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Optimizing Signal-to-Noise Ratio in Flame Atomic Absorption Spectrophotometry Using Sequential Simplex Optimization

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Teaching the strategies of experimental design *should* be a major strategic focus in the undergraduate chemistry curriculum, particularly in the laboratory portion of instruction (*1, 2*). One strategy, sequential simplex optimization, has been part of the arsenal available to analytical chemists for nearly three decades (*3*). It is especially useful in the initial stages of an iterative investigation. While the method of simulated annealing may be better at finding the global optimum (*4*), there are many reasons to teach simplex optimization. In many analytical chemistry applications, finding a local optimum rather than the global optimum is adequate. The mechanics of the simplex method are easily understood. Many variables can be studied simultaneously to determine the few that are likely to affect the result of an experiment. The cost of studying many variables simultaneously is not appreciably different from the cost of studying a single variable. The results of such multivariate experiments generally contain more complete information than "one variable at a time" efforts (*5*).

The importance of the simplex method has been recognized by a steady appearance of articles in this *Journal* (*6–13*). Some describe the rationale and the rules of applying the method (*6–8*). Laboratory experiments include maximizing absorbance in molecular absorbance spectrometry (*6, 9*), absorbance in flame AA (*7*) and hydride AA (*10*), separation by GC (*7, 11*), and yield in synthesis (*8, 12*). The simplex method has also been used to estimate parameters in nonlinear least squares analysis (*13*). Except for one GC experiment (*11*), students investigate only two or three variables. While the simplex method is most easily described and explained using only two variables, it is most useful when there are many potentially active variables. Furthermore, maximizing the signal in spectroscopy (or yield in synthesis) is only part of an effective strategy. A better figure of merit to consider is the signal-to-noise ratio, S/N, which determines the limit of detection and the quality of structural information in a spectrum (*14*). S/N may be maximized by an increase in signal and a decrease in noise.

For flame AA, calcium is an excellent element to study because its behavior in the flame is moderately complex. The standard methods guide states that that maximizing signal and maximizing S/N are accomplished by using different instrumental conditions (*15*). A simplex study of maximizing the Ca AA signal shows the signal increases strongly with increased fuel-to-air ratio (*16*). An interaction of fuel-to-air ratio with flame observation height is also observed.

In the experiment described here, four variables are adjusted simultaneously and the S/N for the flame AA determination of Ca is maximized. The vertical burner position with respect to the light beam (flame observation height), burner horizontal position, and fuel flow rate (fuel-to-air ratio) are variables that might be expected to affect S/N. The fourth variable, volume of water in a graduated cylinder set on a benchtop away from the AA, should not affect S/N. It is included so students can observe the behavior of a clearly unimportant variable when they examine data at the end of the experiment.

Prelaboratory Work—Understanding the Simplex Method

The simplex method is introduced with two hours of lecture and demonstration. After this introduction, students are given three assignments before lab time. They read articles describing the rationale and calculations of the simplex method (*6–8*). They do a tutorial of a spectrophotometric optimization with the MultiSimplex software (Multisimplex KB, Karlskrona, Sweden) that is used during the experiment. They use the MultiSimplex software to determine the design of the five experiments that will make up the initial simplex in the lab work. (The initial conditions are specified so that the flame is rich and the absorbance is measured near the top of the flame. These adjustments ensure that the S/N will be far from maximized.)

The initial experiments can be designed using a corner design (*17*) or a newly described optimal design (*18*). The corner design is easy to describe. When n variables (A, B, …, N) are studied, this initial corner simplex has n edges that are parallel to the experimental factor axes. If this simplex were translated to the origin, it would fit into the corner formed by the intersection of the n orthogonal axes. When the conditions of the first experiment (VA, VB, …, VN, where VN stands for the value of the variable) and the step size for each variable (ΔA, ΔB, …, ΔN) have been established, the conditions of the first $(n+1)$ experiments are determined using Table 1. The optimal design is based on D-optimal linear design, and the first five experiments in Table 2 show an example. If there are 3, 7, or 15 variables, the design is identical with a saturated fractional factorial, but no such simple algorithm exists for other numbers of variables.

The step size is chosen according to specifics of the problem, and it may be adjusted during the optimization process. A

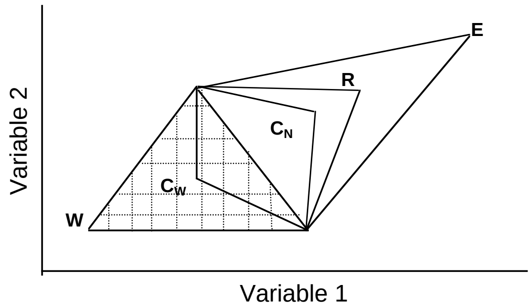

Figure 1. Examples of simplex reflection (R), expansion (E), and two types of contraction (C_R, C_W) operations for a two-variable system. The hatched area is the current simplex.

