Finally, the integrated function $F(C_F)$ results:

$$F(C_F) \equiv K_B C_P \left[\frac{1}{1 + K_B C_F} - \ln\left(C_F + \frac{1}{K_B}\right)\right] + (1 + K_B C_P) \ln C_F = F(C_{F,0}) - kt \quad \text{(Eq. A6)}$$

being obviously C_F expressed as a function of C_P by Eq. A1.

This formal treatment may easily be extended to the case of n identical and independent binding sites present on the protein, simply by substituting C_P by nC_P in these expressions.

The mathematical treatment would be more complicated if nonidentical and possibly cooperative sites were present. Owing to the good agreement between experimental data and the described model, this last case was not considered.

In principle, Eq. A6 implies that the kinetic law for the decomposition of triazenes in the presence of albumin is not first order with respect to triazene concentration. It is easy to realize, by numerical substitution, that the deviation from first-order kinetics is almost negligible in the present case.

For several triazenes, it has been possible to plot the experimental data obtained for each triazene at constant temperature and various C_P values in the form of the general function $F(C_F)$ *versus* t (Eq. A6). All slopes at various C_P values were matched successfully with the value for the kinetic constant, k, in the absence of protein by choosing the same K_B value for each triazene. The same data, plotted according to the simplified form of Eq. 9, provided K_B values in good agreement with those obtained by the more rigorous treatment.

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Extreme Vertexes Design in Formulation Development: Solubility of Butoconazole Nitrate in a Multicomponent System

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Received August 22, 1980, from the *Institute of Pharmaceutical Sciences, Syntex Research, Palo Alto, CA 94304.* Accepted for publication January 7, 1981.

Abstract □ The extreme vertexes design was shown to be an efficient method for the study of mixture problems, for generating points in the factor space that define a region for response surface analysis. By using this method, the solubility of butoconazole nitrate, an imidazole antifungal agent, was studied as a function of four components, polyethylene glycol 400, glycerin, polysorbate 60, and water, whose levels were subject to given constraints. A fifth component, poloxamer 407, was held constant. The design was used to generate 14 points in the region defined by the constraints. The G efficiency of the design, with the assumption of a quadratic model for the response surface, was 79%. By using the solubilities determined at the 14 points and regression analysis, an equation was generated to characterize the response surface. Contour plots of the response surface illustrate the relationship of the solubility as a function of the components, and solubilities calculated at other points (in the region) agree well with the observed data.

Keyphrases □ Extreme vertexes design—formulation development, butoconazole nitrate solubility in a multicomponent system □ Formulation development—extreme vertexes design, butoconazole nitrate solubility in a multicomponent system □ Butoconazole nitrate—solubility, extreme vertexes design in formulation development □ Multicomponent systems—extreme vertexes design in formulation development, butoconazole nitrate solubility

A pharmaceutical formulation generally consists of a mixture of several components, whose levels may often be constrained by factors other than those directly determining its physical properties, such as irritation and cost. In developing this formulation, the component levels must be varied within these constraints to arrive at an optimum formulation with respect to several responses such as solubility, stability, and bioavailability. The conventional

approach is to vary one variable at a time until a satisfactory formulation is developed, which may or may not be the optimum. This process could conceivably involve numerous experiments. Therefore, methods are needed for choosing and determining the adequacy of various groups of possible experimental points.

In this regard, the use of factorial designs has been well established (1). However, classical factorial designs are not suitable for problems involving mixtures because of the constraint $\Sigma X_i = 1$, where X_i is the level of the ith component; *i.e.*, the level of one component is fixed by the levels of the others. Furthermore, the classical factorial approach can result in large numbers of experimental points necessary to complete the design. To overcome these problems, McLean and Anderson (2) proposed the extreme vertexes design for mixture problems with upper and lower bounds. The advantages and disadvantages of this method were discussed and compared with other methods of experimental design (3–6).

The objective of this study was to demonstrate the utility of this design in pharmaceutical formulation development. The method is illustrated here by a solubility study of butoconazole nitrate, an antifungal agent, in a multicomponent system.

THEORETICAL

The general procedure involves constructing, in n-dimensional component space, the hyperpolyhedron that defines the region of all possible formulations. This construction and the shape of the region are based on the constraints put on the various formulation components. The extreme vertexes are then just the collection of the vertexes of this hyperpolyhedron. To them are added the face centroids, the overall centroid, and, if necessary, the edge centroids (midpoints of the edges, generally included only for the longer edges).

