

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/231261925>

Attaining optimal conditions: An advanced undergraduate experiment that introduces experimental design and optimization

ARTICLE *in* JOURNAL OF CHEMICAL EDUCATION · SEPTEMBER 1991

Impact Factor: 1.11 · DOI: 10.1021/ed068p878

CITATIONS

11

READS

12

2 AUTHORS, INCLUDING:



[Hal Van Ryswyk](#)

Harvey Mudd College

24 PUBLICATIONS 578 CITATIONS

[SEE PROFILE](#)

Attaining Optimal Conditions

An Advanced Undergraduate Experiment That Introduces Experimental Design and Optimization

Hal Van Ryswyk and Gerald R. Van Hecke

Harvey Mudd College, Claremont, CA 91711

This experiment is a five-period laboratory exercise in experimental design and optimization performed in our junior laboratory course that integrates synthesis and analysis. We introduce experimental design to undergraduates because such methodology is increasingly important in modern industrial research practice. The well-prepared chemist will need to apply these concepts with facility. While this experiment formally introduces concepts of experimental design and optimization, other pedagogical goals include increasing experience in synthetic techniques and using chromatographic methods for separation and analysis.

Introduction

A chemist consciously and unconsciously makes many decisions about the conditions under which an experiment is done. For example, should the temperature be held constant or varied, or does it even affect experimental outcome? We must know which independent variables do affect experimental outcome and in what manner. To choose the most important variables, we might conduct factor-screening experiments. To determine how important variables affect experimental outcome, we might establish a response surface. Let us briefly introduce these concepts.

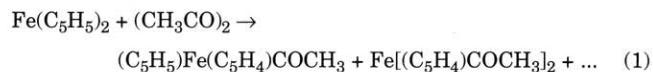
Consider a chemical synthesis. Usually the chemist desires to maximize yield of highly pure product in the shortest time using minimum amounts of reagents. What factors—the statistician's word for independent experimental variables—affect the yield? Clearly, temperature, pressure, reactant ratios, reaction time, and catalyst(s) are all known to affect the outcome of a synthesis, but which factors are the most important may not be known. If it is not known *a priori* which factors have the most effect on synthetic outcome, factor-screening experiments can be initially performed to discover the most significant factors. Such experiments often use the so-called fractional factorial designs of Plackett–Burman or Yates. We will not discuss how to choose the most important factors from a set; readers who face this problem are referred elsewhere (1).

One method to optimize the synthetic outcome is to first establish a response surface to learn how the yield depends quantitatively on the important factors. Then choose factor magnitudes to optimize yield. A specific example of this systematic procedure is presented here.

Before considering details of the systematic procedure, let us comment on why efficiency in establishing the response surface is valuable. Traditionally chemists have been trained to systematically vary one factor at a time, holding all others constant. Although thorough, this approach suffers from two major drawbacks. The cost or time required for the experiments often makes them impractical. Furthermore, if synergistic effects of two or more variables are important or crucial to quantifying the experimental outcome, these effects are very difficult to establish one at a time. A better, systematic method of optimization

that requires minimum effort is provided by response-surface methods.

To illustrate how to develop a response surface and use it to optimize the yield of a synthetic procedure, we have explored the production of acetylferrocene by Friedel-Crafts acylation of ferrocene according to the reaction below.



On the basis of the literature, we took these factors to be the most important experimental variables: temperature T , reactant mole ratio R (acetic anhydride/ferrocene), and time t . A catalyst was used. We assumed that pressure had a minor effect because it varied around the ambient atmospheric pressure of about 0.96 bar. We further used literature data to establish the initial factor values known to give adequate yields of product. A simplex method (2–5) could be used to find initial conditions that produce acceptable yields if we had no idea which initial values produced adequate yields.

The theory section below is organized around five concepts.

A prediction equation is used to fit the data and describe the response surface.

Experimental design determines the number of experiments and the range of experimental variables.

Canonical analysis finds the best yield once the prediction equation has been determined.

