Drug Development and Industrial Pharmacy, 22(9&10), 881-889 (1996)

A Four-Component Study for Estimating Solubilities of a Poorly Soluble Compound in Multisolvent Systems Using a Scheffé-Type Model

Mickey L. Wells,[1,]* Wei-Qin Tong,[1] Jack W. Campbell IV,[1] Ellen O. McSorley,[2] and Michael R. Emptage[2]

[1]*Pharmaceutics, and* [2]*Statistical Services, GlaxoWellcome Inc., 5 Moore Drive, Research Triangle Park, North Carolina 27709*

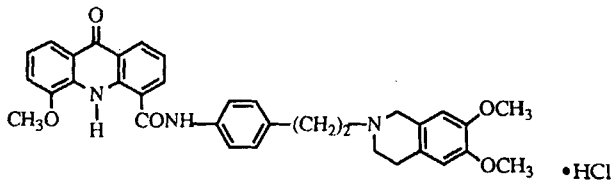
ABSTRACT

The equilibrium solubility of GF120918A, a poorly soluble compound with potential use as a multidrug resistance (MDR) inhibitor of P-glycoprotein, was studied at 25°C in multisolvent systems containing polyethylene glycol 300, polysorbate 80, ethanol, and water. The objective was to determine the feasibility, with respect to solubility, of formulating a concentrated formulation for product presentation in an ampule or vial. Data were fit to a quadratic Scheffé-type model with excellent correlation between the experimentally determined and fitted equilibrium solubilities (R^2 = 0.9875, slope = 1.043). Solubilities greater than 4 mg base/mL at 25°C were determined for mixtures in this study, making it feasible, with regard to solubility, to formulate a concentrated vial or ampule formulation. Maximum solubility, however, was dependent on the ability to adjust the apparent pH to ≤3.5 in the cosolvent/surfactant systems studied.

INTRODUCTION

Many of today's drugs being synthesized and developed for parenteral dosage forms, while venturing into new therapeutic classes with novel mechanisms of action, leave something to be desired with respect to solubility. The compound discussed in this article, GF120918A, is no exception. GF120918A is a compound that shows potential for the reversal of multidrug resistance (MDR) in cancer patients expressed through P-glycoprotein (1). This class of compounds is collectively known as MDR inhibitors. The structure of GF120918A is shown.

*To whom correspondence should be addressed.



GF120918A
MW: 600

Figure 1 shows the pH–solubility profile of GF120918A corrected for buffer and common ion effects. This compound has an equilibrium solubility of >>0.7 mg base/mL in the portion of its pH–solubility curve limited by the solubility of the hydrochloride salt. The objectives of this study were, first, to investigate the feasibility of a cosolvent, surfactant system to increase the solubility of GF120918A in order to place it into an ampule or vial. A second objective was to study how well a Scheffé-type model fits equilibrium solubilities within the experimental design space.

Based on preliminary studies, polyethylene glycol 300 (PEG 300), ethanol, and polysorbate 80 were chosen as the cosolvents and surfactant to study the production of concentrated formulation.

THEORETICAL

Although factorial designs are very useful for studying many variables at several levels, they are not suitable for studying mixtures because of the constraint $\Sigma X_i = 1$, where X_i is the level of the i-th component; i.e., the level of one component is fixed by the levels of the others. Therefore, a Scheffé-type model was used to characterize the solubility in the design space of interest (2,3). This approach has been used and published in previous papers (4–7). Scheffé-type models are particular forms of polynomial equations that can be used with variables that are expressed as proportions of the total mixture. These models are extremely versatile in terms of modeling the linear and nonlinear blending properties, e.g., solubility, of the constituents in the mixtures. The general equation of the quadratic Scheffé-type model used to fit the data from this experiment is:

$$h = \sum_{i=1}^{q} b_i X_i + \sum_{i<j}^{q-1} \sum_{i<j}^{q} b_{ij} X_i X_j$$

where h represents the response, e.g., solubility, X_i and X_j are the fractions of two different components of the mixture out of a total of q components, and b_i and b_{ij} are fitted coefficients for linear and quadratic terms X_i and X_iX_j, respectively.