When developing an experimental design, one generally has a particular model, such as linear, quadratic, or cubic, to be used for the response surface. Given a particular model, a reasonable subset of the derived set of possible formulations must be selected on which to do experimental work. If the full set is small enough, all points can be taken; but when the number of initial points is large, a method is necessary for the rational selection of an experimentally manageable subset of points. Several methods and parameters have been proposed to evaluate the quality and size of the design for a given model. Details of hyperpolyhedron generation, subset selection, and subset evaluation were reported previously (2, 4). The procedure used in this study to determine the design points is as follows.

Table I lists the components, their ranges, and all possible vertexes generated using the method in Ref. 2. Although there are five components, one component, poloxamer 407, was fixed at 0.1 (10%). Therefore, the problem is one of four variables whose sum equals 0.9. From the 32 vertexes generated, there are 10 allowable vertexes where the levels of the components fall within the specified ranges. Normalized distances (2) were calculated between adjacent vertexes of the hyperpolyhedron defined by these 10 allowable vertexes using Eq. 1 (Table II). The normalized distance between the ith and jth points, d_{ij}, is given by:

$$d_{ij} = \sum_{m=1}^{q} [(x_{im} - x_{jm})^2/(b_m - a_m)^2]^{1/2} \qquad \text{(Eq. 1)}$$

where $\mathbf{x}_i$ and $\mathbf{x}_j$ are the q-dimensional vectors defining the two points, b_m and a_m are the upper and lower bounds of the mth component, and q is the number of variables (in this case four). Since points 3 and 29, 4 and 31, 5 and 13, and 6 and 15 are close to each other relative to the other points, they were replaced by their averages to give six extreme vertexes, which define the region in space shown in Fig. 1. This six-point design was then augmented by addition of centroids, using the method for

Table I—All Possible Vertexes for the Given Four-Variable System [a]

Vertex	Component and Range: Polyethylene Glycol 400 (0.1–0.4)	Glycerin (0.1–0.4)	Polysorbate 60 (0–0.08)	Water (0.3–0.7)
1[b]	0.1	0.1	0	0.7
2[b]	0.1	0.1	0.08	0.62
3[b]	0.1	0.4	0	0.4
4[b]	0.1	0.4	0.08	0.32
5[b]	0.4	0.1	0	0.4
6[b]	0.4	0.1	0.08	0.32
7	0.4	0.4	0	0.1
8	0.4	0.4	0.8	0.02
9	0.1	0.5	0	0.3
10[c]	0.1	0.1	0	0.7
11	0.1	0.42	0.08	0.3
12	0.1	0.02	0.08	0.7
13[b]	0.4	0.2	0	0.3
14	0.4	— [d]	0	0.7
15[b]	0.4	0.12	0.08	0.3
16	0.4	— [d]	0.08	0.7
17	0.1	0.1	0.4	0.3
18[c]	0.1	0.1	0	0.7
19	0.1	0.4	0.1	0.3
20	0.1	0.4	— [d]	0.7
21	0.4	0.1	0.1	0.3
22	0.4	0.1	— [d]	0.7
23	0.4	0.4	— [d]	0.3
24	0.4	0.4	— [d]	0.7
25	0.5	0.1	0	0.3
26[c]	0.1	0.1	0	0.7
27	0.42	0.1	0.08	0.3
28	0.02	0.1	0.08	0.7
29[b]	0.2	0.4	0	0.3
30	— [d]	0.4	0	0.7
31[b]	0.12	0.4	0.08	0.3
32	— [d]	0.4	0.08	0.7

[a] All values are fractions of the mixture. Poloxamer 407 was fixed at 0.1. [b] Allowable vertexes. [c] Repeat points. [d] Values could not be calculated as sum of other components >1.

Table II—Normalized Distances between Adjacent Extreme Vertexes

Vertex Pair[a]	Distance
1, 2	1.02
1, 3	1.25
1, 5	1.25
2, 4	1.25
2, 6	1.25
3, 4	1.02
3, 29	0.42
4, 31	0.08
5, 6	1.02
5, 13	0.42
6, 15	0.08
13, 15	1.03
13, 29	0.94
15, 31	1.32
29, 31	1.03

[a] Numbers correspond to points in Table I.

