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Use of the Simplex Method to Optimize Analytical Conditions in Clinical Chemistry

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The optimum analytical conditions with regard to two or more variables can be determined in an unambiguous way by use of the simplex method. The method is particularly applicable to problems involving interacting variables. We describe the application of the simplex technique to the optimization of two procedures on a continuous-flow analyzer (AutoAnalyzer) and to the optimization of chemical variables in the lactate dehydrogenase-catalyzed pyruvate-to-lactate reaction and certain instrumental variables on a centrifugal analyzer (the ENI-GEMSAEC).

Additional Keyphrases: *experimental design • GEMSAEC • centrifugal analyzer • lactate dehydrogenase • AutoAnalyzer • glucose analysis*

A clinical chemist is often faced with the task of determining the optimum handling of several variables in a method, a particularly difficult task when the variables are known to interact. For example, in enzymatic analyses the optimum substrate concentration may change with changes in temperature, pH, concentration and type of buffer, and the like. For example, the pH optimum for measuring alkaline phosphatase activity in serum changes when the substrate concentration is altered (*1*).

The traditional approach for optimizing experimental variables has been to vary one factor at a time and find its optimum while holding all other factors constant. This empirical approach may not provide the unique *combined* optimum of all variables, particularly when several variables must be considered and if the variables are interdependent. Factorial analysis (*2*) is another approach that has been used to find the unique optimum conditions for a method, but it is not popular because many involved calculations are required.

The simplex method described here is much easier to use than factorial analysis. The route to the optimum is clearly marked by a set of straightforward rules; no empiricism is involved. The results from one set of experiments dictate exactly what should be done to reach the optimum.

Here, we present four examples of the application of simplex optimization to problems commonly encountered in the clinical laboratory. The first two involve optimization of the design of AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y. 10591) systems. One is of a simple system in which a $CuSO_4$ solution was sampled. Only two factors were varied, to permit graphical presentation of the progress of the experiment. Our goal was to minimize sample-to-sample interaction.

The second example is the optimization of the "N2b" AutoAnalyzer method for glucose (*3*), in which the goal was the same. Three variables were examined simultaneously.

In the third example, the simplex method was applied to the optimization of pH and of the concentration of three reagents, so as to obtain the maximum reaction rate in the LD-catalyzed[3] pyruvate-to-lactate reaction.

In the last example, we examined instrumental variables on the ENI-GEMSAEC (Electro-Nucleon-

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[3] Nonstandard abbreviations used: LD, lactate dehydrogenase (EC 1.1.1.27); and Tris, tris(hydroxymethyl)aminomethane.

ics, Inc., 368 Passaic Ave., Fairfield, N. J. 07006) centrifugal analyzer and sought the conditions that give the highest precision.

Application of the Simplex Method

The theoretical basis and proof of validity of the simplex method have been described elsewhere (*4–6*). The graphical representation of the simplex technique in the work of Deming and Morgan (*6*) facilitates the understanding of the method. We restrict ourselves here to the application of the simplex method to several clinical analytical problems.

A simplex is a geometric figure having a number of vertices equal to one more than the number of dimensions of the space it occupies. Each "dimension" is an experimental variable such as pH, temperature, time, etc. When two variables are being examined simultaneously, the simplex is a triangle. With three variables, the simplex is three-dimensional, a tetrahedron. With more than three variables, it is not possible to visualize the simplex, because it will have four or more dimensions, but this does not preclude use of the method.

The first step in the method is to define the response to be optimized. The second step is to identify the variables affecting it and the limits of each variable. Assume in a hypothetical system that a maximum response (e.g., reaction rate, absorbance, sensitivity, etc.) is the desired optimum. Assume also that there are two variables involved: A, which can be varied from 0 to 50, and B, which can be varied from 30 to 80. A plot of A vs. B will define a certain area (Figure 1) where the point of maximum response can be found. The simplex method provides a way of finding this point. Assume that three points are chosen on the graph (Figure 1) so that the points form the apices of an equilateral triangle: Point 1 (A = 10, B = 45), 2 (A = 10, B = 55), and 3 (A = 20, B = 50) (see Figure 1). The response at each of these points is measured and the responses are obtained: point 1 = 0.5, 2 = 0.9, and 3 = 1.2. Now, to obtain the coordinates of the fourth experiment, one replaces the point of the current simplex that gave the least desirable result (in this instance, point 1) by its mirror image. The mirror is perpendicular to the graph and intersects points 2 and 3. The coordinates of the fourth experiment (A = 20, B = 60) are shown in Figure 1. Suppose the response obtained at point 4 is 1.0, which is better than that at point 2, but not as good as that at point 3. In the new simplex described by points 2, 3, and 4, the response at point 2 is the least desirable. The second simplex is reflected across the "mirror" through points 3 and 4 to give point 5, which has coordinates A = 30 and B = 55 (Figure 1).