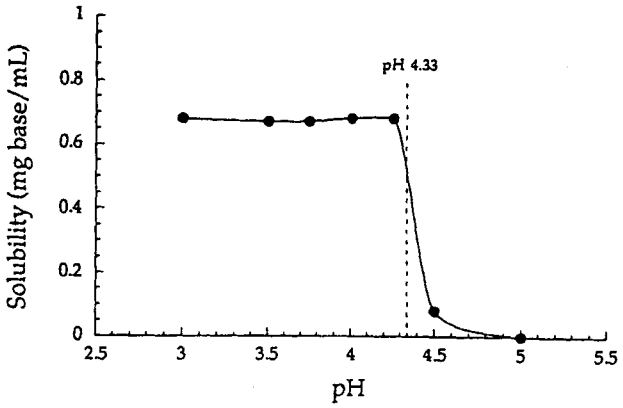


Figure 1. The pH–solubility profile, corrected for buffer and common ion effects, of GF120918A.

EXPERIMENTAL METHODS AND MATERIALS

Excipient Ranges and Systems Studied

A mixture experiment was designed to study the solubility of GF120918A over the following concentration ranges of materials: PEG 300: 0–67.1% w/w (Mallinckrodt, Lot E822-68/1189-JM), polysorbate 80: 0–10.8% w/w (Sigma Chemical Co., Lot 91H0685), 95% v/v ethanol USP: 0–31% w/w (Aaper Alcohol & Chemical Co., Lot 92611UA), and water: 32.9–100% w/w (Millipore Milli-Q Plus Ultra-Pure Water System). The ranges for these excipients were based on ranges used in currently marketed parenteral products. The hyperpolyhedron that defines all possible formulations using the above concentration restrictions is given in Fig. 2. Table 1 lists the components, their concentrations, and location in the design space. Twenty-one different mixtures were used to characterize the design space. The center point was determined in triplicate to provide a measure of variability in the solubility determinations.

Preparation of Samples

The appropriate amounts of PEG 300, polysorbate 80, ethanol, and water for a particular mixture were weighed into 25-mL Erlenmeyer flasks. The total volume for each mixture was approximately 10 mL. Next, 174 μL of glacial acetic acid (J. T. Baker, Lot E09833) was added to each mixture. At this intermediate point, the apparent pH of the mixture was taken. Each mixture

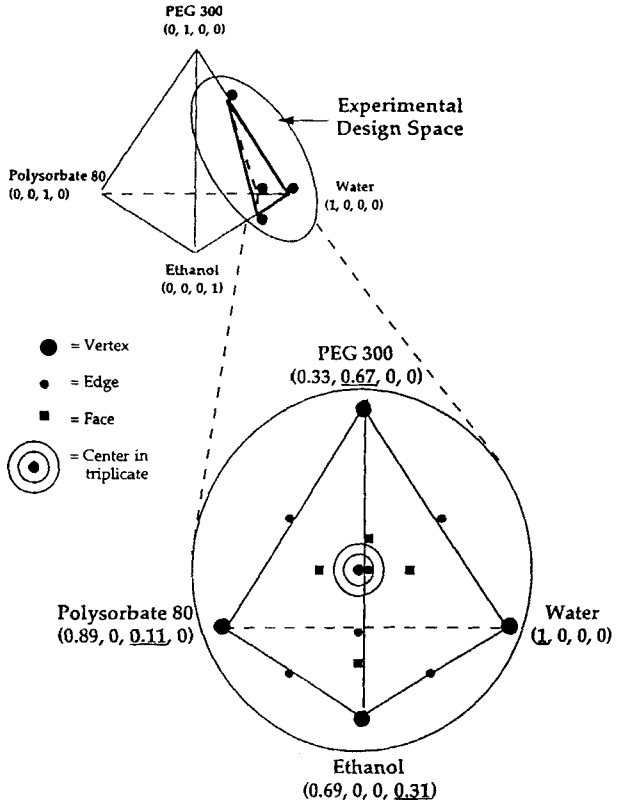


Figure 2. The hyperpolyhedron that defines all possible formulations using the concentration restrictions for this study (numbers given in terms of % w/w).

was then titrated to pH 3.5 with N/2 sodium hydroxide (Fisher Chemical, Lot 903803-24), if necessary. A pH of 3.5 was chosen based on the pH–solubility profile (see Fig. 1) and acceptable pH ranges for small-volume parenterals (8). Approximately 100 mg of GF120918A was added to each mixture and the flask was stoppered. These flasks were all placed in the same shaking water bath at a temperature of 25°C and shaking level of 3 (Neslab Exacal Shaking Bath, Model EX700, Neslab MTP-5 Programmer, Neslab DR-2 Digital Readout, Newington, NH). Samples were withdrawn and analyzed by reversed-phase high-performance liquid chromatography (HPLC) weekly, for up to 4 weeks until the change in concentration from the previous week was $< 10\%$. This value was then taken as the equilibrium solubility in that mixture. Data were statistically analyzed using RS/1 (BBN Software Products Corp., Cambridge, MA).

