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Simplex Optimization of Reaction Conditions with an Automated System

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An automated system for the optimization of experimental conditions was applied to the reaction of carboxylic acid. This system consists of a laboratory robotic system, a spectrophotometer, and a personal computer which are interfaced with each other. The optimum conditions were obtained according to the super modified simplex algorithm. This model reaction involved 3 or 4 parameters, and was optimized after 28 experiments at most. The effects of the initial simplex as well as the feasibility and efficiency of the system are discussed.

Keywords——automated optimization; simplex method; laboratory robot; sodium valproate

Laboratory robotic systems have been used in the area of analytical chemistry for routine work such as sample preparation and determination, and several reviews have appeared.[1] In these applications, the robot executes a large amount of work according to the established procedure, and reduces the input of human labor with its inherent errors. Laboratory robots should be useful for the development of analytical methods.[2]

In the process of establishment of an analytical procedure the selection of the optimum conditions takes a large amount of time and labor. In order to reduce them, some systematic strategies have been designed for the optimization. One of the most widely used methods is to hold all but one parameter constant while searching for the optimum value of that parameter. This method, however, does not always lead to the true optimum conditions, because it neglects the possibility of interaction between parameters. The simplex optimization method overcomes this defect, and some instrumental conditions such as flame spectrophotometry[3] and inductively coupled plasma[4] are optimized with the use of the simplex algorithm.

This method, however, is not effective in the optimization of reaction conditions, probably because it requires rather large numbers of experiments. Kleeman and Bailey[5] optimized the reaction of blue tetrazolium and α-ketol steroid involving five factors with the variable size simplex method. In their work 58 experiments with different conditions were involved. It is not easy to perform a large number of experiments with different conditions manually. We constructed an automated system for the optimization of the experimental conditions of a color developing reaction.[6] In this system a less-intelligent laboratory robot was interfaced to a desk-top computer for calculating and making decisions. The reaction and measurement procedure were performed automatically by the robot, and the optimum conditions were found by the computer according to the modified super modified simplex (SMS2) algorithm. This system was applied to the simple reaction of phosphotungstic acid and phenothiazines, and the combination of two parameters, the amount of phosphotungstic acid and the reaction time, was optimized.

In the present paper, this system is applied to the reaction of carboxylic acid to give hydroxamate.[7–9] This reaction was divided into two steps. In the first step, carboxylic acid reacts with hydroxylamine perchlorate (HAP) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) to form hydroxamic acid. In the second, iron hydroxamate is

formed with ferric perchlorate (FP), developing a violet color (λ_{max} 538 nm). The procedure of this reaction is fairly complicated and 3 or 4 parameters have to be optimized. The feasibility of applying the automated system to this model reaction was investigated, and the effect of the initial conditions is also discussed.

Simplex Method

The algorithm employed in this optimization system is the modified super modified simplex (SMS2) method described by Parker *et al.*[4] In this section the simplex algorithm is introduced briefly. The simplex method was introduced by Spendley *et al.*[10] in 1962, and some variations have been contrived by many authors; they have been summarized by Betteridge *et al.*[11]

Simplex is a geometrical figure with $n+1$ vertices in n-dimensional space. In the simplex optimization method, simplex moves towards the optimum in the n-dimensional variable space in which response can be measured for each point. The basic rule for the movement of simplex involves discarding the vertex that gives the worst response and replacing it with a point that is expected to give a better response. This procedure is iterated until the response is not improved any more. The rule for location of next vertex was modified in variations of the simplex method.

The super modified simplex (SMS) was introduced by Routh *et al.*[3] In this algorithm, the location of the next vertex is determined by fitting the responses to a second-order polynominal at the worst vertex, the centroid of the remaining vertices, and the reflected point of the worst through the centroid, and by predicting the location that might give the best response. In SMS2, the average of the responses of the remaining vertices is used instead of the measured response at the centroid. These procedures allow the new vertex a large freedom of location, whereas the number of possible locations is restricted to four in the modified simplex method of Nelder and Mead.[12] This large freedom reduces the number of movements of simplex until it attains the optimum point.