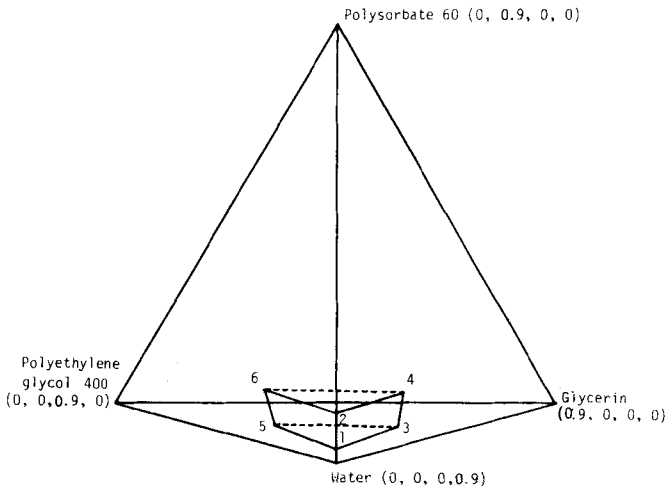

Figure 1—*Region defined by the extreme vertexes.*

Table III—Final Design Used for Solubility Determinations and Their Respective Solubilities [a]

Number	Polyethylene Glycol 400	Glycerin	Polysorbate 60	Water	Solubility, mg/ml
Vertexes					
1	0.1	0.1	0	0.7	3.0
2	0.1	0.1	0.08	0.62	7.3
3	0.15	0.4	0	0.35	4.9
4	0.11	0.4	0.08	0.31	8.4
5	0.4	0.15	0	0.35	8.6
6	0.4	0.11	0.08	0.31	12.7
Centroids					
7	0.1	0.1	0.04	0.66	5.1
8	0.4	0.13	0.04	0.33	10.8
9	0.13	0.4	0.04	0.33	6.6
10	0.216	0.216	0	0.468	4.4
11	0.203	0.203	0.08	0.414	7.9
12	0.255	0.255	0.08	0.31	9.4
13	0.275	0.275	0	0.35	5.8
Overall centroid					
14	0.21	0.21	0.04	0.44	6.3

[a] All values for the components are in fractions. Poloxamer 407 was fixed at 0.1.

centroids as in Ref. 2. Two face centroids, five edge centroids, and the overall centroid were calculated and added to the vertexes obtained above to result in the 14-point design shown in Table III.

For mixture experiments, the quadratic model (Eq. 2) is the most adequate for modeling the response surface (3). The response Y, in this case the solubility, is given by:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{1 \le i < j} \beta_{ij} X_i X_j \qquad \text{(Eq. 2)}$$

where β is the fitted coefficient for each term and the X's are the formulation component variables. (The quadratic model for mixture does not contain any squared terms due to the constraint $\Sigma X_i = 1$. In this case, a constant term is included since one component, poloxamer 407, is held constant.)

For a given model for characterizing the response surface, one can determine the quality or efficiency of the experimental design. One such criterion is the G or global efficiency (4), which is defined by:

$$\%G = \frac{100p}{nv} \qquad \text{(Eq. 3)}$$

where p is the number of parameters in the model, n is the number of points in the design, and v is $\max[\mathbf{x}(\mathbf{X}'\mathbf{X})^{-1}\mathbf{x}']$. The $\mathbf{x}$ is the p-dimensional vector of the model $\mathbf{Y} = \beta\mathbf{x}$ (*e.g.*, for the quadratic model discussed, $\mathbf{x}$ has the elements 1, X_i, X_iX_j); $\mathbf{X}$ is the nxp matrix of the $\mathbf{x}$'s. For the quadratic model ($p = 11$ here since one component is constant), the values of v and the G efficiency of the 14-point design described are 0.989 and 79%, respectively. High efficiencies are generally not practical, and an efficiency of 50% or greater is ordinarily acceptable (4). Therefore, this design was used to generate the solubility data and response surface.

EXPERIMENTAL

Materials—Polyethylene glycol 400[1] (I), glycerin[2] (II), polysorbate 60[2] (III), and poloxamer 407[3] (IV) were used as received.

Solubility Determinations—The drug was equilibrated in the respective solution for up to 7 days. At least two time points were taken for the solubility determinations. The solutions were filtered or centrifuged, and an aliquot of the filtrate or supernate was suitably diluted with methanol and assayed spectrophotometrically.