Quantitative Method for GF120918A Analysis

The concentration of GF120918A was determined by reversed-phase HPLC using a mobile phase composed of 25% tetrahydrofuran (Baxter, Lot BC108)/75% aqueous trifluoroacetic acid (0.1% v/v) (J. T. Baker, Lot E296), a flow rate of 1.0 mL/min., an injection volume of 20 μL, an an analytical wavelength of 235 nm. The typical retention time for GF120918A was 14 min. The HPLC system used for the analysis included the following items: Digital Color Monitor, Digital VAXstation 3100, Digital LJ250 Companion Color Printer, Waters (Milford, MA) 510 HPLC Pump, Waters System Interface Module, Waters LAC/E, Waters 715 Ultra WISP Sample Processor, Waters 490E Programmable Multiwavelength Detector and a Keystone Scientific, Inc. (Bellefonte, PA) HPLC column: BDS Hypersil® C18, 250 × 4.6 mm, 5-μ particle size, 120-Å pore size.

RESULTS AND DISCUSSION

For purposes of statistical modeling, the concentrations of the various components were normalized such that the lowest concentration of a component was assigned a value of 0 and the highest a 1. For example, mixture number 1 in Table 1 would be designated a concentration of 1 with respect to PEG 300 at a concentration of 67.1% w/w and 0 with respect to water at a concentration of 32.9% w/w.

The equilibrium solubilities for mixtures 1–21 in Table 1 were fit to a quadratic Scheffé-type model. Table 2 gives the least squares summary analysis of variance (ANOVA) for this fitted quadratic Scheffé-type model. One notices in Table 2 that the residual lack of fit term is very significant (F ratio = 37.49).

Upon plotting the studentized residual versus the mixture number, Fig. 3, mixture number 1 falls far off the residual = 0 line. Normally one expects the residuals in this plot to fall between −2 and 2 (8). During sample preparation, mixture number 1 was the only mixture that could not be adjusted to an apparent pH of 3.5 because of the high concentration of PEG 300. Mixture number 1 had pHs of 4.33 and 4.03 for the initial and 4-week time points, respectively. The pH change for all the remaining mixtures was less than 6% from the initial to the 4-week time point. It appears that the pH of mixture number 1 is high enough that the compound's solubility is limited by the free base rather than the salt. One can envision this by extrapolating the

Table 1

The Components, Their Concentrations, and Location in the Design Space for This Study

Mixture[a]	Point Type	% w/w PEG 300	% w/w Polysorbate 80	% w/w Water	% w/w Ethanol
1	Vertex	67.1	0	32.9	0
2	Vertex	0	10.8	89.2	0
3	Vertex	0	0	69.0	31.0
4	Vertex	0	0	100.0	0
5	Edge	34.6	5.3	60.1	0
6	Edge	36.3	0	49.4	14.3
7	Edge	33.9	0	66.1	0
8	Edge	0	5.4	80.1	14.5
9	Edge	0	5.4	94.6	0
10	Edge	0	0	85.4	14.6
11	Face	23.7	3.7	63.1	9.5
12	Face	23.6	3.5	72.9	0
13	Face	24.7	0	65.9	9.4
14	Face	0	3.8	86.5	9.7
15	Center	17.9	2.8	71	8.3
16	Center	17.9	2.8	71	8.3
17	Center	17.9	2.8	71	8.3
18	Inside Point	35.4	1.8	57.9	4.9
19	Inside Point	12.1	5.5	77.6	4.8
20	Inside Point	12.5	1.9	70.8	14.8
21	Inside Point	12	1.8	81.4	4.8

[a]All solutions except mixture number 1 contained 0.30 M acetate buffer adjusted to pH 3.48 ± 0.06. Mixture number 1 contained 0.30 M acetate buffer at pH 4.33.

aqueous pH-solubility curve given in Fig. 1 to the cosolvent/aqueous system of mixture number 1. The dotted line in Fig. 1 marks pH 4.33, which is in the pH range where the solubility is limited by the free base. This coupled with the fact that the pK_a of a weak base is usually lowered in mixed solvent systems makes this the likely cause of mixture number 1 being an outlier in the initial statistical analysis of the data (10). Previous

