4 factorial with four center points, was designed to investigate the effect of four formulation components that had been identified as the most likely to affect turbidity and cloud point. When lack-of-fit tests for the first order linear model indicated that a second-order model would provide a better approximation to the response surface in the experimental region, a central composite design with six center points was run in order to fit second-order linear models. Within the experimental region of the second-order design, a simple first-order linear model with the concentration of one component was adequate to describe the cloud point response function; turbidity was fitted with a three component second-order response equation. Experimental runs were performed to confirm the optimum combination of components predicted by the fitted response functions. Response surface methodology provided an efficient approach to development of a nonionic surfactant solution with turbidity less than 4 ppm and cloud point greater than 60°C while maintaining the concentration of formulation ingredients within ranges suitable for oral administration.

TABLE 1
Formulation to be Optimized

Component	Concentration (% w/v)
Drug 1 (slightly soluble)	0.3
Drug 2 (water soluble)	0.3
Drug 3 (water soluble)	0.6
Oleic Acid	0.25
Alcohol USP	1.5
Lactic Acid 50% Solution	0.07
Polysorbate 80	2.5
Butylated Hydroxytoluene	0.01
Propylene Glycol	10.0
Sucrose Invert Medium	65.0
Sodium Hydroxide 50% Solution	qs to pH 5.3-5.5
Purified Water	qs

INTRODUCTION

The addition of two water-soluble drug salts to an optically clear nonionic surfactant solution that had been used to solubilize a third, very slightly water-soluble drug resulted in a formulation, listed in Table 1, that was cloudy at room temperature and separated readily on heating. Turbidity, a fractional decrease in the intensity of light as it passes through a solution (1) and cloud point, the temperature at which the turbidity of a nonionic surfactant solution abruptly increases on heating (2), were measurable properties of the formulation that could be used to quantitate its clarity and resistance to separation on heating. The formulation's turbidity was to be less than four parts per million (ppm) to insure its clarity. A minimum cloud point of 60°C was desired to insure the physical stability of the formulation under most shipping and storage conditions. It was necessary to keep the concentration of formulation components within ranges that would be acceptable for oral administration.

EXPERIMENTAL

Sample Preparation - Samples were prepared in 100 mL quantities by wetting the very slightly water-soluble drug with Alcohol USP in which butylated hydroxytoluene had been dissolved, adding oleic acid and lactic acid 50% solution and stirring until the drug had completely dissolved. Polysorbate 80 was added to the solution, which was stirred until uniformly mixed. The drug solution was added to a separate solution of sucrose invert medium, propylene glycol and purified water and stirred. The water-soluble drug salts were added to the resulting solution and mixed until completely dissolved. The pH of the formulations was adjusted, if necessary, to between 5.3 and 5.5 with sodium hydroxide, followed by addition of purified water to volume.

Turbidity Measurement - Turbidity was determined using a Monitek turbidimeter[1] which was calibrated with a 10 ppm turbidity standard[2] before each use. Eight dram screw-cap vials[3] were used to hold samples for turbidity measurement, and results were recorded in ppm.

Cloud Point Determination - A Mettler Thermal Analysis System[4] was used to measure cloud point. Samples of the solutions were placed in capillary tubes[5], then inserted in a furnace[6] equipped with a light source and photoresistors to measure light transmitted through the solutions. A central processor[7] was used to control heating of the samples from 30°C to their cloud point at 1.0°C/minute. Cloud point data was recorded on a thermal printer[8] in degrees Celsius.

Statistical Methods - Response surface methodology (3) was used to optimize the turbidity and cloud point of the liquid formulation. The objective was to achieve turbidity less than 4 ppm, and cloud point not less than 60°C.

The results of previous experiments and literature concerning solublization with nonionic surfactants suggested that Alcohol USP, polysorbate 80, propylene glycol and sucrose invert medium were the components most likely to affect the turbidity and cloud point of the surfactant solution. A first-order experimental design was set up to investigate the effects of the four components on turbidity and cloud point in the region covered by the following ranges:

Component	Concentration (% w/v)
Alcohol USP	1 - 5
Polysorbate 80	2 - 4
Propylene Glycol	5 - 25
Sucrose Invert Medium	25 - 65

The concentrations of the other formulation components were held constant. The first-order design used was a 2^4 factorial design (4) with four center points. The

TABLE 2
Composition of Samples for the First-Order Experimental Design With Center Points

Component	Concentration (% w/v)
Drug 1 (slightly water soluble)	0.3
Drug 2 (water soluble)	0.6
Drug 3 (water soluble)	0.3
Butylated Hydroxytoluene	0.01
Oleic Acid	0.25
Lactic Acid 50%	0.06
Sodium Hydroxide 50% Solution	qs to pH 5.3-5.5
Purified Water	qs to 100 mL

Preparation Order	Concentration (% w/v)				Response Variables	
	Alcohol USP	Polysorbate 80	Propylene Glycol	Sucrose Invert Medium	Turbidity (ppm)	Cloud Point (°C)
1	1	2	5	25	> 200	67.1
10	5	2	5	25	111	72.6
7	1	4	5	25	3.6	80.2
5	5	4	5	25	3.8	84.1
18	1	2	25	25	2.8	82.7
9	5	2	25	25	3.0	81.7
12	1	4	25	25	2.3	100.2
6	5	4	25	25	2.6	102.2
4	1	2	5	65	157	--
13	5	2	5	65	94	45.6
11	1	4	5	65	8.8	50.4
15	5	4	5	65	8.1	51.6
17	1	2	25	65	19.3	52.9
14	5	2	25	65	26	52.8
8	1	4	25	65	1.6	62.5
3	5	4	25	65	1.8	67.2
2	3	3	15	45	3.7	71.4
16	3	3	15	45	4.5	72.2
19	3	3	15	45	7.8	72.1
20	3	3	15	45	7.5	72.1

first-order design with the observed results is listed in standard order in Table 2. Results were analyzed using a first-order linear model, and lack-of-fit tests were performed to determine the adequacy of the first-order linear models.