As the simplex is moved or flipped over, the coordinates and conditions of the next experiment are defined. By rejecting the least-desirable response in each triangle and flipping the triangle away from that vertex, the triangle is made to move toward the point of optimum response.

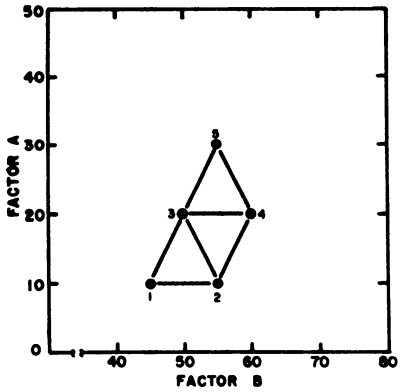

Fig. 1. Progress of simplex in sample problem

The size of the original triangle will determine how precisely the optimum point is located. If the increments in A and B, *i.e.*, the step size, approximate the experimental error, the simplex is too small. In this case, the simplex will tend to wander erratically both before and after reaching the optimum. In fact, the optimum point may never be found. With a simplex that is too large, the point of maximum response will probably be overshot and may never be found. For best results, the step size and hence the size of the simplex should be at least as large as two to three times the error of each measurement.

Three additional rules are necessary to prevent failure of the method under certain conditions. These rules have been discussed in detail by Deming and Morgan (*6*) and will only be listed here:

(1) If the most recently obtained vertex of a simplex produces the least-desirable experimental result, discard the second-from-the-worst vertex in determining the coordinates of the next vertex.

(2) If a vertex has been retained in $k + 1$ simplexes, where k is the number of variables under consideration, redetermine the experimental result at that vertex before continuing.

(3) If the calculated coordinates at a new point lie outside the boundaries of one or more of the variables, making it impossible to do an experiment under the predicted conditions, assign an undesirable response to that point and continue.

When the simplex reaches the area of optimum response, application of the rules forces it to circle in the immediate vicinity of the optimum.

If more than three variables are being investigated, the simplex will have more than three dimensions, and then it is handled by calculation. To calculate the value of a variable at the new point, the values of that variable at each of the retained vertices of the previous simplex are added and the sum is multiplied by $2/k$. The value of the same variable at the rejected vertex of the previous simplex is then subtracted from the product to yield the desired value of that variable at the new point. This procedure is repeated for each variable in turn. A convenient format for setting up this calculation for any number of

Table 1. Sample Format Used to Calculate the Coordinates of a New Vertex

	Vertex no.	Factor A	Factor B
Retained vertices	2	10	55
	3	20	50
Sum of retained coordinates		30	105
Coordinates times 2/k[a]		30	105
Above less coordinates of discarded vertex 1	1	−10	−45
Coordinates of new vertex	4	20	60

[a] k is the number of variables.

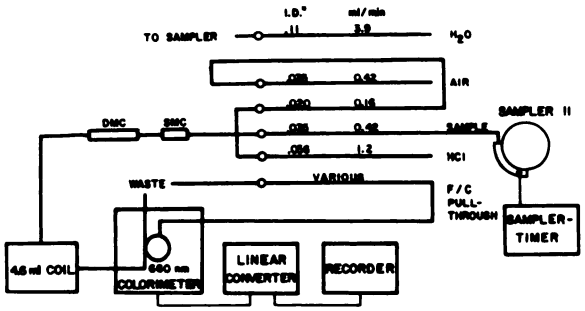

Fig. 2. Flow diagram for simplified continuous-flow system

variables has been presented by Long (*5*) and is illustrated in Table 1.