Table 1. Generalized Initial Corner Design for Simplex Optimization Using *n* Variables

Experiment	Variable [a]				
	A	*B*	*C*	…	*N*
1 [b]	V*A*	V*B*	V*C*	…	V*N*
2	V*A* + Δ*A*	V*B*	V*C*	…	V*N*
3	V*A*	V*B* + Δ*B*	V*C*	…	V*N*
⋮	⋮	⋮	⋮		⋮
n + 1	V*A*	V*B*	V*C*	…	V*N* + Δ*N*

[a] V*A*, V*B*, etc. is the value and Δ*A*, Δ*B*, etc. is the step size of the indicated variable.
[b] Experiment 1 is the reference design.

Table 2. Progress of the Optimization Using an Optimized Initial Design

Exp.	Burner Measure/ turns CCW		Fuel Flow/ $L\ min^{-1}$	Water Vol/ mL	S/N	Comment
	Height	Horizontal				
1	1.50	1.25	3.25	40	20	Initial, W for simplex 3
2	0.50	1.25	2.75	40	27	Initial
3	1.50	1.25	2.75	60	30	Initial
4	1.50	0.75	2.75	40	18	Initial, W for simplex 2
5	0.50	0.75	3.25	60	17	Initial, W for simplex 1
6	2.00	1.50	2.50	30	28	Reflection for simplex 2
7	1.25	1.88	2.85	45	40	Reflection for simplex 3
8	1.13	2.44	2.93	48	67	Successful expansion for simplex 3

NOTE: The reference design is: burner height = 1 turn counterclockwise (CCW); flame horizontal position = 1 turn CCW; fuel flow = 3 L/min; water volume = 50 mL. The corresponding step size values are 1.0 turn CCW; 0.5 turns CCW; 0.5 L/min; 20 mL.

large step size gives rapid progress toward the optimum and is appropriate if the experimenter believes the optimum conditions are very different from the current conditions. The optimum may be missed, however, if the simplex takes too large a jump. A small step size gives slow progress and is used when the current experiments are believed to be near the optimum. The experiment described here begins with a relatively large step size, and the optimization algorithm has the ability to change step size according to the success or failure of new experiments to move toward larger S/N.

The rules guiding the progress of simplex optimization are described in detail by Leggett (*7*). They can be summarized as follows, for an investigation of *n* variables where the results of the (*n* + 1) experiments in the current simplex are known. The experimental design giving the worst result is identified as W. The design of the next experiment to be done, R, is determined by reflecting W through the centroid of the *n* designs of all other experiments in the current simplex, as shown schematically in Figure 1.[1] The new simplex includes the new reflection (R) design and the best *n* designs of the previous simplex. There are three possible branches after the reflection experiment is done and evaluated.

1. If the reflection result is neither especially favorable nor especially unfavorable, this vertex is kept as part of the new current simplex. A new reflection design is then determined. The simplex size is unchanged.
2. If a result obtained with the reflection design is especially favorable, the next experiment will have the design described by the expansion vertex (E). If the result obtained with the expansion design is especially good, this vertex is kept, and simplex size increases. The result is acceleration toward the optimum. If the result of the expansion design experiment is not the best, the expansion design is dropped from the simplex and the reflection point is kept. The simplex size does not change.
3. If a reflection result is especially unfavorable, the next experiment has the design described by one of two contraction operations (C_R, C_W). This causes the simplex size to decrease.

This general guide can be applied to the data in Table 2. Of the first five experiments, experiment 5 gives the smallest S/N and therefore the coordinates for the least favorable vertex are:

$$W = (0.5, 0.75, 3.25, 60)$$

The centroid, P, of the four other vertices is calculated as:

$$P = ((1.5+0.5+1.5+1.5)/4, (1.25+1.25+1.25+0.75)/4, (3.25+2.75+2.75+2.75)/4, (40+40+60+40)/4))$$

or

$$P = (1.25, 1.125, 2.875, 45)$$

The difference (P – W) is

$$(1.25 - 0.5, 1.125 - 0.75, 2.875 - 3.25, 45 - 60)$$

so

$$(P - W) = (0.75, 0.375, -0.375, -15)$$

The reflection design is calculated as $R = P + (P - W)$, so

$$R = (1.25 + 0.75, 1.125 + 0.375, 2.875 + (-0.375), 45 + (-15)) = (2.00, 1.50, 2.50, 30)$$

Experiment 6 is performed at these conditions and the resulting S/N of 28 is good, but not good enough to warrant an expansion calculation. The simplex size is unchanged. Experiment 4 has the worst result in simplex #2, and the reflection point design for experiment 7 is (1.25, 1.88, 2.85, 45). The S/N for this point is 40, the best observed thus far, so experiment 8 is done at the expansion vertex ($E = P + 2(P - W)$) with design (1.13, 2.44, 2.93, 48). Since the resulting S/N = 67 is the best observed, the expansion design is maintained in the current simplex, which has increased in size.