Table 2

Least Squares Summary ANOVA (n = 21)

Source	*df*	Sum Sq.	Mean Sq.	*F* Ratio	Significance
Total (corr.)	20	14.33852			
Regression	9	13.24113	1.47124	14.75	0.0001
Linear	3	9.58337	3.19446	32.02	0.0000
Nonlinear	6	3.65776	0.60963	6.11	0.0050
Residual	11	1.09740	0.09976		
Lack of fit	9	1.09093	0.12121	37.49	0.0263
Pure error	2	0.00647	0.00323		

Note. R^2 = 0.9235; R^2-adj. = 0.8608.

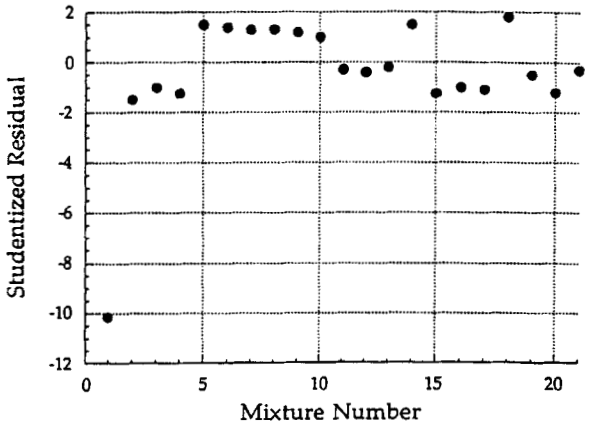


Figure 3. Studentized residuals (n = 21) of equilibrium solubilities of mixtures from Table 1.

studies of this nature have either been able to adequately adjust the pH of the mixtures studied (7) or have not had to be buffered to a particular pH, possibly because the pK_a was sufficiently removed from the pHs of the mixtures studied (5,6). Figure 3 is a textbook example of the utility of examining residual plots when applying statistical models to data.

Mixture number 1 was removed from the modeling and another quadratic Scheffé-type model was fit to the data for mixture numbers 2–21. Tables 3, 4, and 5 give the least squares components ANOVA, least squares coefficients, and least squares summary ANOVA, respectively, for the fitted quadratic Scheffé-type model. From those tables, it is seen that PEG 300, polysorbate 80, and ethanol were significant at the $p < 0.01$ level at increasing the equilibrium solubility. One notices that

Table 3

Least Squares Components ANOVA (n = 20)

Source	*df*	Sum Sq.	*F* Ratio	Significance
P	1	18.7473	1921.00	0.0000
P80	1	11.7408	1203.00	0.0000
E	1	9.4081	963.90	0.0000
W	1	0.2870	29.41	0.0003
P·P80	1	1.0300	105.50	0.0000
P·E	1	0.0917	9.40	0.0119
P·W	1	0.8643	88.55	0.0000
P80·E	1	0.4937	50.58	0.0000
P80·W	1	0.0486	4.98	0.0497
E·W	1	0.3077	31.53	0.0002
Residual	10	0.0976		

Note. P = PEG 300; P80 = polysorbate 80; E = ethanol; W = water.

Table 4

Least Squares Coefficients (n = 20)

Term	Coeff.	Std. Error	*T* value	Significance
P	8.107857	0.485860		
P80	3.122868	0.097156		
E	2.972743	0.097154		
W	0.846184	0.097161		
P·P80	-9.440617	0.919037	-10.27	0.0001
P·E	-2.817435	0.919104	-3.07	0.0119
P·W	-8.645194	0.918720	-9.41	0.0001
P80·E	-3.022757	0.425023	-7.11	0.0001
P80·W	0.948602	0.424941	2.23	0.0496
E·W	-2.386028	0.424935	-5.62	0.0002

Note. No. cases = 20; R^2 = 0.9993; RMS error = 0.0988; Resid. *df* = 10; R^2-adj. = 0.9985; Cond. No. = 16.92. P = PEG 300; P80 = polysorbate 80; E = ethanol; w = Water.