RESULTS AND DISCUSSION

The results of the solubility measurements for the different solutions are given in Table III. The solubilities varied from 3 to 12.7 mg/ml and formed an acceptable range for response surface analysis. By using regression analysis[4], the data were fitted to the quadratic model defined by Eq. 2; Table IV gives the equation and regression parameters for the solubility equation:

Table IV—Regression Parameters for the Solubility Equation [a]

Variable	Regression Coefficient	Standard Error	Probability >F
Intercept	7.66		
I	41.1	2.3	0.0001
III	30.6	6.1	0.0015
Water	−4.2	1.2	0.0088
(I) (II)	−59.9	4.8	0.0001
(I) (III)	−39.3	11.7	0.0121
(I) (water)	−74.2	6.5	0.0001
(III) (water)	23.3	9.4	0.0427

[a] I is polyethylene glycol 400, II is glycerin, and III is polysorbate 60 ($r^2 = 0.998$, $SE = 0.12$, $n = 14$).

$$\text{solubility} = 7.66 + 41.1\,(\text{I}) + 30.6\,(\text{III}) - 4.2\,(\text{water}) - 59.9\,(\text{I})(\text{II}) - 39.3\,(\text{I})(\text{III}) - 74.2\,(\text{I})(\text{water}) + 23.3\,(\text{III})(\text{water}) \qquad \text{(Eq. 4)}$$

This eight-parameter model gives the best fit to the data with all coefficients significant at the 95% confidence level. As might be expected, both polyethylene glycol 400 and polysorbate 60 have strong linear effects, which also were observed in solubility measurements with independent components. Several two-factor nonlinear terms show synergistic or antagonistic relationships between the different components and the solubility; however, a mechanistic interpretation of these terms must be done with caution since some coefficients are cross-correlated with the linear terms.

The equation, however, can be used for two purposes: (*a*) to calculate solubilities for any combination of components within the defined region, and (*b*) to generate contour plots of the response surface for delineating the behavior of the system within the region. Figure 2 shows a plot of calculated *versus* observed solubilities for some points within the region. The agreement is excellent over the entire solubility range; therefore, the equation may be used for calculating solubilities in any formulation of interest, which is of considerable value in development work.

Contour plots are useful for observing trends within the system, and they also provide a choice of alternative formulations for a given value of the response. Since this is a four-variable system, the effect of all four variables cannot be simultaneously represented. Hence, contour plots were made at the point of maximum solubility within the region, holding one component constant. The maximum solubility of 12.8 mg/ml is at polyethylene glycol 400 = 0.4, glycerin = 0.12, polysorbate 60 = 0.08, and water = 0.3, which is one vertex of the region. Figure 3 shows one such contour plot holding polysorbate 60 at 0.08. The equation below the figure describes the contours and is generated from the original equation by substituting polysorbate 60 = 0.08. The triangle in the center is the region of interest.

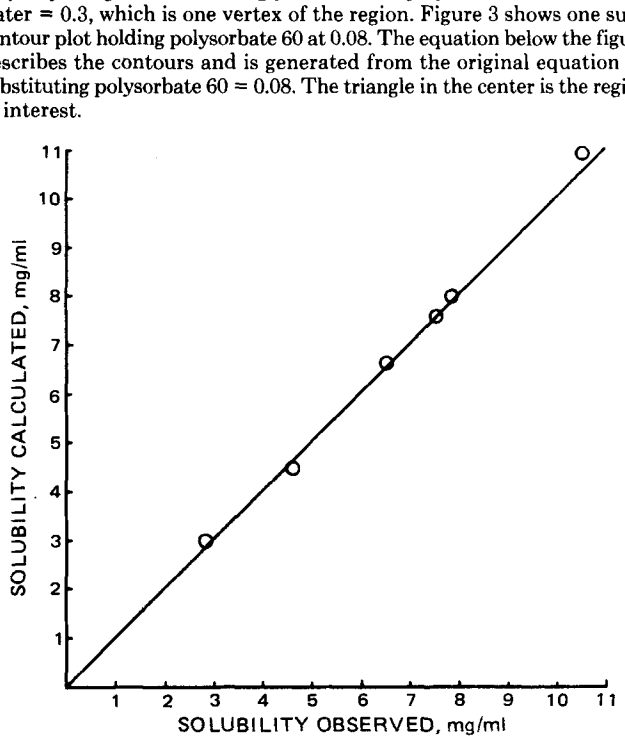

Figure 2—*Observed* versus *calculated solubilities (using the regression equation in Table IV).*

[1] Union Carbide Corp.
[2] Emery Industries.
[3] Pluronic F127, BASF Wyandotte Corp.
[4] Maximum R^2 improvement method, SAS Institute Program.