Application to Continuous-Flow Analysis

Interaction or longitudinal mixing on the AutoAnalyzer limits the rate at which determinations can be carried out, and a goal of method optimization should be to find conditions of minimum interaction. The primary factors that affect interaction are the sample-to-wash ratio, the rate at which the solution flows through the flowcell, and the fraction of the stream that is air. Thiers and others (*7–11*) have described mathematical models from which the interaction can be calculated under certain circumstances. The simplex method does not permit the calculation of interaction, but rather it leads to the point of minimum interaction by experimentation.

Simplified Continuous-Flow System

The manifold of the simple system is shown in Figure 2. An AutoAnalyzer Sampler II, a Pump II, a 4.6-ml coil, 2-mm (i.d.) coil, a colorimeter, and a recorder were used. A Dynacon (Dynacon Research and Systems, Inc., Palisades, N. Y. 10964) No. LC-172 single-channel linear converter was used to amplify and linearize the signal from the colorimeter and to permit direct reading from the chart in concentration units. Sampling rate and the sample-to-wash ratio were controlled by a No. 4016 Sampler-Timer (Gilford Instrument Laboratories Inc., Oberlin, Ohio 44074) in place of the cam on the Technicon sampler. The Gilford timer permits setting sample-to-wash ratios from 1:6 to 6:1, in eleven steps. A sampling rate of 100 samples per hour was used here. The flowcell pull-through rate was varied by changing the tube size on the manifold. The rate of solution delivery to the colorimeter was held constant at 1.62 ml/min.

The diluent stream was 125 mmol/liter HCl containing 0.5 ml of surfactant (Brij-35) per liter. We were looking for the combination of the sample-to-wash ratio and flowcell pull-through rate that gave the least interaction. Samples containing 25, 100, and 25 mmol of $CuSO_4$ per liter were run, in that order, to produce peaks 1, 2, and 3, respectively. The percent interaction (%I) of peak 2 with peak 3 was calculated by dividing the difference of peak heights 1 and 3 by the value of peak 2 and multiplying by 100.

The results of these experiments are shown in Table 2 and the movement of the simplex toward the optimum area is shown in Figure 3. Since the percent sample (%S)—*i.e.*, [volume sample/(volume sample + volume wash)] × 100—and percent pull-through (%PT)—i.e., (volume of liquid going through flow cell/volume of liquid reaching flowcell) × 100—were not continuously variable owing to the limitations of the sampler timer and the available tube sizes, it was not always possible to perform an experiment at the exact conditions required by the simplex method. Values shown for %S and %PT in Table 2 and those plotted in Figure 3 are the values actually used.

The coordinates of the first three points were chosen with a step size substantially larger than the experimental error of each measurement. Based on data from earlier experiments, the starting position of the simplex was chosen in an area away from the point of minimum interaction. The %I was found at points 1–3 and the simplex was reflected away from the highest %I. Reflection of the simplex away from the highest %I was continued until the simplexes with vertices 9, 10, and 11, and 9, 11, and 12 (Figure 3) were reached where the second-from-worst response had to be rejected (Rule 1) to prevent the simplex from becoming stranded. Because vertex 9 had been retained in three simplexes, the determination of %I at the conditions of vertex 9 was repeated (Rule 2), and the %I is indicated beside 9a in Table 2.

The highest %I in simplex 9, 12, 13 is at vertex 12. When reflecting away from vertex 12, the new vertex is nearly identical to the conditions described at vertex 7, i.e., the simplexes had circled vertex 9 and the conditions of lowest %I were defined somewhere in the area of the hexagon about vertex 9. The point of lowest %I could have been found more precisely by defining a simplex smaller than simplex 7, 9, 13 within the hexagon (Figure 3) and proceeding as before.