Ridge analysis optimizes when there is no maximum on the response surface.

Adequacy of fit concerns statistical tests for the goodness of the prediction equation.

Theory

Prediction Equation

We assume that the yield of acetylferrocene (y) can be modeled as

$$y = f(t, T, R) + \varepsilon \quad (2)$$

where ε is the residual in each run, that is, the difference between the value of $f(t, T, R)$ and y . In the region around the conditions of maximum yield, we approximate f to be quadratic in the experimental factors.

Equation 3 is the prediction equation that defines the fitted response surface

$$y = b_0 + \sum_{i=1}^k b_i x_i + \sum_{i=1}^k b_{ii} x_i^2 + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} x_i x_j + \varepsilon \quad (3)$$

where k is the number of variables, and the x_i 's are the experimental factors. In our example, $k = 3$ and x_1 , x_2 , and x_3 are the experimental factors—reaction time, temperature, and mole ratio.

Four assumptions are implicit in eq 3.

A function $y = f(t, T, R)$ exists but is unknown. The variables involved are quantitative and continuous, and the function is single-valued.

The function f can be approximated in the region of interest by a low-order polynomial.

The independent variables $x_1 \dots x_k$ are controlled in the experimental process and measured with negligible error.

The residuals are statistically independent and normally distributed.

Further comments about eq 3 are worthwhile. The equation is quadratic in the factors due to the third and fourth terms on the RHS. Moreover, the fourth term accounts for any binary synergistic effects. By the quadratic model, third-order ($x_1 x_2 x_3$) effects, are excluded. Remember that eq 3 is a linear model to the response surface because the fitting coefficients (b 's) are all first-order, even though quadratic factor terms appear. Thus, linear rather than nonlinear algebra methods can be used to solve for the b coefficients.

Our experimental goal is not to explore eq 1 over the entire possible factor space, but to fit the response surface in the region of maximal yield. We believe that using a second-order polynomial to approximate the true, unknown surface is justified in the small region around the maximum yield.

If a series of m experiments are performed, then the m yields can be related to the independent variables in a matrix format as

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \boldsymbol{\epsilon} \quad (4)$$

where

$$\mathbf{y} = \begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ \vdots \\ y_m \end{bmatrix}, \quad \boldsymbol{\beta} = \begin{bmatrix} b_0 \\ b_1 \\ b_2 \\ b_3 \\ b_{11} \\ b_{22} \\ b_{33} \\ b_{12} \\ b_{13} \\ b_{23} \end{bmatrix}, \quad \boldsymbol{\epsilon} = \begin{bmatrix} \epsilon_1 \\ \epsilon_2 \\ \epsilon_3 \\ \vdots \\ \epsilon_m \end{bmatrix} \quad (5)$$

Here \mathbf{y} is a column vector of the m yields, $\boldsymbol{\beta}$ is a column vector of the b coefficients of eq 3, \mathbf{X} is derived from the experimental design matrix of the independent variables (vide infra), and $\boldsymbol{\epsilon}$ is a column vector of the residuals, that is, the differences between the experimental yield and what is predicted by the first four terms of eq 3. We wish to estimate the coefficients (vector $\boldsymbol{\beta}$) to minimize the square of the residuals $\boldsymbol{\epsilon}'\boldsymbol{\epsilon}$. Such a minimization corresponds to a least-squares procedure.

Lower-case Greek and Roman letters in boldface represent column vectors, and upper-case Roman italic letters in boldface represent $(k \times m)$ or $(m \times m)$ matrices. All other standard variables are in italics. An apostrophe indicates a matrix transpose; a superscripted negative one indicates a matrix inverse.

The methods of linear algebra allow ready solution for the $\boldsymbol{\beta}$ vector, also known as the least-squares estimator.

$$\boldsymbol{\beta} = (\mathbf{X}'\mathbf{X})^{-1}\mathbf{X}'\mathbf{y} \quad (6)$$

It is assumed that $\mathbf{X}'\mathbf{X}$ is nonsingular and thus has an inverse. It is crucial to establish the pseudo design matrix \mathbf{X} discussed in the next subsection.