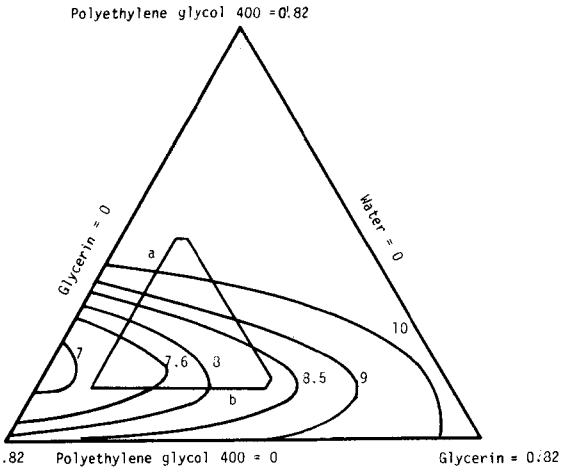

Figure 3—*Contour plot at polysorbate 60 = 0.08. Solubility = 10.1 + 38.0 (I) − 2.3 (water) − 59.9 (I)(II) − 74.2 (I)(water).*

The contour plot allows the formulator to choose any desirable solubility value and to determine the corresponding mixture. More importantly, it gives an overall picture of the response surface and the variation of the solubility as a function of the components. This, in turn, can be used to design a more robust formulation, that is, a region where the solubility does not vary greatly with small variations in component concentrations. Minor variations in production, therefore, would not result in drastic effects on the product. In addition, the following observations may be made from Fig. 3:

1. If one moves along the line glycerin = 0.1 (line a in Fig. 3) toward increasing polyethylene glycol 400, the solubility increases more slowly at lower polyethylene glycol 400 concentrations but increases more rapidly at higher levels.

2. If one moves along the line polyethylene glycol 400 = 0.1 (line b in Fig. 3) and replaces glycerin by water, the solubility changes only marginally. Hence, in changing the mixture from 40% glycerin to 10% glycerin by replacing with water, there is a change in solubility of only ~1 mg/ml. This information could be very useful if one were also concerned, for example, with the viscosity of the solution. The viscosity could be decreased considerably without compromising the solubility significantly.

Similar contour plots can be drawn holding different variables constant. These plots are not included here, but the interpretations are analogous to those made earlier.

From this analysis, it is evident that the extreme vertexes method in formulation development is a powerful approach to characterizing a formulation with a minimum number of experiments. (Factorial designs can be used for mixture problems only if one neglects the nth component, generally the least responsive, in generating the design. However, this approach may not give an optimum design.) This approach assumes even greater significance when more than one response must be studied since it allows optimization of the formulation with respect to the several responses simultaneously. This is easily done by overlapping contour plots of the different responses and choosing the region that satisfies all response criteria. An additional feature of the method is that one could first perform experiments only at the vertexes; if these experiments gave acceptable values for for the response, the design could be completed. Otherwise, the experimental region could be shifted to another area where the response might be more favorable, and the process could be repeated.

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Radioimmunoassay of Flunisolide in Human Plasma

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Received March 4, 1980, from the *Department of Analytical and Metabolic Chemistry, Syntex Research, Palo Alto, CA 94304.* Accepted for publication January 15, 1981.

Abstract □ A simple radioimmunoassay was developed for the measurement of flunisolide in human plasma or serum. Plasma extraction was not required. Antiserums were produced in rabbits by immunization against the flunisolide 21-hemisuccinate–bovine serum albumin conjugate. Cross-reactivities were determined for cortisol and a major metabolite of flunisolide and were 0.02 and 0.06%, respectively. Assay sensitivity is in the 100–200-pg/ml range. Accuracy studies gave regression lines of $y = 1.06x$, $r = 1.00$, for a 0.1-ml plasma aliquot and $y = 0.99x$, $r = 0.99$, for a 0.2-ml plasma aliquot. The accuracy of the method was estimated to be at least ±15%. The method was used to determine plasma concentration–time profiles in human subjects after the administration of a 1.0-mg iv dose.

Keyphrases □ Flunisolide—radioimmunoassay, human plasma or serum, plasma concentration–time profiles □ Corticoids—flunisolide, radioimmunoassay, human plasma or serum, plasma concentration–time profiles □ Radioimmunoassay—flunisolide, human plasma or serum

Flunisolide (6α-fluoro-11β,16α,17,21-tetrahydroxypregna-1,4-diene-3,20-dione cyclic 16,17-acetal with acetone, I) is a fast acting corticoid designed for use in the treatment of allergic rhinitis, asthma, and other respiratory disorders in humans (1–4).

Since the intended use of this drug was for the treatment of respiratory and upper respiratory disorders, its administration in an inhalation dosage form was a requirement. Since the amount of drug delivered by inhalation is generally small, the plasma levels achieved can be expected to be low.

This paper describes a selective and sensitive radioimmunoassay for the measurement of flunisolide in plasma or serum. This method was developed to provide data for the determination of certain pharmacokinetic parameters in humans, *e.g.*, plasma clearance, elimination half-life, and

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