Table 2. Movement of Simplex in Optimization of Simplified ($CuSO_4$) Continuous-Flow System[a]

Vertex no.	% Sample	% Pull-through	Response, % interaction	Vertices retained from previous simplex
1	25.0 —	24.0 —	37.3	—
2	25.0 —	34.0 —	22.4	—
3	33.3 —	29.0 —	27.2	—
4	33.3 (33.3)	40.1 (39.0)	19.2	2, 3
5	25.0 (25.0)	45.7 (45.1)	13.5	2, 4
6	33.3 (33.3)	51.2 (51.8)	12.0	4, 5
7	25.0 (25.0)	56.8 (56.8)	5.4	5, 6
8	33.3 (33.3)	63.0 (62.3)	5.1	6, 7
9	25.0 (25.0)	69.1 (68.6)	4.0	7, 8
10	33.3 (33.3)	75.3 (75.3)	4.2	8, 9
11	25.0 (25.0)	82.7 (82.4)	4.2	9, 10
12	14.3 (16.7)	77.2 (76.5)	6.9	9, 11
9a	25.0	69.1	3.8	
13	14.3 (14.3)	63.0 (63.6)	5.4	9a, 12
14	85.7 —	40.1 —	36.8	—
15	85.7 —	51.2 —	29.0	—
16	75.0 —	45.7 —	28.1	—
17	75.0 (75.0)	56.8 (56.8)	27.3	15, 16
18	66.6 (64.3)	51.2 (51.3)	24.8	16, 17
19	66.6 (66.6)	63.0 (62.3)	20.8	17, 18
20	60.0 (58.2)	56.8 (57.4)	20.3	18, 19
21	60.0 (60.0)	69.1 (68.6)	18.6	19, 20
22	54.5 (53.4)	63.0 (62.9)	15.5	20, 21
23	54.5 (54.5)	75.3 (75.3)	14.2	21, 22
24	45.5 (49.0)	69.1 (69.2)	8.4	22, 23
25	45.5 (45.5)	82.7 (81.4)	9.2	23, 24
26	33.3 (36.5)	75.3 (76.5)	4.4	24, 25
27	33.3 (33.3)	63.0 (61.7)	5.0	24, 26

[a]Bracketed values are those predicted by the simplex but which could not always be obtained experimentally. The response at vertex 9 was redetermined since this vertex had been retained in 3 simplexes (Rule 2). The new response is listed at vertex 9a. The response at 9a was used in the decision-making process of subsequent simplexes to move toward the optimum. A new simplex was started at vertices 14, 15, and 16. The % sample, % pull-through, etc., are defined in the text.

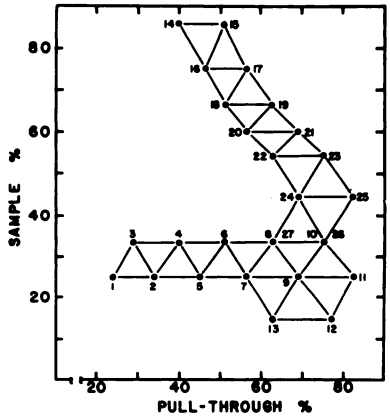

Fig. 3. Progress of simplex in optimization of simplified ($CuSO_4$) continuous-flow system

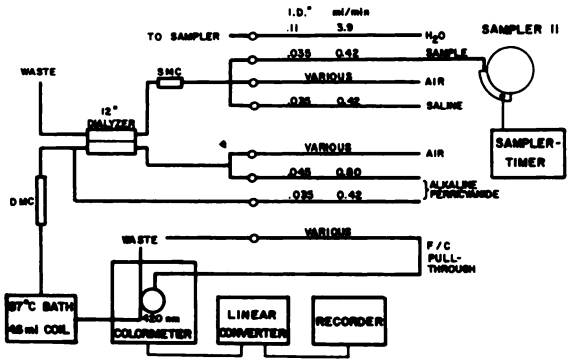

Fig. 4. Flow diagram for glucose AutoAnalyzer

A second set of experiments was started at points 14, 15, and 16 to see if the same optimum area would be found in a new search. The search progressed as shown in Figure 3. Vertices 26 and 27 came very close to vertices 10 and 8. Rejection of vertex 24 in simplex 24, 26, 27 landed us on simplex 8, 9, 10 found in the first search, and the subsequent results were identical to the first search. The same hexagon about point 9 was found as before.