Knowing $\boldsymbol{\beta}$ provides the b coefficients of eq 3, but we really want the values of the experimental factors that describe the maximum yield. The maximum in y occurs for

those x_k values for which derivatives $(\partial y / \partial x_1), \dots, (\partial y / \partial x_k)$ are simultaneously zero. If such a maximum exists, it will be called the stationary point \mathbf{x}_S and can be found from

$$\mathbf{x}_S = -\mathbf{B}^{-1}\mathbf{b}/2 \quad (7)$$

where

$$\mathbf{x}_S = \begin{bmatrix} x_{S1} \\ x_{S2} \\ \vdots \\ x_{Sk} \end{bmatrix}, \quad \mathbf{b} = \begin{bmatrix} b_1 \\ b_2 \\ \vdots \\ b_k \end{bmatrix}, \quad \mathbf{B} = \begin{bmatrix} b_{11} & b_{12}/2 & \cdots & b_{1k}/2 \\ b_{12}/2 & b_{22} & \cdots & b_{2k}/2 \\ \vdots & \vdots & \ddots & \vdots \\ b_{1k}/2 & b_{2k}/2 & \cdots & b_{kk} \end{bmatrix} \quad (8)$$

Experimental Design

The goal of the experimental design methodology is to establish an adequate response surface in the minimum number of experiments. The stationary point is found by studying the response surface. A range of values that encompasses the stationary point is chosen for the experimental factors. The stationary point is not sought by continued change of variables until the goal is reached. (Such a procedure is actually the essence of simplex methods.) The major conceptual break with one-at-a-time methodology is that one experiment may involve simultaneous changes in two or more, even all, variables relative to a previous experiment. The number of experiments should be adequate to estimate the $\boldsymbol{\beta}$ vector. Also, circumstances permitting, there should be replications to test experimental consistency.

The factor-design method is used to select values for experimental variables, beginning with scaling, or encoding, them. To encode, we might set the low level of each variable to -1 , the high level to $+1$, and the initial level to 0 . In this experiment, time, temperature, and mole ratio were encoded as shown in eqs 9–11.

$$x_1 = \frac{t - 120 \text{ s}}{90 \text{ s}} \quad (9)$$

$$x_2 = \frac{T - 100 \text{ }^{\circ}\text{C}}{15 \text{ }^{\circ}\text{C}} \quad (10)$$

$$x_3 = \frac{R - 10}{7} \quad (11)$$

The zero-level corresponds to the initial values: $t = 120 \text{ s}$, $T = 100 \text{ }^{\circ}\text{C}$, and $R = 10$. Efficient use of this procedure requires good zero-level or starting-point values. The starting point is obtained from the literature, from experience, or from experiments carried out before the design experiments to determine it. A good initial point locates the stationary point by interpolation *within* the range of factors selected to define the response surface. The range of the experimental values used, also called the factor space volume, can be varied by manipulation of the encoding equations. For example, setting the high level at $+2$ covers a different factor space volume than $+1$.

In this three-variable experiment, if we set each factor to each of the three coded values $(-1, 0, +1)$ there are 3^k or 27 total combinations to explore. Such a design is known as a 3-level, 3-factor, complete factorial design. However, as k increases, it becomes impractical to perform 3^k experiments. (If $k = 4$, then 81 experiments are required.) therefore, we construct a smaller factorial design augmented by additional experiments to get a design combination called a composite design.