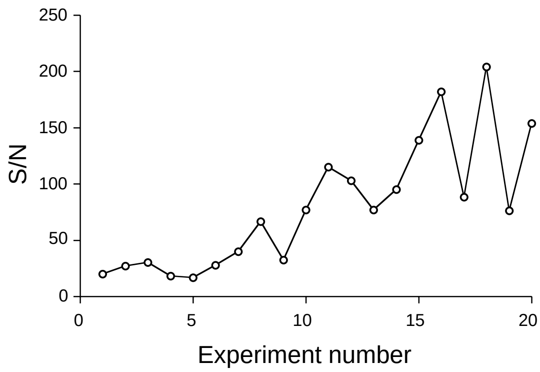

Figure 2. Signal-to-noise ratio trend. The design of experiments 1 to 5 is determined by the initial simplex. The design of experiments 6 and above is calculated using variable size simplex optimization calculations.

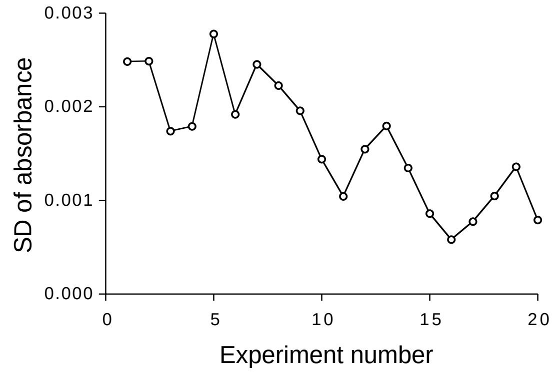

Figure 4. Noise trends (median standard deviation calculated from 5 sets of 20 replicated net absorbance measurements).

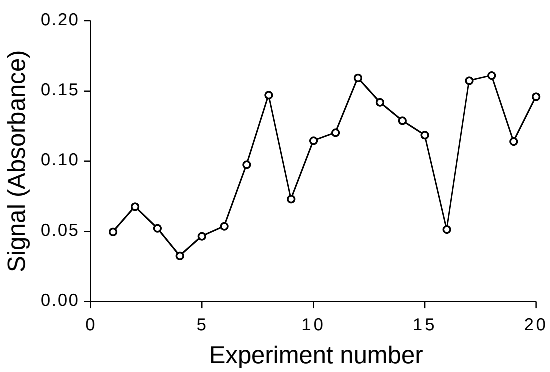

Figure 3. Average signal trend.

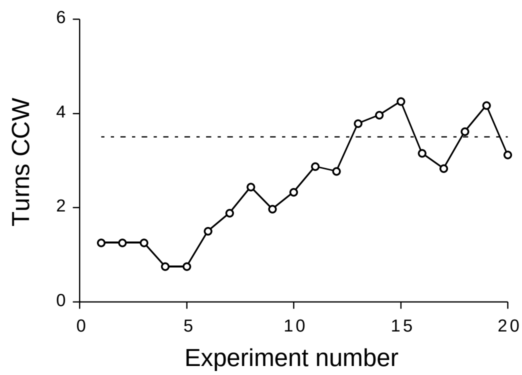

Figure 5. Sequential setting of burner horizontal adjustment. Optical alignment corresponds to approximately 3.5 turns counterclockwise, represented by the dotted line. Each full turn moves the burner head 1.1 mm horizontally.

Experimental Procedure

A flame atomic absorption spectrometer (Perkin Elmer 3300) is used with an air–acetylene flame and a Ca–Mg–Zn hollow cathode lamp. The wavelength is 422.7 nm, slit width 0.7 nm, and air flow 10 L/min. The software (Perkin Elmer WinLab) is set so that 20 integrated 0.5-s absorbance measurements are averaged. Average signal, standard deviation, and % RSD are displayed and stored to disc.[2] Because the flame is susceptible to air currents, traffic flow past the instrument should be minimized.