Table 5

Least Squares Summary ANOVA (n = 20)

Source	*df*	Sum Sq.	Mean Sq.	*F*- Ratio	Significance
Total (Corr.)	20	131.6773			
Regression	10	131.5797	13.1580	1348.00	0.0000
Linear	4	127.7808	31.9452	3273.00	0.0000
Nonlinear	6	3.7989	0.6332	64.87	0.0000
Residual	10	0.0976	0.0098		
Lack of fit	8	0.0911	0.0114	3.52	0.2398
Pure error	2	0.0065	0.0032		

Note. R^2 = 0.9993; R^2 adj. = 0.9985.

the lack of fit term is no longer significant in Table 5 and that the adjusted R^2 has increased from 0.8608 to 0.9985 from Table 2 (n = 21) to Table 5 (n = 20).

Using the fitted quadratic Scheffé-type model given in Table 4, fitted concentrations were determined for all mixtures in Table 1 with the exception of mixture number 1. Figure 4 shows that the model did an excellent job fitting the response surface of equilibrium solubilities that were experimentally determined (R^2 = 0.9875, slope = 1.043).

Figure 5, 6, 7, and 8 show contour plots of equilibrium solubilities in the design space studied. These contour lines were generated using the n = 20 fitted quadratic Scheffé-type model given in Table 4. Figure 5 shows that the highest solubilities (>4 mg base/mL) occur at high concentrations of PEG 300. However, this area is an extrapolation of the study since mixture number 1 was not included in fitting this model because the apparent pH of the mixture could not be adjusted to 3.5. Therefore, the apparent pH values of the mixtures after addition of the glacial acetic acid, but before addition of any N/2 sodium hydroxide, were fit to a Scheffé-type model. This analysis would allow one to delineate exactly where the apparent pH of the mixtures is = 3.5, this pH separating the area where the Scheffé-type model for solubility is applicable in Figs. 5–8 (apparent pH ≤ 3.5) and where the Scheffé-type model is an extrapolation and not real (apparent pH > 3.5).

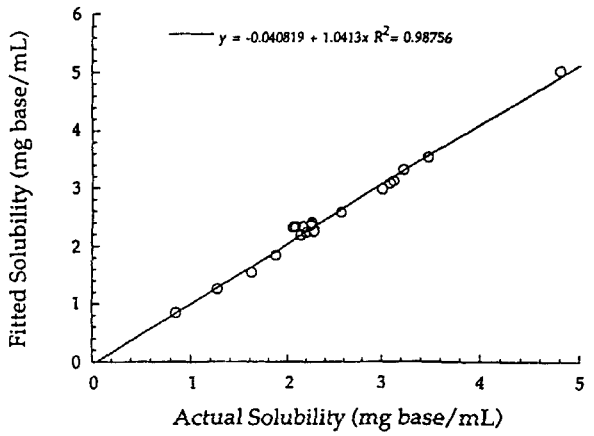


Figure 4. Correlation between experimentally determined and fitted equilibrium solubilities for the quadratic Scheffé model (n = 20).

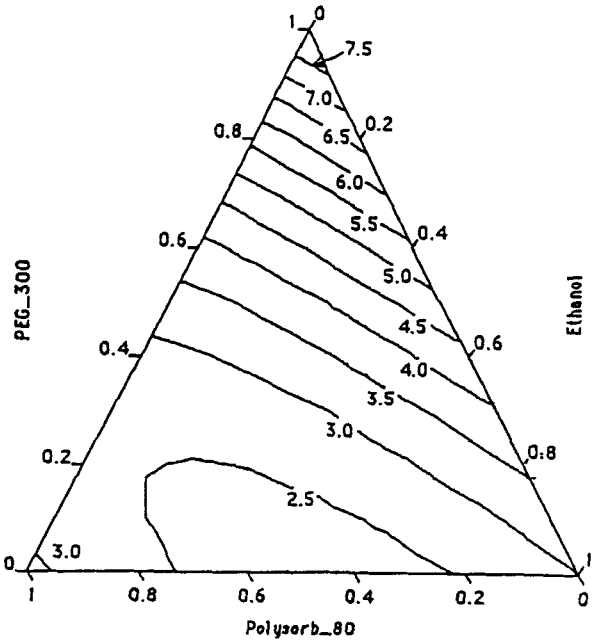


Figure 5. Contour plot of GF120918A equilibrium solubilities at 25°C based on the quadratic Scheffé-type model (n = 20), tetrahedron face 1 of 4.