When significant lack of fit was detected, second-order experimental designs were implemented in order to fit second-order linear models. Components that did not have statistically significant effects on turbidity or cloud

TABLE 3
Composition of Samples for the Second-Order Experimental Design

Component	Concentration (% w/v)
Drug 1 (slightly water soluble)	0.3
Drug 2 (water soluble)	0.6
Drug 3 (water soluble)	0.3
Butylated Hydroxytoluene	0.01
Oleic Acid	0.25
Lactic Acid 50%	0.06
Alcohol USP	2.0
Sodium Hydroxide 50% Solution	qs to pH 5.3-5.5
Purified Water	qs

Preparation Order	Concentration (% w/v)			Response Variables	
	Polysorbate 80	Propylene Glycol	Sucrose Invert Medium	Turbidity (ppm)	Cloud Point (°C)
12	3.7	17	49	3.1	75.7
7	4.3	17	49	2.8	73.5
5	3.7	23	49	3.9	80.5
4	4.3	23	49	3.1	83.7
2	3.7	17	61	6.0	62.0
11	4.3	17	61	3.4	69.5
8	3.7	23	61	3.5	69.9
9	4.3	23	61	1.8	70.8
17	3.5	20	55	4.9	73.4
16	4.5	20	55	3.3	74.6
13	4.0	15	55	4.5	81.9
15	4.0	25	55	5.1	69.5
18	4.0	20	45	3.3	80.7
14	4.0	20	65	3.2	59.9
1	4.0	20	55	2.3	67.7
3	4.0	20	55	3.8	74.9
6	4.0	20	55	2.9	72.7
10	4.0	20	55	2.4	74.8
19	4.0	20	55	3.5	70.4
20	4.0	20	55	3.2	72.8

point in the first-order experiment were not considered in the second-order design, and were held constant. Ranges used in the second-order design were also modified to allow more focus on the region where turbidity was no more than 4 ppm and cloud point was no less than 60°C according to the fitted first-order linear model. The second-order experimental design, a central composite design with six center points, is shown in Table 3.

Second-order models were fitted to the observed responses from the second-order experiment. Stationary points of the fitted response surfaces were determined and canonical analysis (5) was used to describe the nature of the response surfaces. When there were no significant second-order terms and no significant lack-of-fit, first-order linear models were fitted to the data. Optimum combinations of components were then determined from the fitted response functions. Experimental runs using the predicted optimum combinations were performed for confirmation. Due to the subjective nature of the measurement readings, it was of interest to assess the sources of variation through variance component analysis (6) based on the results of the confirmatory runs.

Models were fitted to the data using PROC GLM, and analyses of second-order models were performed using PROC RSREG. Variance component analysis was performed using PROC NESTED. PROC GLM, PROC RSREG, and PROC NESTED are statistical procedures in SAS (7). For convenience, analyses were performed using coded independent variables rather than actual values. Model terms with p-values of < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Four parts per million was selected as the maximum desirable turbidity for the oral solution because haziness could be readily detected in formulations with turbidity greater than 4 ppm by visual examination. To insure that the liquid would remain physically stable under most shipping conditions, a cloud point of at least 60°C (140°F) was desired for the formulation. Phase separation generally occurs several degrees above the cloud point of nonionic surfactant solutions (2, 8).

The results of previous experiments, and literature concerning solubilization with nonionic surfactants suggested that Alcohol USP, polysorbate 80, propylene glycol and sucrose invert medium were the ingredients most likely to affect the turbidity and cloud point of the liquid. In the first-order experimental design, concentration ranges for these four ingredients were selected to allow the formulation of a liquid that would be acceptable for oral administration. At least 1.0% w/v Alcohol USP was needed to wet the slightly soluble drug so that it dissolved rapidly in oleic and lactic acids; however, no more than 5.0% w/v Alcohol USP was to be included in the formulation. Although polysorbate 80 and

TABLE 4
First-Order Model Parameter Estimates and Test Statistics

Response: Log_{10} (Turbidity)			
Source	Parameter Estimate	t-test Statistic	p-value
Alcohol USP	-0.014	-0.169	0.87
Polysorbate 80	-0.495	-5.871	< 0.01
Propylene Glycol	-0.415	-4.928	< 0.01
Sucrose Invert Medium	0.125	1.482	0.16
Response: Cloud Point			
Source	Parameter Estimate	t-test Statistic	p-value
Alcohol USP	1.462	1.790	0.10
Polysorbate 80	6.538	8.000	<0.01
Propylene Glycol	7.012	8.582	<0.01
Sucrose Invert Medium	-15.588	-19.076	<0.01

propylene glycol are necessary for the development of physically stable formulations, minimizing their concentrations improved the palatability of the liquid. A range of 25 to 65% w/v was chosen for sucrose invert medium in the first-order experiment because its effect on turbidity and cloud point over a broad concentration range was of interest. Sucrose invert medium concentrations of 45 to 65% w/v would actually produce the best-tasting formulations.