The design we will illustrate here is called a 2-level, 3-factor, full factorial with a star, commonly called a central composite design. It codes only variables with high and low values, requiring 2^k experiments. There are also experi-

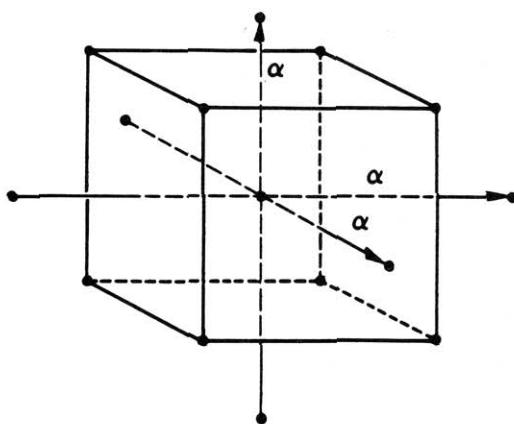


Figure 1. A central composite design for $k = 3$. Cube vertices correspond to a full 2^3 factorial design. Axial points and the center point(s) correspond to the star design augmentation. Vertices allow estimation of first-order and synergistic two-factor interaction terms, whereas axial points allow estimation of pure quadratic effects. Varying α alters the factor space volume. The center point is the initial or zero-level point, but it also can be used for replication, and hence precision estimates.

ments at the initial or center of the design space and along the principal axes. Figure 1 illustrates the factor space volume for this central composite design. The 2^3 factorial aspect of the design is illustrated by eight cube corners. Six axial points constitute the star augmentation in which the center point is the initial point, as well as any replicate points. This central composite design requires 15 points instead of 27 with the 3^3 full factorial design, providing considerable efficiency.

The embodiment of the experimental design method is the design matrix \mathbf{D} and the derived matrix \mathbf{X} , given below as eq 12. Each column represents the values of the factors in the following order: 1, x_1 , x_2 , x_3 , x_1^2 , x_2^2 , x_3^2 , x_1x_2 , x_1x_3 , x_2x_3 . The design matrix \mathbf{D} is within \mathbf{X} (columns 2–4).

$$\mathbf{X} = \begin{bmatrix} 1 & -1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & -1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 & -1 \\ 1 & -1 & 1 & -1 & 1 & 1 & 1 & 1 & -1 & 1 \\ 1 & -1 & 1 & 1 & 1 & 1 & 1 & 1 & -1 & -1 \\ 1 & 1 & -1 & -1 & 1 & 1 & 1 & 1 & -1 & -1 \\ 1 & 1 & -1 & 1 & 1 & 1 & 1 & 1 & -1 & 1 \\ 1 & 1 & 1 & -1 & 1 & 1 & 1 & 1 & 1 & -1 \\ 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 1 & -\alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 & 0 \\ 1 & \alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 & 0 & 0 \\ 1 & 0 & -\alpha & 0 & 0 & \alpha^2 & 0 & \alpha^2 & 0 & 0 \\ 1 & 0 & \alpha & 0 & 0 & \alpha^2 & 0 & \alpha^2 & 0 & 0 \\ 1 & 0 & 0 & -\alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 \\ 1 & 0 & 0 & \alpha & 0 & 0 & \alpha^2 & 0 & 0 & 0 \end{bmatrix} \quad (12)$$

The first eight rows of \mathbf{D} correspond to all possible values for the coded variables in the 2^3 factorial design (the cube vertices of Fig. 1). The last seven rows are the star augmentation (the remaining points in Fig. 1). The arbitrary modifying factor α is selected to aid exploration of the response surface or to make calculations easier. The columns to the right of these three are derived from the design matrix by appropriate manipulation of the columns in the design matrix corresponding to the x_1 , x_2 , and x_3 factors. For example, the column corresponding to x_2x_3 (the last column) is obtained by multiplying the column for x_2 by that for x_3 (in \mathbf{D}). Once the design matrix and the experimental values

of the factors have been selected, the requisite experiments (15 in this composite design) are performed. The β vector, calculated from \mathbf{X} and \mathbf{y} , allows the canonical and ridge analyses to be performed as discussed below.