The designs of the five initial experiments are calculated with respect to a “reference design”. Both the corner design (Table 1) and the optimal design (Table 2) have been used successfully. Our reference design gives measurements of absorbance near the top of a fuel-rich flame, which has been adjusted a few millimeters out of horizontal alignment with the light beam. The S/N observed for 2 ppm Ca, typically 20 to 30, is far from the maximum attainable, approximately 200. For each experiment, students adjust the burner height, burner horizontal position, and fuel flow within boundary constraints.[3] Students allow the flame temperature to stabilize for one minute. (They wait ten minutes for the first experiment when the burner must warm from room temperature to operating temperature.) They also adjust the volume of water in a 100-mL graduated cylinder set on a lab bench away from the AA. They are then do the following.

1. Aspirate water and zero the instrument.
2. Aspirate the 2 ppm Ca solution and make the set of 20 absorbance measurements. Record the average absorbance signal and the % RSD value.
3. Repeat the step 2 four more times to get five values of % RSD.
4. Place the aspirator tubing in the R.O. water.
5. Determine the median of the five values of % RSD. Calculate the median signal-to-noise ratio (100 ÷ median %RSD) and enter this value in the optimization program.
6. Use the optimization program to calculate the design of the next experiment to be done.
7. Do steps 1–6 until the S/N is greater than 200 or until 20 experiments have been done.

Simplex optimization calculations are done with an Excel add-in, MultiSimplex (Multisimplex KB, Karlskrona, Sweden). A variable-size simplex is used so that progress toward the optimum may accelerate. Previous generations of students have successfully used a DOS program Simplex-V (Statistical Programs, Houston, TX) and a locally produced simplex program written in the Basic programming language, with equally good results.

During a three-hour lab period, students can get S/N results for approximately 20 sets of conditions (15 vertices

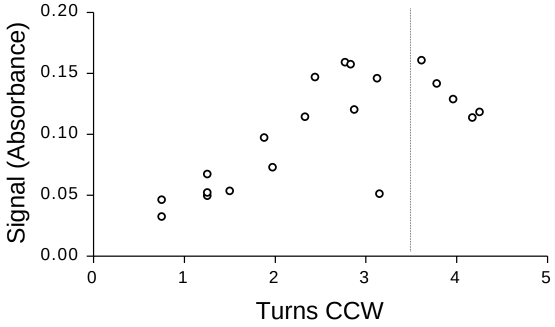

Figure 6. Signal as a function of burner horizontal adjustment.

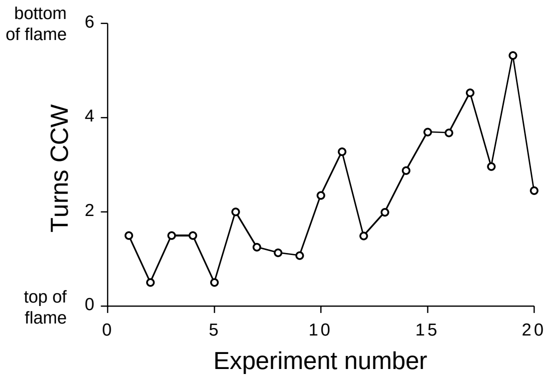

Figure 7. Sequential setting of burner height adjustment. Zero turns counterclockwise probes the top of the flame. Five turns counterclockwise probes the base of the flame. Each full turn moves the burner head 4 mm vertically.

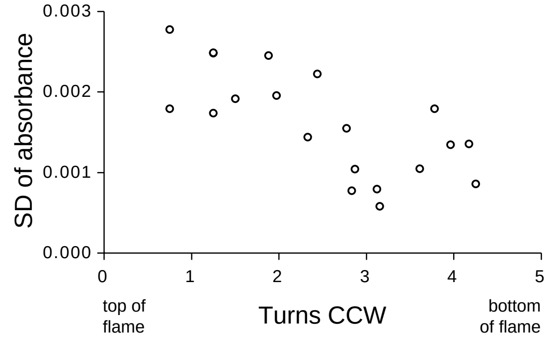

Figure 8. Noise as a function of burner height.

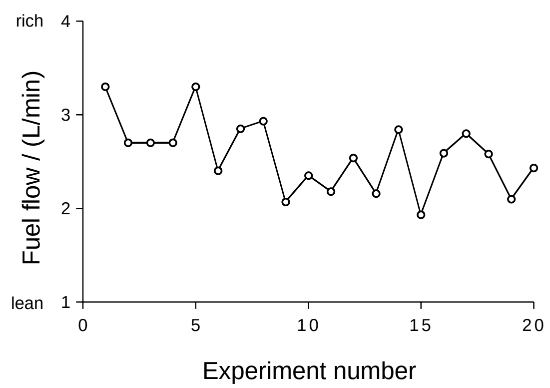

Figure 9. Sequential acetylene flow rates. A value of 3.6 corresponds to a yellow, fuel-rich flame. A value of 1.6 corresponds to a blue, lean flame.

beyond the initial simplex).