Data from the first-order experiment shown in Table 2 suggested the need for a transformation of the response variable, turbidity, due to nonnormality. A common logarithmic transformation was used and the transformed data was analyzed using a first-order linear model. Parameter estimates for the turbidity and cloud point models and their corresponding test statistics for testing H_0: parameter = 0 are given in Table 4. Results indicate that Alcohol USP and sucrose invert medium do not have significant effects on turbidity. No transformation was needed on the cloud point data. All components except alcohol have statistically significant effects on cloud point.

Lack-of-fit tests were performed to determine the adequacy of the first-order linear models. Results are shown in Table 5. Quadratic terms were marginally significant ($p = 0.06$) for the $\log_{10}$(turbidity) model, and severe lack of fit was detected in the cloud point model. This apparent lack of fit suggested that a better

TABLE 5
Lack-of-Fit Tests for the First-Order Linear Models

Response: Log_{10} (Turbidity)				
Source	Degrees of Freedom	Sum of Squares	F-value	p-value
Model	4	6.9269	-	-
Error	15	1.7041	-	-
Lack of Fit	12	1.6259	5.19	0.10
Interaction	11	1.3827	4.82	0.11
Quadratic	1	0.2432	9.32	0.06
Pure Error	3	0.0782		

Response: Cloud Point				
Source	Degrees of Freedom	Sum of Squares	F-value	p-value
Model	4	4406.73	-	-
Error	14	137.31	-	-
Lack of Fit	11	136.90	91.04	< 0.01
Interaction	10	127.81	93.50	< 0.01
Quadratic	1	9.09	66.50	< 0.01
Pure Error	3	0.41		

approximation to the response surface could be achieved by using a second-order model.

Contour plots of predicted turbidity and predicted cloud point from reduced linear models are given in Figures 1, and 2A to 2C. Figure 1 shows that predicted turbidity is less than 4 ppm in the upper right hand corner of the plot, corresponding to higher concentrations of polysorbate 80 and propylene glycol. Figures 2A to 2C show that cloud point increases with increasing polysorbate 80 and propylene glycol concentration, but decreases with increasing sucrose invert medium concentration. Since high levels of sucrose invert medium were desired to produce a palatable formulation, the region with the greatest sucrose invert medium concentration and cloud point over 60°C was identified on the plots. At 65% w/v sucrose invert medium, cloud point was greater than 60°C only in the upper right hand corner of the plot. This indicated that the more fruitful regions were in the upper right hand corner of the plots, and that the region considered in the first-order experiment was too large. It was decided that the second-order experiment for fitting the second-order models should focus on the region of interest, resulting in narrower ranges of the components under consideration.

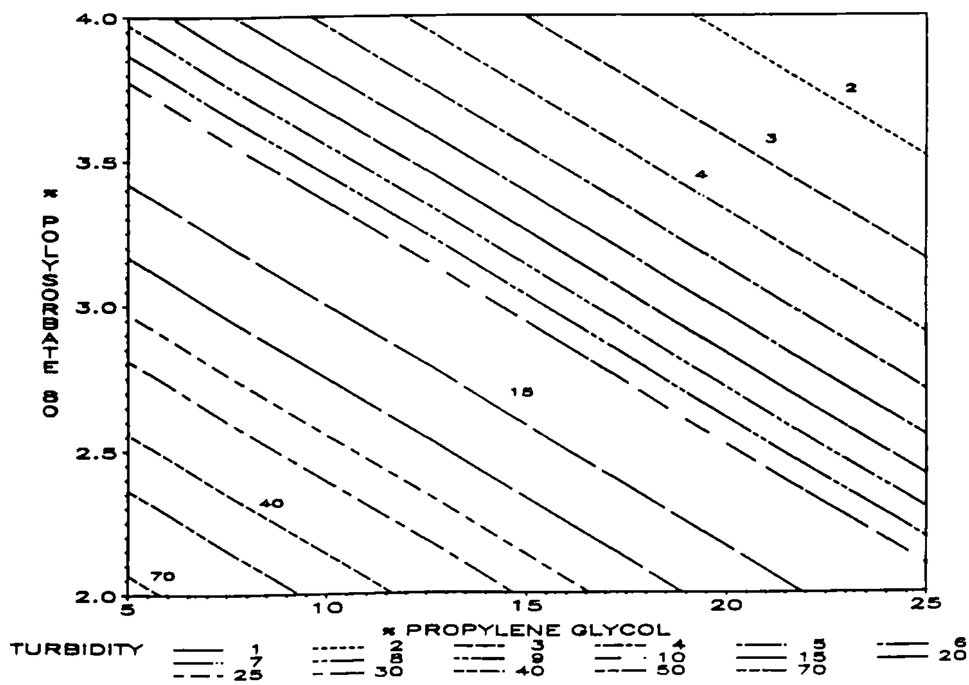

FIGURE 1
Contour Plot of Predicted Turbidity From the First-Order Linear Model.

Alcohol USP was not included as a factor in the second-order experimental design (Table 3) because it did not have a statistically significant effect on turbidity and cloud point. The concentration of Alcohol USP was fixed at 2.0% w/v, since this concentration allowed rapid dissolution of the slightly soluble drug during production of the liquid.