Glucose Analyzer

The manifold supplied by Dynacon for the N2b alkaline ferricyanide method for glucose is shown in Figure 4. A small-bore 10-mm debubbler flowcell (Dynacon No. 19B15) was used and the air and flowcell pull-through lines were changed as required by the experiment. Three variables were studied: %S, %PT, and percent air (%A)—i.e., [(volume of air slugs)/(volume of air + volume of solution in stream)] × 100. We sought the combination that would give the lowest %I. The %I at each condition was determined by analyzing 50, 300, and 50 mg/100 ml aqueous glucose solutions, in that order, at 140 samples per hour (Table 3). The coordinates of vertex 1 were those recommended by Dynacon, and the coordinates of vertices 2–4 were calculated by using Long's procedure (*5*), with a step size of 10 for each factor.

The progress of the simplex in this example is straightforward. Rule 1 was applied in calculating the coordinates of vertices 12 and 14 (Table 3).

Interpretation of the results in this example is not as easy as in the two-factor example, where the progress could be followed graphically. The experiment was stopped when two consecutive vertices duplicated earlier points. However, the vertices that were duplicated had been obtained much earlier in the experiment. Also, the duplicated points were not those that gave the best response. Three points were found (vertices 7, 9 and 12, Table 3) where a %I of approximately 0.5% was measured. The optimum ranges at points 7, 9, and 12 are 50–55 %S, 67–77 %PT, and

Table 3. Data Obtained for Simplex Optimization of Glucose AutoAnalyzer[a]

Vertex no.	% Sample	% Pull-through	% Air	Response, % interaction	Vertices retained from previous simplex
1	50.0 —	76.0 —	11.9 —	2.5	—
2	60.0 (60.0)	76.0 (76.0)	11.9 (11.9)	3.4	—
3	54.5 (55.0)	83.6 (84.7)	11.9 (11.9)	2.2	—
4	54.5 (55.0)	77.9 (78.9)	20.0 (20.1)	1.3	—
5	45.5 (46.0)	83.6 (82.3)	15.0 (17.3)	1.8	1, 3, 4
6	54.5 (53.0)	87.8 (87.4)	20.0 (19.4)	1.5	3, 4, 5
7	50.0 (48.5)	76.6 (82.6)	24.0 (24.8)	0.6	4, 5, 6
8	60.0 (60.5)	77.9 (77.9)	32.0 (27.7)	1.4	4, 6, 7
4a	54.5 —	77.9 —	20.0 —	1.2	—
9	54.5 (55.2)	67.8 (67.1)	32.0 (30.7)	0.5	4a, 7, 8
10	45.5 (46.0)	71.0 (70.3)	20.0 (18.7)	0.9	4a, 7, 9
11	45.5 (45.5)	65.7 (65.7)	32.0 (30.7)	1.1	7, 9, 10
7a	50.0 —	76.6 —	24.0 —	0.7	—
12	54.5 (54.5)	71.0 (69.1)	38.0 (38.7)	0.6	7a, 9, 11
13	60.0 (60.5)	77.9 (77.9)	32.0 (30.7)	1.7	7a, 9, 12
14	— (55.2)	— (77.2)	— (20.7)	—	7a, 9, 13

[a] Bracketed values are those predicted by the simplex but which could not always be obtained experimentally. The coordinates of vertex 13 are the same as those of vertex 8 and the calculated coordinates of vertex 14 are very close to those of vertex 4.
An "a" after a vertex number has the same meaning as in Table 2.

24–38 %A. The major difference between the optimum found by the simplex and the conditions recommended by Dynacon is the amount of air in the stream. We found that an increase in the air from 12% to 38% decreased the interaction by a factor of about five.

Application to Chemical Variables in Kinetic Enzyme Analysis

In 1964, the International Union of Biochemistry recommended that enzyme activities be expressed in terms of micromoles product formed per minute and "*that other conditions be optimal*" (*12*). Optimum conditions implies those that give the fastest rate, but some caution should be exercised in using conditions that give the fastest rate, because the resulting system may not be very stable or may give poor accuracy. A three-dimensional drawing could be made of a system involving three variables such as pH, substrate concentration, and co-factor concentration. At a given condition of the three variables, the enzyme activity could be measured and the activity then be represented as a point in the defined three-dimensional space. High enzyme activity would be represented by peaks and low activity by valleys. The conditions giving the highest activity could lie on a ridge or sharp peak, which would make the system very sensitive to a change in concentration of one of the variables and hence be rather unstable. Ideally, the optimum should lie on a plateau. For example, McComb and Bowers (*13*) found that in choosing between two aminoalcohol buffers in the determination of alkaline phosphatase, the one that gave a lower observed activity in the pH 9.8–10 range actually gave a more stable system (*13*). Nevertheless, it should be possible to find in a direct way the conditions that yield the fastest rate. The stability of the system and the precision of the method can be evaluated after the presumed optimum is found.