Canonical Analysis

The stationary point specified by \mathbf{x}_s in eq 7 is not necessarily a maxima; it could be a minimum or saddle point on the fitted response surface. To explore the local surface topology in greater detail, we perform a canonical analysis, which translates the experimental factor coordinate system from its initial origin to the stationary point. Also, the axes may be rotated to emphasize constant-yield contours on the response surface. Thus, the prediction equation in the new coordinates has the form

$$y_s = y_s + \lambda_1 w_1^2 + \lambda_2 w_2^2 + \dots + \lambda_k w_k^2 \quad (13)$$

where y_s is the estimated response at the stationary point. In eq 13 the λ_i 's are constants and the w_i 's are mutually orthogonal linear combinations of the original experimental factor axes x_i . By the old coordinates y_s may be estimated as

$$y_s = b_0 + \frac{1}{2} \mathbf{x}_s' \mathbf{b} \quad (14)$$

To perform coordinate transformation, while keeping the information on the stationary point, the determinantal equation must hold true

$$|\mathbf{B} - \lambda \mathbf{I}| = 0 \quad (15)$$

where the implied polynominal in λ is of order k . (\mathbf{I} is the identity matrix, and the λ_i 's are eigenvalues of the matrix \mathbf{B} .) The canonical analysis consists of noting the signs of the solutions to the k th order polynominal provided by eq 15. The signs of the λ_i 's reflect the character of the stationary point. If all λ_i 's < 0 , the stationary point is a maximum. If all λ_i 's > 0 , the stationary point is a minimum. When the λ_i 's differ in sign, the stationary point is a saddle point.

If canonical analysis implies that a saddle point exists, modifying the experimental conditions to move in the direction of the w_i axis with the largest positive λ_i allows the experimenter to reach a better (perhaps satisfactory) response, even though there is no maximum in the response surface.

Ridge Analysis

In addition to the sign analysis, the relative magnitudes of the λ_i 's also characterize the response surface. Consider a system with $k = 2$ and $\lambda_1 > \lambda_2 > 0$. Since both λ 's are positive, the stationary point is a minimum. However, note the difference in sensitivity of the response surface when moving in the direction of w_1 or w_2 . As one moves along w_2 from the stationary point in either direction ($\pm w_2, w_1 = 0$), there is little change in the response because the surface is almost flat in this direction. An excursion of equal length along either direction of the w_1 axis ($\pm w_1, w_2 = 0$) will show a much greater response enhancement because the surface is steep in this direction.

Adequacy of Fit

How well the experimental data are modeled by eq 3 can be seen most easily by calculating the residuals for each run. The residual $(\mathbf{y} - \mathbf{X}\beta) = \epsilon$ is also the absolute error of the fit of eq 3 to the data. All of the above calculations were predicated on minimizing $\epsilon' \epsilon$. More sophisticated testing of the model can be accomplished using ANOVA procedures (6). They allow explicit tests for lack-of-fit, and also provide for testing incremental augmentation of the model, that is, selectively adding high-order terms and checking the fit. If

an estimate can be made of the total variance for all data (σ^2), the variance associated with each b coefficient can be obtained from the diagonal elements of the matrix $(\mathbf{X}'\mathbf{X})^{-1}\sigma^2$. For example, $\sigma^2(b_0) = [(\mathbf{X}'\mathbf{X})^{-1}\sigma^2]_{11}$. The total variance can be estimated by

$$\sigma^2 = \frac{\mathbf{y}'\mathbf{y} - \mathbf{b}'\mathbf{X}'\mathbf{y}}{m - k} \quad (16)$$

Experimental

The design matrix (eq 12) is used to calculate experimental values for t , T , and R using encoding eqs 9–11. A value of 1.216 is used for α , which is explained by Meyers (7).

For the synthesis, place 0.2 g (0.001 mol) of ferrocene and 0.1 mL of 85% phosphoric acid catalyst in a small test tube, which is then placed in a heating block at the desired temperature, using a water, steam, or oil bath. After the tube and its contents have come to thermal equilibrium with the block, add the appropriate amount of acetic anhydride. Stopper and shake. Then immediately replace the tube in the block. At designed intervals quench the reaction by pouring the contents of the tube on about 5 g of crushed ice. Allow the red-brown mixture to stand (with occasional stirring) for 5 min. Then filter. Wash the solid with 5 mL of cold water and air-dry. Repeat this procedure for each of the 15 experiments.