The second-order experimental region covered the following ranges:

Component	Concentration Range (% w/v)
Polysorbate 80	3.5 - 4.5
Propylene Glycol	15 - 25
Sucrose Invert Medium	45 - 65

The second-order model analysis for turbidity is given in Table 6. A second-order model was fitted to the data and the three components were found to

(a)

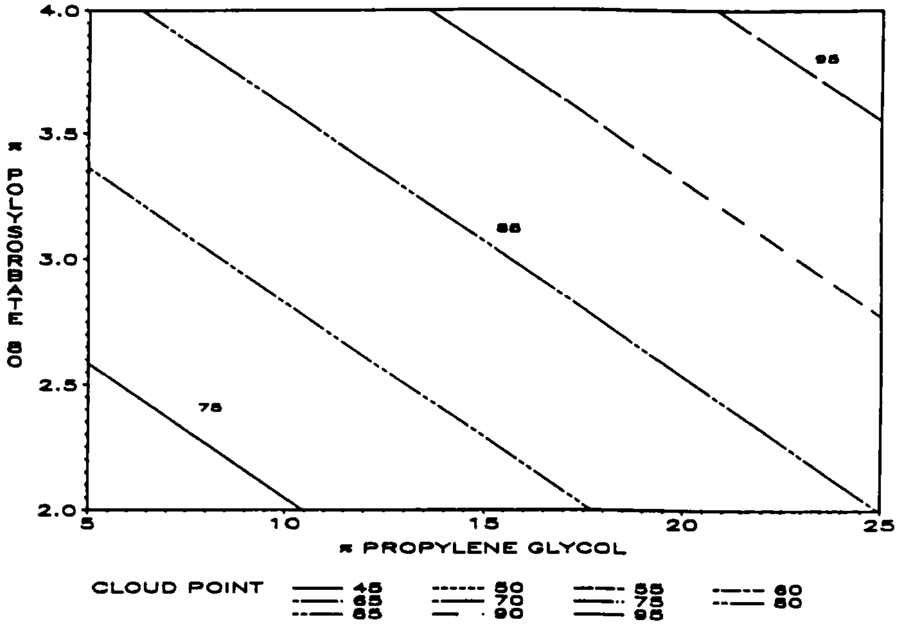

FIGURE 2A
Contour Plot of Predicted Cloud Point From the First-Order Model; Sucrose Invert Medium = 25 % w/v.

(b)

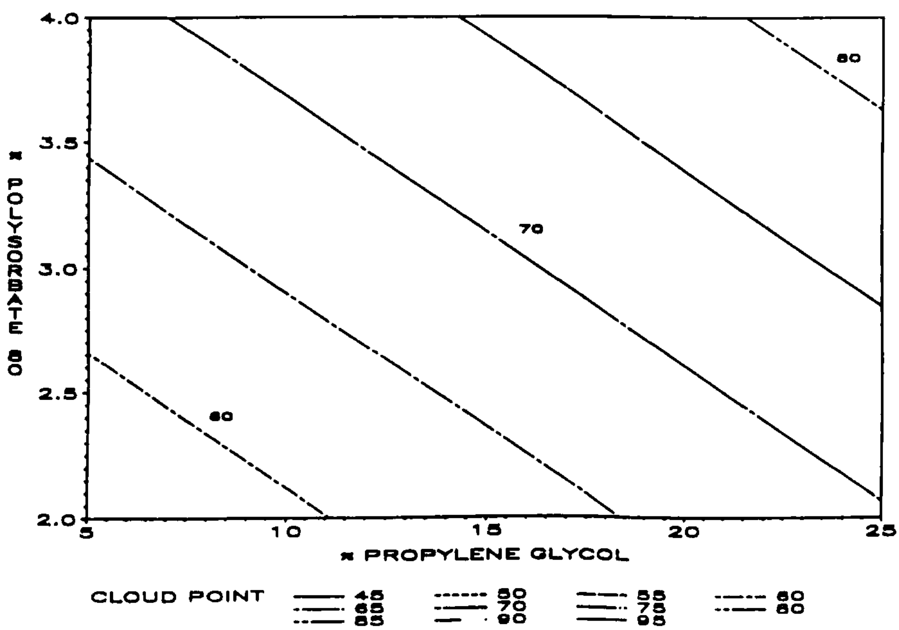

FIGURE 2B
Contour Plot of Predicted Cloud Point From the First Order Model; Sucrose Invert Medium = 45 % w/v.