Results

Progress of the simplex optimization is generally, but not always, toward an increased S/N. The students must know that the data cannot be viewed one experiment at a time. If they do not recognize this, they invariably stop their work to tell the instructor that the experiment is “not working”. By the end of the lab, students see a 4- to 10-fold improvement in S/N (Fig. 2). The details of the results differ among groups, but signal generally increases significantly (Fig. 3) and noise always decreases (Fig. 4) over the course of the experiments. It is worthwhile to note that an immediate and rapid increase in S/N is typically observed in experiments 6 through 10 or 12, followed by a small number of unsuccessful experiments. This is followed by another series of experiments with designs giving increased S/N. By experiment 15 or 20, the simplex begins to wander around the maximum, with very little improvement through 30 experiments.

Students identify the active variables by making a variety of plots. Sometimes an important variable will clearly trend in one direction in the successful experiments immediately after the initial simplex (*16*). For example, the optimization procedure guides the burner into horizontal alignment over experiments 6–13 (Fig. 5), which causes the signal to increase (Fig. 6). There is a somewhat later trend in the burner height adjustment, which moves the flame upward with respect to the light beam (Fig. 7). In this case, the increased S/N is due to a decrease in noise near the base of the flame (Fig. 8). This is consistent with the visual observation of flicker in the top half of the flame. A trend toward lower fuel flow rate (leaner flame) is suggested (Fig. 9), but the details of trends in signal and noise are not clear. This is not surprising, given the reported interaction of fuel-to-air ratio with observation height in the flame when absorbance was measured (*16*). Measurements with the instrument used in this experiment show that the maximum signal is near mid-flame in a fuel-rich flame, whereas it is near the base of the flame in a lean flame. The formation of the refractory CaO is responsible for the decrease in signal with height in the lean flame. The volume of water in the graduated cylinder invariably changes randomly.

Conclusions and Extensions

This experiment generates responses of the sort “I can’t believe it—it really worked!” The notion of “automatic” design applied to the preliminary phase of an investigation is new to the students, although they have done a factorial design experiment previously (*19*). There are caveats, however. Doing the experiment well is difficult. There is considerable chance for gross error if care is not taken in adjusting the settings of the instrument. Flame fluctuation due to excessive air motion in even 1 measurement of 20 will inflate the standard deviation, causing a decrease in S/N. (Doing five determinations of S/N and using the median works well in all but a very crowded laboratory.) The lack of a real-time trend in data can distract students from the larger picture of a complete data set. However, when the data are graphed and the trends reveal themselves, students' eyes get wide and they become believers.

There are opportunities to build upon their observations and to expand the range of applicability of the simplex method. The use of simplex optimization for maximizing the Ca signal (only) has been reported in the research literature (*16*). Those results can be compared to the signal results generated by the students. Simplex optimization is used for adjusting the shim power settings to homogenize the magnetic field in modern NMR spectrometers. A searchable index of applications in chemistry and biotechnology is available on the Web (*20*).

Calcium is commonly present in natural waters at sufficiently high concentration that the AA determination can be done, often after dilution. For diluted ground water, the analysis can be done with high reliability if the S/N of the measurement is very high. No doubt, modern students find this sort of analysis more satisfying than a hardness titration. Our preferred use of the high S/N available in flame AA determination of calcium is in the determination of calcium in snow. The calcium concentration of snow in our area is about 0.1 to 0.5 ppm. Flame AA, with a detection limit of about 0.02 ppm Ca, is the method of choice to do the determination. Following the idea used by John Walters (*21*), instrumental methods students write a protocol for Ca determinations based on their results. This protocol will be used by students in the introductory analytical chemistry lab to do the analyses for a study of Ca distribution in snow around the city.

Acknowledgment

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Note

1. Visualization of the progress of simplex optimization is typically shown with only two variables and a triangular simplex to keep to a two-dimensional figure. In practice, simplex optimization is best used where there are many more than two variables and where there are consequently three or more dimensions to visualize. If two variables are being studied, a design other than simplex optimization will be more appropriate.
2. Previous classes have used a manually controlled AA and a simpler software system. The experiment worked comparably.
3. Boundary conditions for each variable are given to avoid a physically undesirable condition like flashback or a sooty flame. *When an experimental design is outside of the boundary, the experiment is not done*. A very poor response is entered into the simplex software, which calculates another experiment that will be within the allowable range of conditions.

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