The LD-catalyzed conversion of pyruvate to lactate has been studied extensively in an effort to find the optimum conditions (*14–17*). Gay et al. (*15*) showed that the optimum pyruvate concentration depends on the pH. In the presence of interdependent variables, it is fortuitous if the unique optimum is found by an empirical approach. We studied the pyruvate-to-lactate reaction with a manually operated Gilford Model 220 ultraviolet spectrophotometer equipped with a cuvet positioner and thermostatting bath set at 25 °C as described elsewhere (*18*). All reagents were from Sigma Chemical Co., St. Louis, Mo. 63178. The mean LD activity of the lot of Versatol-E (General Diagnostics Co., Morris Plains, N. J. 07950) that was used had been established by the stopped-flow method (*20*) over a 30-day period with 90 determinations as 953 ± 33 (SD) units/ml [these are the enzyme units as defined by Henry et al. (*14*)].

Tris acid, Tris base (*19*), sodium pyruvate, and NADH were combined in 1-cm quartz cuvets to give a volume of 2.9 ml. Control serum (Versatol-E), 0.1 ml, was added and mixed in, and the change in absorbance at 340 nm was recorded for 5 min. The activity was calculated from the zero-order slope of absorbance vs. time.

We sought the conditions that would give the fastest rate. Four variables (pH, and the concentrations

Table 4. Data for Simplex Optimization of the LD Reaction[a]

Vertex no.	pH	Tris buffer, mmol/liter	Pyruvate, mmol/liter	NADH, mmol/liter	Response, activity in units/ml	Vertices retained from previous simplex
1	7.40	50	0.91	0.17	924	—
2	7.70	50	0.91	0.17	946	—
3	7.55	76	0.91	0.17	950	—
4	7.55	59	1.39	0.17	893	—
5	7.55	59	1.03	0.24	975	—
6	7.55	59	0.49	0.20	1065	1, 2, 3, 5
7	7.78	72	0.76	0.22	945	2, 3, 5, 6
8	7.52	83	0.68	0.24	1095	3, 5, 6, 7
9	7.30	66	0.80	0.20	935	3, 5, 6, 8
10	7.41	58	0.59	0.27	1125	5, 6, 8, 9
5a	7.55	59	1.03	0.24	950	
11	7.72	64	0.60	0.28	1140	5a, 6, 8, 10
6a	7.55	59	0.49	0.20	1070	
12	7.55	73	0.15	0.26	750	6a, 8, 10, 11
13	7.55	80	0.52	0.33	1075	8, 10, 11, 12
8a	7.52	83	0.68	0.24	1060	
14	7.55	69	1.04	0.30	935	8a, 10, 11, 13
15	7.60	53	0.70	0.35	865	10, 11, 13, 14
10a	7.41	58	0.59	0.27	1110	
16	7.59	59	0.16	0.32	785	10a, 11, 13, 15
11a	7.72	64	0.60	0.28	1150	
17	7.54	77	0.24	0.25	915	10a, 11a, 13, 16
18	7.52	81	0.82	0.24	1060	10a, 11a, 13, 17
13a	7.55	80	0.52	0.33	1040	
19	7.56	65	1.02	0.31	915	10a, 11a, 13a, 18
20	7.55	54	1.00	0.22	930	10a, 11a, 18, 19
21	7.54	63	0.48	0.20	—	10a, 11a, 18, 20

[a] The vertices are listed in the order in which the experiments were performed and the concentrations listed are those in the final 3-ml reaction mixture including 0.1 ml of the pooled serum. An "a" after a vertex number has the same meaning as in Table 2.

of Tris buffer, pyruvate, and NADH) were examined simultaneously. The coordinates of vertex 1 (Table 4) are the conditions in current use in our laboratory for the estimation of LD. The coordinates of vertices 2 through 5 were calculated by Long's method (*5*) with the following step sizes: 0.3 for pH, 30 mmol/liter for the buffer, 0.59 mmol/liter for the pyruvate, and 0.09 mmol/liter for the NADH concentration. The simplex was moved from its first position away from the lowest LD activity found and the coordinates of the new vertex were calculated according to the format given in Table 1.