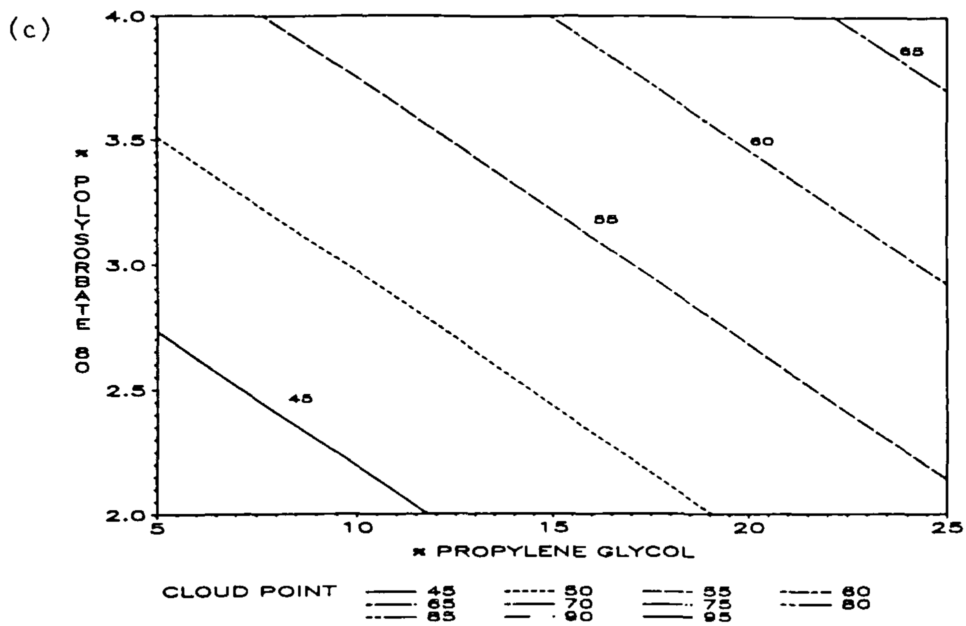

FIGURE 2C
Contour Plot of Predicted Cloud Point From the First-Order Model; Sucrose Invert Medium = 65% w/v.

exhibit statistically significant effects on turbidity. Canonical analysis resulted in the response function being expressed in three new variables w_1, w_2, and w_3 which correspond to the principal axes of the contour system. Contour plots for predicted turbidity from the fitted second-order equation are given in Figures 3A to 3C. Based on the canonical form of the fitted response function, turbidity should decrease as one moves away from the stationary point along the w_3 axis, and turbidity should increase as one moves away from the stationary point along the w_1 or w_2 axes. This indicated that the stationary point was a saddlepoint, and that one must move only along the w_3 axis in order to minimize turbidity. The combination of components that yielded the lowest predicted turbidity (1.10 ± 1.07) in the experimental region is 4.5% w/v polysorbate 80, 22% w/v propylene glycol, and 65% w/v sucrose invert medium.

A second-order model was similarly fitted to the cloud point data. Results of the analysis are given in Table 7. The only significant term in the model was the linear term corresponding to the concentration of sucrose invert medium.

TABLE 6
Second-Order Model Analysis for Turbidity

Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	F-ratio	p-value
Model	9	14.16	3.25	0.04
Error	10	4.84	-	-
Lack of Fit	5	3.05	1.70	0.29
Pure Error	5	1.79		

Parameter Estimates

Parameter	Estimate	Standard Error	p-value
Intercept	3.045	0.283	< 0.01
Polysorbate 80	-0.595	0.189	0.01
Propylene Glycol	-0.148	0.189	0.45
Sucrose Invert Medium	0.120	0.189	0.54
$(\text{Polysorbate 80})^2$	0.242	0.186	0.22
$(\text{Propylene Glycol})^2$	0.494	0.186	0.02
$(\text{Sucrose Invert Medium})^2$	-0.064	0.186	0.74
Polysorbate 80 * Propylene Glycol	0.050	0.246	0.84
Polysorbate 80 * Sucrose Invert Medium	-0.400	0.246	0.13
Propylene Glycol * Sucrose Invert Medium	-0.650	0.246	0.02

Stationary Point:

	Coded Value	Actual Value (% w/v)
Polysorbate 80	0.834	4.25
Propylene Glycol	-0.227	19.32
Sucrose Invert Medium	-0.508	51.95

Predicted Turbidity at the Saddle Point (± Standard Error): 2.78 ± 0.308

Canonical Form of the Second-Order Equation:

$$\text{Turbidity} = 2.78 + 0.672w_1^2 + 0.272w_2^2 - 0.272w_3^2$$

Within the experimental region of the second design (a central composite design), the simple first-order linear model with the concentration of sucrose invert medium adequately described the cloud point response function. The first-order model analysis of the cloud point data is presented in Table 8. A plot of the response function with its 95% prediction limits is given in Figure 4. The first-order fitted model shows cloud point decreasing as the concentration of sucrose

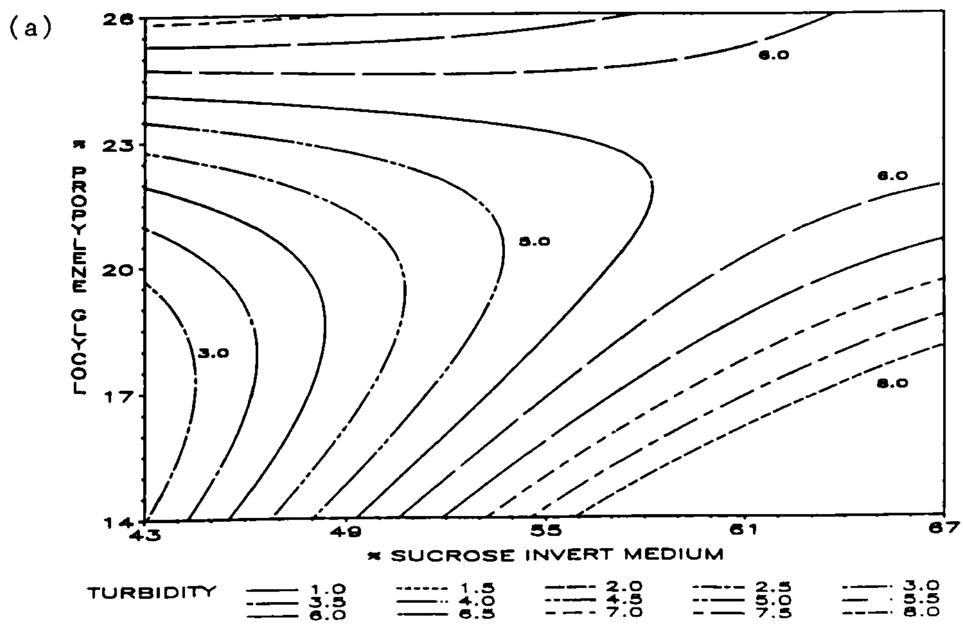

FIGURE 3A
Contour Plot of Predicted Turbidity from the Second-Order Linear Model; Polysorbate 80 = 3.4 % w/v.