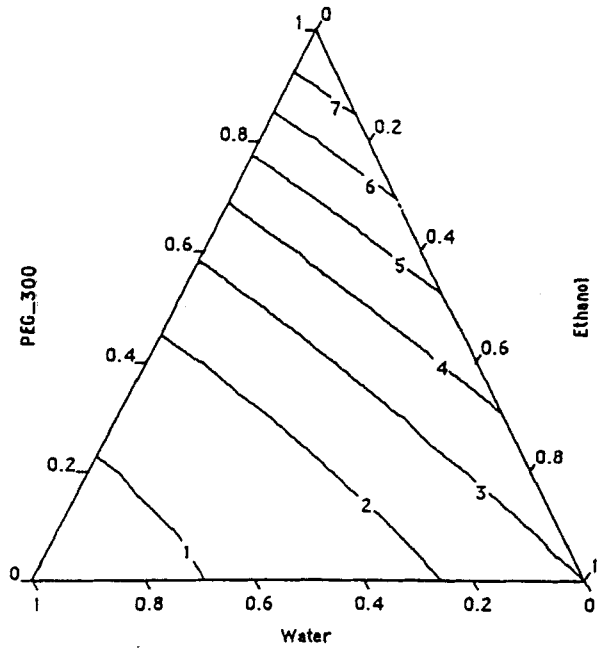


Figure 6. Contour plot of GF120918A equilibrium solubilities at 25°C based on the quadratic Scheffé-type model (n = 20), tetrahedon face 2 of 4.

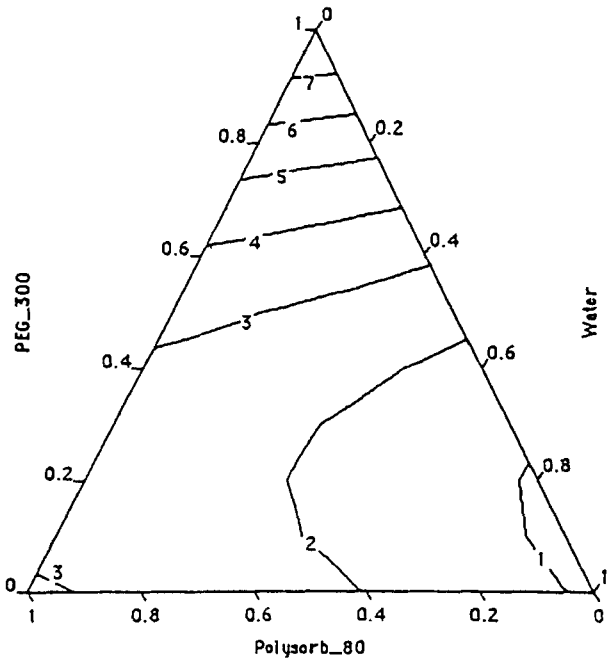


Figure 7. Contour plot of GF120918A equilibrium solubilities at 25°C based on the quadratic Scheffé-type model (n = 20), tetrahedon face 3 of 4.

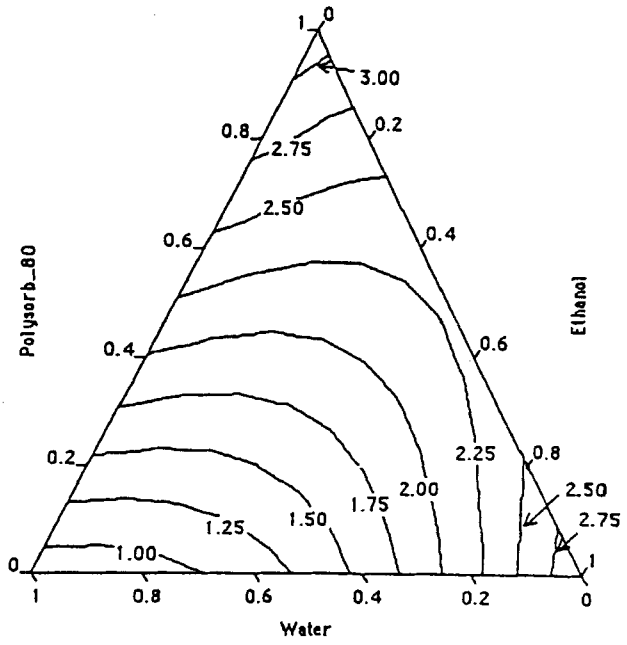


Figure 8. Contour plot of GF120918A equilibrium solubilities at 25°C based on the quadratic Scheffé-type model (n = 20), tetrahedon face 4 of 4.