The products were analyzed using HPLC (8) with a C₁₈ column and isocratic 3:1 methanol–water solvent eluent at 1.2 mL/min with UV detection at 254 nm. (With UV detection the response factor for acetylferrocene is about three times that of ferrocene at 254 nm.) The yields are assembled into the response vector \mathbf{y} , and the matrix \mathbf{X} is computed. \mathbf{b} and the b coefficients are found using eq 6. The adequacy of the model can be ascertained by calculating the residuals and using eq 16. From the b coefficients of the model, the vector \mathbf{b} and matrix \mathbf{B} are calculated, and eq 7 is used to find the stationary point. A canonical analysis is then done using eq 15 to find the eigenvalues of \mathbf{B} , and the nature of the stationary point is determined. If time permits, students are encouraged to perform multiple runs at the center of the factor space with all factors coded to zero, to estimate reproducibility.

Results and Discussion

Students typically find optimal yields of acetylferrocene around 80%. The actual conditions vary from student to student, but most students report coded and uncoded variables similar to those shown in the table. A typical prediction equation in coded variables is given by eq 17. Since the eigenvalues obtained from canonical analysis of the stationary point described in the table are all negative (-0.212, -0.126, -0.143), the stationary point is a maximum. Figure 2 shows a pseudo-three-dimensional plot of the yield response surface vs. reaction time and mole ratio, at the constant, optimum temperature 102 °C.

$$y = 0.665 + 0.173 x_1 + 0.030 x_2 - 0.047 x_3 \\ - 0.193 x_1^2 - 0.135 x_2^2 - 0.152 x_3^2 \\ + 0.016 x_1 x_2 - 0.066 x_1 x_3 - 0.011 x_2 x_3 \quad (17)$$

Stationary Point as Derived from Eq 17 for Optimal Production of Acetylferrocene Via Eq 1.

| Experimental Factor | Encoded Value | Laboratory Value |
|---------------------|---------------|------------------|
| x_1 | 0.500 | 165 s |
| x_2 | 0.152 | 102 °C |
| x_3 | -0.269 | 8.1 mole ratio |

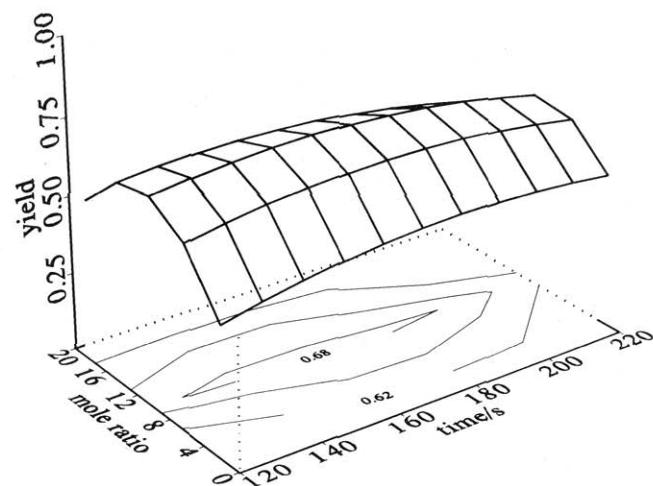


Figure 2. Response surface for the production of acetylferrocene from ferrocene and acetic anhydride at ambient pressure with phosphoric acid catalyst at a constant reaction temperature of 102 °C (optimum temperature). This surface is described by eq 17. The axes have been labeled with the actual experimental variables using eqs 9–11.

Typical reproducibility at the design center point is $\pm 3\%$, and the uncertainty in the HPLC analysis for any single run is $\pm 1\%$. Yields obtained with scale-up are lower than those suggested by the small-scale optimization experiments, sometimes by a factor of 2. Students are asked to evaluate this difference, considering the practical demands of scaling up a reaction. These include different mixing efficiencies and different heat transfer rates due to changes in the surface-to-volume ratio with a change in size of the reaction vessel.