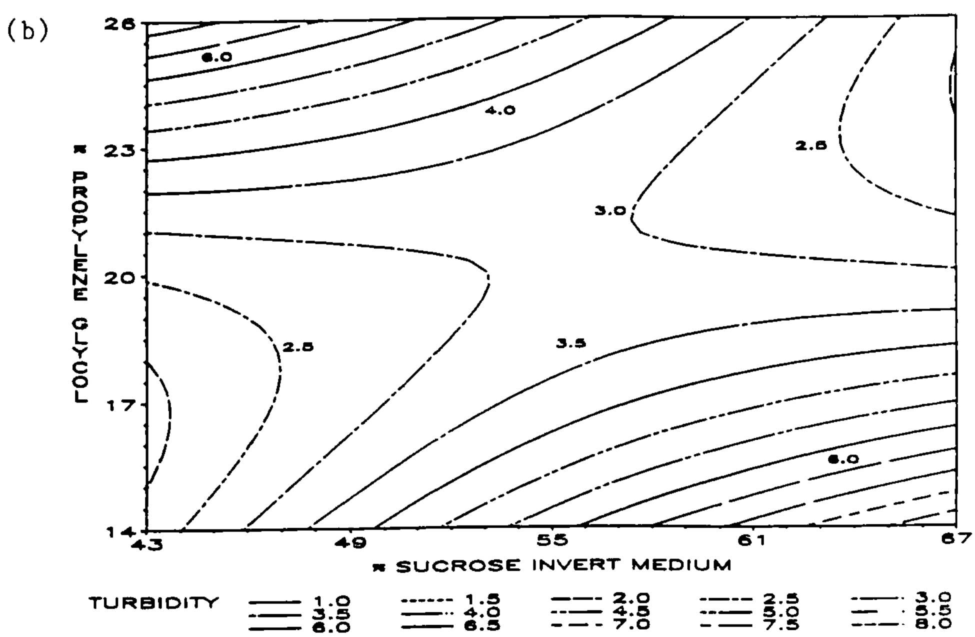

FIGURE 3B
Contour Plot of Predicted Turbidity From the Second-Order Linear Model; Polysorbate 80 = 4.0 % w/v.

(c)

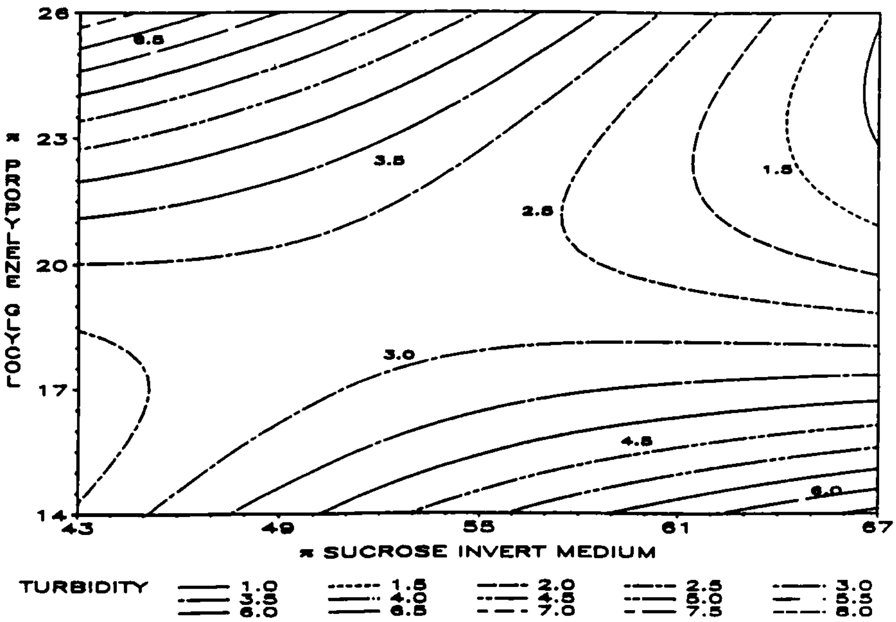

FIGURE 3C
Contour Plots of Predicted Turbidity From the Second-Order Linear Model; Polysorbate 80 = 4.3 % w/v.

invert medium increases. Since a high concentration of sucrose invert medium was desired, inverse regression (12) was applied to the reduced model to determine the highest concentration of sucrose invert medium at which one can be 95% confident that cloud point will be greater than 60°C. The highest concentration of sucrose invert medium at which one can be 95% confident that cloud point is at least 60°C is 61.2% w/v. With 61.2% w/v sucrose invert medium, turbidity is minimized by setting polysorbate 80 at 4.4% w/v and propylene glycol at 21.2% w/v.