Tables 6, 7, and 8 give the least squares components ANOVA, least squares coefficients, and least squares summary ANOVA, respectively, for the fitted quadratic Scheffé-type model to the apparent pH for all mixtures in Table 1 (n = 19 because one mixture was done in triplicate). Since the 5% critical value of an $F_{10,9}$ distribution is 5.26 and the observed F ratio is 46396.14, which is far more than 10 times the critical value, the

Table 6

Least Squares Components ANOVA (n = 19)

Source	*df*	Sum Sq.	*F* Ratio	Significance
P	1	19.07	50353.01	0.0000
P80	1	8.08	21335.60	0.0000
W	1	6.95	18340.00	0.0000
E	1	8.74	23071.10	0.0000
P·P80	1	0.03	86.61	0.0000
P·W	1	0.15	387.08	0.0000
P80·W	1	0.00	0.14	0.7182
P·E	1	0.01	16.66	0.0027
P80·E	1	0.00	3.37	0.0995
E·W	1	0.00	12.98	0.0057

Note. P = PEG 300; P80 = polysorbate 80; E = ethanol; w = Water.

Table 7

Least Squares Coefficients (n = 19)

Term	Coeff.	Std. Error	*T* value	Significance
P	4.289	0.01911	224.39	0.0000
P80	2.792	0.1911	146.07	0.0000
W	2.588	0.01911	135.43	0.0000
E	2.903	0.01911	151.89	0.0000
P·P80	-0.757	0.08138	-9.31	0.0000
P·W	-1.601	0.08138	-19.67	0.0000
P80·W	-0.030	0.08138	-0.37	0.7182
P·E	-0.332	0.08138	-4.08	0.0027
P80·E	-0.149	0.08138	-1.84	0.0995
E·W	-0.293	0.08138	-3.60	0.0057

Note. P = PEG 300; P80 = polysorbate 80; E = ethanol; w = Water.

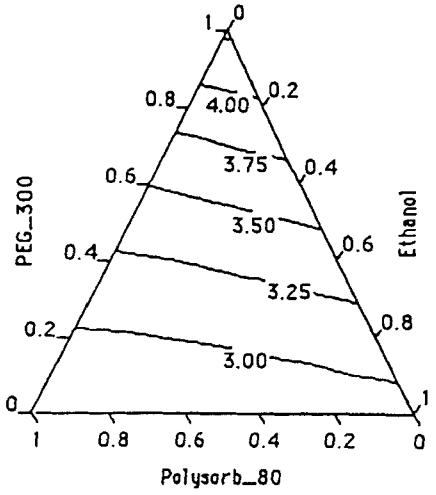


Figure 9. Contour plot of cosolvent/surfactant mixtures' apparent pH based on the quadratic Scheffé-type model (n = 19), tetrahedon face 1 of 4.

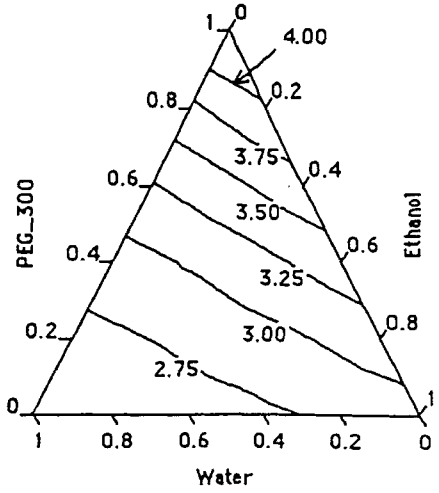


Figure 10. Contour plot of cosolvent/surfactant mixtures' apparent pH based on the quadratic Scheffé-type model (n = 19), tetrahedon face 2 of 4.

model appears to fit the data extremely well. Contour plots of apparent pH for the design space studied are given in Figs. 9, 10, 11, and 12. One can determine exactly where the Scheffé-type model for solubility applies (i.e., apparent pH ≤ 3.5) by overlaying Figs. 5 and 9, and likewise for Figs. 6 and 10, 7 and 11, and 8 and 12, respectively. In doing so, one sees that the Scheffé-type model for solubility applies in all areas except at very high concentrations (>40% w/w) of PEG 300.