The LD activity was redetermined at the conditions of a vertex that had been retained in five simplexes (Rule 2). The results are given as vertices 5a, 6a, 8a, 11a, and 13a in Table 4. The conditions at vertex 5 and 5a were the same, 6 and 6a were the same, etc. An estimate of the reproducibility of the LD activities can be made from the duplicate values. In all cases, the duplicate values agreed to within ±3%.

The maximum activity of 1150 units/ml was found at vertex 11a, and it was 25% greater than the activity at vertex 1. For the five vertices at which the highest enzyme activity was found (6, 8, 10, 11, 13), the pH ranged from 7.41 to 7.72, the buffer from 58 to 83 mmol/liter, the sodium pyruvate from 0.49 to 0.68 mmol/liter, and the NADH from 0.20 to 0.33 mmol/liter. None of these ranges include the values that had been determined earlier by a conventional optimization technique (*20*). The optimum ranges found for pH, buffer concentration, and NADH concentration are quite broad, in agreement with the findings of Henry et al. (*14*). The optimum range found for pyruvate concentration is relatively narrow, also in agreement with that found by Henry et al. (*14*), and by Hill (*16*).

The simplex here does not seek out the maximum and lock into that area, as might be supposed. Instead, it finds the area of maximum activity at various times throughout the experiment and then moves on to an area of lower activity. This behavior is a consequence of two factors: the large step sizes chosen in the original simplex, and the relative insensitivity of the LD reaction to changes in the pH, buffer, and NADH concentrations. A smaller simplex started in the immediate area of the optimum would have defined the optimum conditions more precisely.

Application to Optimization of Instrumental Variables

Centrifugal analyzers are well suited for kinetic enzyme determinations (*21, 22*). We studied certain instrumental variables in the LD-catalyzed pyruvate-to-lactate reaction on the ENI-GEMSAEC centrifugal analyzer. The optimum chemical reaction conditions found in the previous experiment were used (Table 4, vertex 11). The same freshly pooled human serum with about 300 units of LD activity per milliliter was used for all the samples, and all the experiments with the GEMSAEC were performed on the same day. The Teflon distribution disks were loaded (with the Rotoloader) with 0.020 ml of serum, followed by 0.100 ml of buffer wash into well B and 0.480 ml of reagent (pyruvate, NADH, buffer) into well C.

In the kinetic determination of enzyme activity, the GEMSAEC user can elect to have the first reading taken between 5 and 999 s after mixing of serum and reagents, he can take readings every 5 to 99 s, and he can take up to 20 readings on the same sample. When the instrument is used for kinetic enzyme analyses, the absorbancies for the successive readings on each cuvet are stored in the computer memory, and the best fit of the curve of absorbance vs. time is calculated with a least-squares curve-fitting algorithm. A loss of zero-order kinetics is flagged on the print-out.

Absorbance readings can be taken at short time intervals, but the resulting absorbance difference between readings is small and the relative error in the measured absorbance change is large (*22*). As the time between readings is increased, there is a practical limit to the number of absorbance readings that can be taken.

We used the number of readings taken and the time between readings as variables in the simplex optimization with the goal of minimizing the within-run coefficient of variation (CV). The time to the first reading was held at 120 s throughout.

In each experiment, the same pooled serum was loaded into each of the 15 sample positions of the Teflon disk. After the LD activity was determined with a desired reading interval and number of readings, the CV of the 15 results was calculated and the simplex was moved away from the vertex with the highest CV.

The results are summarized in Table 5 and the progress of the simplex is shown in Figure 5. The initial position of the simplex was chosen well away from the manufacturer's recommended conditions of 60 s between readings and four readings.

The progress of the simplex toward the optimum was straightforward. The simplex was found to circle around vertex 11, which was taken to be the optimum. The conditions producing the lowest CV were found to be 70 s between readings and five readings. The mean LD values for vertex 11 and the six vertices surrounding it agreed very well. There was much more fluctuation in the mean LD activity at some of the earlier vertices where the total observation time was shorter.