A follow-up experiment was performed to confirm the predicted optimal combination of components. In addition to this optimal combination of components, another combination consisting of 4.5% w/v polysorbate 80, 22% w/v propylene glycol, and 65% w/v sucrose invert medium was investigated. Three determinations of turbidity and cloud point were performed on each of ten samples of the two most desirable formulations. Results shown in Table 9

TABLE 7
Second-Order Model Analysis for Cloud Point

Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	F-ratio	p-value
Model	9	481.3	2.53	0.08
Error	10	211.5		
Lack of Fit	5	173.4	4.54	0.06
Pure Error	5	38.1		

Parameter Estimates

Parameter	Estimate	Standard Error	p-value
Intercept	72.23	1.87	< 0.01
Polysorbate 80	0.84	1.25	0.52
Propylene Glycol	0.26	1.25	0.84
Sucrose Invert Medium	-5.60	1.25	< 0.01
$(\text{Polysorbate 80})^2$	0.59	1.23	0.64
$(\text{Propylene Glycol})^2$	1.21	1.23	0.35
$(\text{Sucrose Invert Medium})^2$	-0.74	1.23	0.56
Polysorbate 80 * Propylene Glycol	-0.15	1.63	0.93
Polysorbate 80 * Sucrose Invert Medium	0.92	1.63	0.58
Propylene Glycol * Sucrose Invert Medium	-0.72	1.63	0.67

TABLE 8
Fitting First-Order Model to Cloud Point Data from the Central Composite Design

Analysis of Variance

Source	Degrees of Freedom	Sum of Squares	F-ratio	p-value
Model (Sucrose Invert Medium)	1	424.6	28.5	< 0.01
Error	18	268.2		
Lack of Fit	13	230.1	2.3	0.18
Pure Error	5	38.1		

Parameter	± Standard Error
Intercept	72.94 ± 0.86
Sucrose Invert Medium	-5.60 ± 1.05

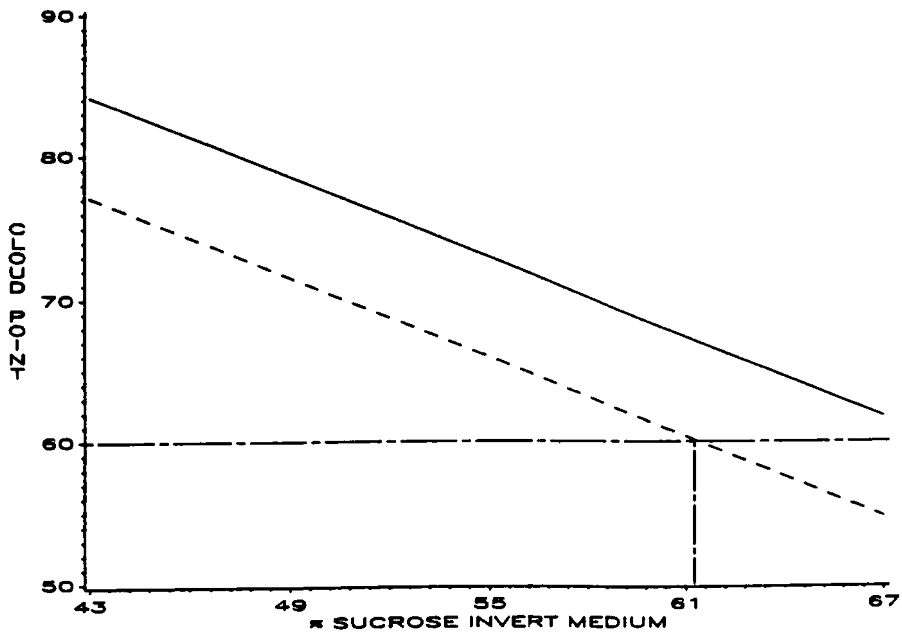

FIGURE 4
Response Function for Cloud Point with 95% Lower Prediction Limits.

corroborated analytical results obtained through response surface methods. Variance component analyses of the confirmatory runs, Table 10, showed that turbidity measurement variation is about eight times the "between samples" variation. This may be because each sample was divided into three separate vials for the repeat turbidity measurements, and the measurements are somewhat dependent on the clarity and cleanliness of the vials. Variation in cloud point data was about evenly attributable to measurement variability and sampling variability. Summary statistics for the confirmatory experiment are given in Table 11. No statistically significant differences in turbidity and cloud point were observed between the two combinations.

The concentration of sucrose invert medium should be set at 61.2% w/v to insure with 95% confidence that the formulation will have an acceptable cloud point and will be the best-tasting product possible. Minimum turbidity at 61.2% w/v sucrose invert medium in the experimental region can be achieved by setting polysorbate 80 at 4.4% w/v and propylene glycol at 21.2% w/v. This

TABLE 9
Results of Confirmatory Runs

Component	Optimal Combination (% w/v)		Second Combination (% w/v)	
Polysorbate 80	4.4		4.5	
Propylene Glycol	21.2		22.0	
Sucrose Invert Medium	61.2		65.0	
Sample	**Turbidity (ppm)**	**Cloud Point (°C)**	**Turbidity (ppm)**	**Cloud Point (°C)**
1	2.3	64.3	2.0	63.4
	2.3	63.6	2.0	66.7
	2.4	65.2	2.0	66.1
2	2.4	65.2	2.3	65.9
	2.5	64.9	2.2	64.5
	2.3	65.3	2.3	66.1
3	2.5	63.8	2.1	63.0
	2.2	64.6	2.1	64.2
	2.3	65.0	2.1	64.1
4	2.3	66.6	2.1	65.2
	2.3	66.6	2.2	64.9
	2.3	66.8	2.1	64.4
5	2.1	61.5	2.3	65.6
	2.4	61.6	1.7	66.3
	2.0	62.4	1.9	65.1
6	2.1	67.1	2.5	64.8
	2.2	63.5	2.1	64.3
	2.2	64.0	4.9	65.2
7	1.9	65.8	2.6	61.4
	1.9	63.0	2.3	63.7
	2.0	63.2	1.4	63.4
8	2.1	64.4	2.1	59.5
	2.0	66.1	2.1	64.2
	2.0	65.1	2.2	62.7
9	1.8	66.8	2.3	62.1
	1.7	65.0	2.1	61.8
	1.9	66.0	2.2	60.3
10	1.8	64.2	2.2	60.8
	1.9	65.3	3.1	62.6
	3.8	63.3	3.4	62.6