This experiment is scheduled for five laboratory periods.

Day 1: Lay out and execute the 15 runs of the 2^k fractional factorial composite design.

Day 2: Analyze the products by quantitative HPLC.

Day 3: Do computations.

Days 4 and 5: Verify the optimal conditions predicted by the response surface analysis by scaling up to produce about 1 g of pure acetylferrocene.

Since even optimal synthesis yields a mixture of products, thin-layer chromatography is used to find the best solvent system for separation by preparative-size column chromatography. The products are characterized by melting point, IR, and ¹H NMR. Typical conditions for the scale-up and chromatography are given elsewhere (9).

Computers or calculators that invert matrices and find roots of polynomials are required for efficiency. (An advanced scientific calculator such as a Hewlett-Packard 15c can do both.) The computations described here were done using Innosoft's MATHLIB package¹ that allows interactive matrix manipulation. Alternatively, many spreadsheet programs (Lotus 1-2-3, Quattro) can handle matrices and solve some polynomials.

These experimental design concepts coupled with response-surface methodology could be used with a wide range of experiments in undergraduate curricula. This acetylferrocene synthesis was selected to introduce the methodology due to the ease of carrying out the reactions, the simplicity of the quantitative HPLC analysis, and the general pedagogical value of the variety of chromatographic techniques illustrated.

¹ MATHLIB is a product of Innosoft International, Inc., Claremont, CA.

Summary

This experiment works very well in an integrated laboratory such as ours. It illustrates experimental design, optimization by response-surface methodology, scale up, and three different forms of chromatography. The students found this first encounter with experimental design a challenge and a real opportunity to realize the type of research that is increasingly commonplace in industry.

Acknowledgment

We thank the students of the 1989 and 1990 junior laboratory classes who worked diligently and with good humor on the first trials of this experiment. One of those students, Katrina F. Hughes, did the ANOVA analyses to verify the adequacy of the experimental model.

Bibliography

- Box, G. E. P.; Draper, N. R. *Empirical Model-Building and Response Surfaces*; John Wiley: New York, 1987.
Deming, S. N. "Quality by Design. Part I." *Chemtech*, **1988**, 560.
Deming, S. N. "Quality by Design. Part II." *Chemtech*, **1989**, 52.
Deming, S. N. "Quality by Design. Part III." *Chemtech*, **1989**, 249.
John, P. W. M. *Statistical Design and Analysis of Experiments*; Macmillian: New York, 1971.
Myers, R. H. *Response Surface Methodology*; Allyn and Bacon: New York, 1971.

Literature Cited

1. Box, G. E. P.; Hunter, W. G.; Hunter, J. S. *Statistics for Experimenters. An Introduction to Design, Data Analysis, and Model Building*; Wiley: New York, 1978; Chapter 13.
2. Shavers, C. L.; Parsons, M. L.; Deming, S. N. *J. Chem. Educ.* **1979**, 56, 307–309.
3. Stieg, S.; Nieman, T. A. *Anal. Chem.* **1980**, 52, 796–800.
4. Ryan, R. B.; Barr, T. L.; Todd, H. D. *Anal. Chem.* **1980**, 52, 1460–1467.
5. Morgan, S. L.; Deming, S. N. *Anal. Chem.* **1974**, 46, 1170.
6. Box, G. E. P.; Draper, N. R. *Empirical Model Building and Response Surfaces*; Wiley: New York, 1987; pp 50–74.
7. Meyers, R. H. *Response Surface Methodology*; Macmillian: New York, 1971; pp 126–139.
8. McKone, H. T. *J. Chem. Educ.* **1980**, 57, 380.
9. Angelici, R. J. *Synthesis and Techniques in Inorganic Chemistry*; University Science: Mill Valley, CA, 1986; pp 157–168.