Table 5. Data for Simplex Optimization of Instrumental Parameters on the GEMSAEC[a]

Vertex no.	Time between readings, s	No. of readings	Mean LD activity, units/ml	Coefficient of variation	Vertices retained from previous simplex
1	30	3	308	11.6	—
2	40	3	336	9.1	—
3	35	4	344	9.7	—
4	45	4	306	6.9	2, 3
5	50	3	327	6.1	2, 4
6	55	4	294	3.7	4, 5
7	60	3	334	3.5	5, 6
8	65	4	289	2.4	6, 7
9	70	3	302	3.8	7, 8
10	75	4	279	2.5	8, 9
11	70	5	280	1.7	8, 10
8a	65	4	292	2.4	
12	60	5	286	2.3	8a, 11
13	65	6	281	2.6	11, 12
14	75	6	289	2.3	11, 13
11a	70	5	293	1.6	
15	80	5	291	2.3	11a, 14
SR	60	4	298	3.1	—

[a] "SR" refers to the settings recommended by the manufacturer. An "a" after a vertex number has the same meaning as in Table 2.

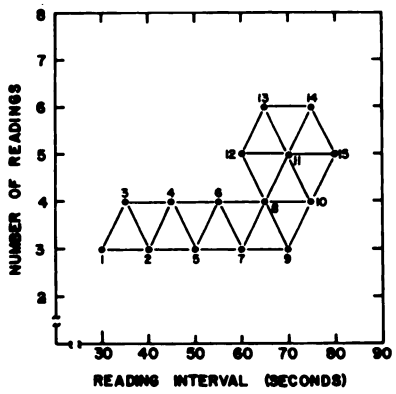

Fig. 5. Progress of simplex in optimization of GEMSAEC parameters

Conclusions

The validity of the simplex method is well established (*4–6*). Here, it was not our intention to prove its validity, but rather to show that the simplex technique is a useful tool for optimizing the conditions of analytical methods in clinical chemistry.

With the simple AutoAnalyzer system in which we used $CuSO_4$ solutions, we found the same optimum area when the search for the optimum (minimum

%I) was started from two different points well away from the optimum. In the AutoAnalyzer method for glucose on the Dynacon system, we were able to find conditions by use of the simplex technique under which interaction between samples was reduced five-fold and also to find conditions permitting a faster rate of analysis. We found a combination of pH and reagent concentrations in the determination of serum LD activity that gave an increase of 25% in the observed LD activity when compared to other conditions (*20*) that had been determined by an empirical approach. In the examination of instrumental parameters on the GEMSAEC, we found that the conditions predicted by the simplex gave a CV that was significantly smaller than the CV obtained under the manufacturer's recommended conditions.

In both the LD and GEMSAEC experiments, we found the optimum conditions for each at one level of enzyme activity. The chemical conditions for the LD reaction were optimized at about 1000 units/ml and the instrument settings for the GEMSAEC at about 300 units/ml. An LD activity of 1000 units/ml was chosen to magnify the effect of small changes in reagent concentration and pH on the observed LD activity. With the GEMSAEC, the LD activity was chosen to be at or near the upper limit of normal, which is 300 units/ml. At the borderline between normal and abnormal, a method should have the highest precision attainable.

Have we found the unique optimum for each of the methods we studied? For the simple AutoAnalyzer system ($CuSO_4$) and the GEMSAEC, we have found (or have come very close to) the optimum. The areas within which the optimum points are located are determined easily from the two-dimensional figures of the simplex movements (Figures 3 and 5). In the examination of the glucose and LD methods, the region near the optimum was found more than once in each study. There is some doubt whether the exact optimum has been found in these two cases; however the reduction in %I in the case of glucose and the increase in the observed activity for LD over that found under the previously used conditions is significant.

The major advantage of the simplex technique is the speed by which the optimum conditions for a method can be found. With the GEMSAEC for example, we were able to locate the optimum instrumental settings after performing only 18 experiments. There are certainly many more than 18 combinations of time between readings and number of readings possible so that an empirical search would have required many more experiments.

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