TABLE 10
Confirmatory Experiment: Variance Component Analysis

Turbidity (ppm) Source	Degrees of Freedom	Sum of Squares	Mean Square	Variance Component
Between Combinations	1	0.15	0.15	-
Between Samples	18	5.69	0.32	0.03
Between Measurement	40	9.11	0.23	0.23

Cloud Point (°C) Source	Degrees of Freedom	Sum of Squares	Mean Square	Variance Component
Between Combinations	1	10.67	10.67	-
Between Samples	18	115.65	6.42	1.73
Between Measurement	40	48.93	1.22	1.22

TABLE 11
Summary Statistics for Confirmatory Experimental Runs

Component	Optimal Combination (% w/v)		Second Combination (% w/v)	
Polysorbate 80	4.4		4.5	
Propylene Glycol	21.2		22.0	
Sucrose Invert Medium	61.2		65.0	
	Turbidity (ppm)	Cloud Point (°C)	Turbidity (ppm)	Cloud Point (°C)
Mean	2.2	64.7	2.3	63.8
Standard Deviation	0.37	1.50	0.61	1.85
Median	2.2	64.95	2.15	64.2
Mode	2.3	65	2.1	62.6
Minimum	1.7	61.5	1.4	59.5
Maximum	3.8	67.1	4.9	66.7
Interquartile Range	0.3	2.3	0.2	2.6

combination of components yielded a predicted turbidity value of 1.93 ppm (s.e. = 0.61), and a cloud point of 67.12°C (s.e. = 1.39). A formulation with this composition would be acceptable for oral administration.

The inverse linear relationship between cloud point and the concentration of sucrose invert medium in the formulations within the experimental region for the second-order design correlates with characteristics of nonionic surfactant solutions that are discussed in the literature. Cloud point is dependent on the structure of the nonionic surfactant and the composition of the aqueous solution (4). For a given nonionic surfactant, changes in an aqueous solution that decrease its solubility, decrease the solution's cloud point. In the formulation, increasing sucrose invert medium would decrease the amount of water that is available to solubilize the hydrophilic portion of the polysorbate 80 molecules, leading to lower cloud points.

A complex relationship among the concentrations of polysorbate 80, propylene glycol and sucrose invert medium and the turbidity of the formulations was predicted by the second-order model for turbidity. More experimentation would be necessary to develop a probable mechanism for this relationship.

CONCLUSIONS

Response surface methodology provided an efficient approach to optimization of the turbidity and cloud point of the nonionic surfactant solution. The formulation goals of turbidity less than 4 ppm and cloud point greater than 60°C were met, and the concentrations of polysorbate 80, propylene glycol and sucrose invert medium were maintained within ranges that were desirable for an oral liquid. The optimized formulation was suitable for flavoring and probe stability studies.

An inverse linear relationship between sucrose invert medium concentration and the cloud point of the formulation was determined within the experimental region for the second-order model. The second-order model that was fitted to turbidity data indicated that a complex relationship among the concentrations of polysorbate 80, propylene glycol and sucrose invert medium within the experimental region determined the turbidity of the formulation.

NOTES

1. Model 151, Monitek, Inc., Hayward CA.
2. Monitek Turbidity Standard, Monitek, Inc., Hayward CA.
3. 25 x 95 mm Vials, Kimble Division, Owen-Illinois, Inc., Toledo OH.
4. Model FP800 Thermosystem, Mettler Instrument Corp., Hightstown NJ.
5. Melting point tubes, Mettler Instrument Corp., Hightstown NJ.
6. FP81 MBC Cell, Mettler Instrument Corp., Hightstown NJ.
7. FP80 Central Processor, Mettler Instrument Corp., Hightstown NJ.
8. GA44 Thermal Printer, Mettler Instrument Corp., Hightstown NJ.

REFERENCES

1. A. Cammarata, A. Martin and J. Swarbrick, "Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences," Third Edition, Lea and Febiger, Philadelphia, 1983.
2. M. Bourrel and R.S. Schechter, "Microemulsions and Related Systems: Formulation, Solvency, and Physical Properties," Marcel Decker, Inc., New York, 1988.
3. R.H. Myers, "Response Surface Methodology," Allyn and Bacon, Boston, 1976.
4. P.W.M. John, "Statistical Design and Analysis of Experiments," The Macmillan Company, New York, 1971.
5. G.E.P. Box and N.R. Draper, "Empirical Model-Building and Response Surfaces," John Wiley and Sons, New York, 1987.
6. G.E.P. Box, W.G. Hunter and J.S. Hunter, "Statistics for Experimenters," John Wiley and Sons, New York, 1978.
7. "SAS User's Guide: Statistics," Version 5 Edition, SAS Institute Inc., Cary, North Carolina, 1985.
8. L.M. Prince, "Microemulsions," Academic Press, Inc., New York, 1977.
9. N.R. Draper and H. Smith, "Applied Regression Analysis," Second Edition, John Wiley and Sons, New York, 1981.