CONCLUSIONS

PEG 300, polysorbate 80, and ethanol all increase the equilibrium solubility of GF120918A at 25°C with respect to water. Solubilities greater than 4 mg base/mL drug were determined for mixtures in this study, mak-

Table 8

Least Squares Summary ANOVA (n = 19)

Source	*df*	Sum Sq.	Mean Sq.	*F* Ratio	Significance
Model	10	175.74	17.57	46396.14	0.0000
Error	9	0.00	0.00		
Total	19	175.74			

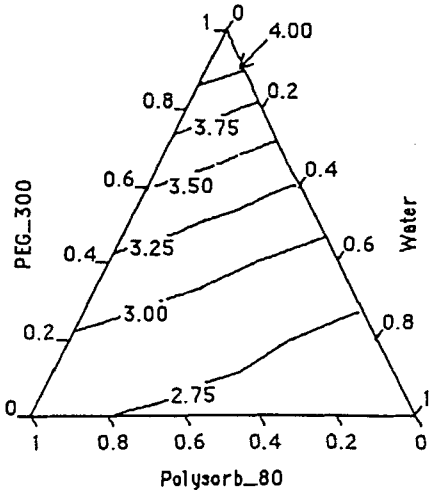


Figure 11. Contour plot of cosolvent/surfactant mixtures' apparent pH based on the quadratic Scheffé-type model (n = 19), tetrahedon face 3 of 4.

ing it feasible, with regard to solubility, to formulate a concentrated vial or ampule formulation. Maximum solubility, however, was dependent on the ability of adjusting the apparent pH to ≤3.5 in the cosolvent/surfactant systems studied. Presumably this is necessary so

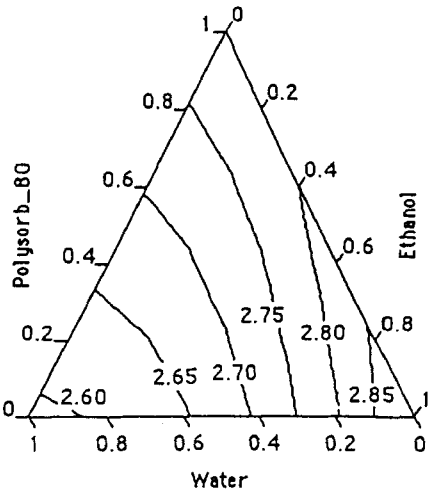


Figure 12. Contour plot of cosolvent/surfactant mixtures' apparent pH based on the quadratic Scheffé-type model (n = 19), tetrahedon face 4 of 4.

the solubility of the drug is limited by the salt rather than the free base.

The fitted quadratic Scheffé-type model did an excellent job modeling the equilbrium solubilities of GF120918A within the experimental design space. The importance of examining residual plots when statistically modeling data was evident from this study.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Mark Patrick, Elizabeth Steere, and Dorothea Roberts from the Analytical Sciences Department of GlaxoWellcome Inc. for providing the analytical method making this work possible.

REFERENCES

1. F. Hyafil, C. Vergely, P. Du Vignaud, and T. Grand-Perret, Cancer Res., 53, 4595–4602 (1993).
2. J. A. Cornell, J. Biopharm. Stat., 1, 303 (1991).
3. J. A. Cornell, *Experiments with Mixtures: Designs, Models, and the Analysis of Mixture Data*, 2nd ed., Wiley, New York, 1990.
4. A. B. Ochsner, R. J. Belloto, Jr., and T. D. Sokoloski, J. Pharm. Sci., 74(2), 132 (1985).
5. R. J. Belloto, Jr., A. M. Dean, M. A. Moustafa, A. M. Molokhia, M. W. Gouda, and T. D. Sokoloski, Int. J. Pharm., 23, 195 (1985).
6. S. T. Anik and L. Sukumar, J. Pharm. Sci., 70(8), 897 (1981).
7. M. A. Moustafa, A. M. Molokhia, and M. W. Gouda, J. Pharm. Sci., 70(9), 1172 (1981).
8. P. P. DeLuca and J. C. Boylan, in *Pharmaceutical Dosage Forms: Parenteral Medications*, Vol. 1 (K. E. Avis, L. Lachman, and H. A. Lieberman, eds.), Marcel Dekker, New York, 1984, p. 155.
9. N. Draper and H. Smith, *Applied Regression Analysis*, 2nd ed., John Wiley & Sons, Inc., New York, 1981, p. 144.
10. A. Albert and E. P. Sergeant, *The Determination of Ionization Constants*, 2nd ed., Chapman and Hall, 1971, p. 35.