Experimental

Instrumentation——The Zymate II laboratory robotic system (Zymark) was used. It is composed of a robotic arm and two exchangeable hands, a general purpose hand and a syringe hand. The general purpose hand has rigid fingers to grip a test tube and transport it. The syringe hand is equipped with a 5 ml gas-tight syringe, and adds or takes a liquid sample quantitatively. Other major components included a master laboratory station that supplied liquids and a vortex mixer station. All these stations are controlled by the Zymate controller. Other instruments used were a water bath (P-80, Taiyo Kagaku Kogyo) to heat the reaction mixture, and a UV-VIS spectrophotometer (UV-160, Shimadzu) with a sipper unit for measurement of absorbance. The spectrophotometer was interfaced to the desk-top computer *via* a GP-IB interface.

The analytical procedures were programmed in EASYLAB, that was designed to control the movement of the robot. An external desk top computer (PC-9801, NEC), programmed in N88BASIC, controlled the sipper, and calculated the next combination of parameters based on the absorbance data transmitted from the spectrophotometer. The Zymate controller and the external computer communicated through the remote control interface (Zymark) and RS-232C interface of the external computer.

Reagents——Sodium valproate (Kyowa Hakko Kogyo) was used without further purification. DCC, HAP solution and FP were reagents for carboxylic acid analysis (Kanto Kagaku). Ethanol was of guaranteed reagent grade.

Solutions——All solutions but FP solution were prepared with ethanol. The concentration of sodium valproate solution was 5 mM and that of DCC solution was 0.5 M. HAP solution was diluted with an equal volume of ethanol (0.5 M). FP was dissolved in ethanol containing 1 N perchloric acid at a concentration of 0.025 mM.

Reaction Procedure——The reaction procedure described in ref. 7 was modified for the robotic operation. The volume of the reaction mixture was kept constant in all experiments independent of the added volume of the reagents. In this way the added volume can be used as a substitute for the concentration of the reagent in the reaction mixture. The robotic procedure was as follows: (1) One milliliter of the sodium valproate solution was added to a test tube and specified volumes of HAP and DCC solutions (parameters 1 and 2) were added. (2) The required volume of ethanol to give a volume of 6 ml of the reaction mixture was added to the test tube by the master laboratory station. (3) The test

tube was vortexed and placed in the water bath at 50 °C for the specified length of time (parameter 3). (4) A 2 ml aliquot of the mixture was transferred to another test tube and a specified volume of FP solution (parameter 4) was added. (5) The required volume of ethanol to give a volume of 5 ml of the mixture was added and the mixture was vortexed. After 5 min the absorbance was determined. (6) The absorbance was measured for the reagent blank in which 1 ml of ethanol was substituted for the sodium valproate solution and treated concurrently with the sample. (7) The net absorbance, that is, the difference between the absorbance values for sample and reagent blank solutions, was computed.

Grid Search Study——A set of the three parameters, the volumes of HAP and DCC solutions and the reaction time, was transmitted to the robot from the external computer, and the robot measured the absorbance according to the reaction procedure. This procedure was repeated with different combinations of parameters. The volumes of HAP and DCC were varied from 0.1 to 2 ml, and the reaction time from 2 to 20 min.

Optimization Procedure——(1) The number of parameters to be optimized and their boundary values were inputted to the desk-top computer. (2) The initial conditions for starting simplex were inputted to the desk top computer and transmitted to the robot. (3) Sample and reagent blank were prepared with the specified conditions according to the Reaction Procedure, and the net absorbance was determined and transmitted to the computer. The responses at all vertices were compared and the reflected point of the worst vertex was located. (5) The response was determined at the reflected point. (6) The computer calculated the new conditions and transmits them to the robot. (7) The procedures (3)—(7) were repeated. Each time the computer received an absorbance value at a new vertex, it evaluated the value and decided whether the simplex had reached the optimum, and generated new conditions or terminated the movement of the simplex.

Results

The precision of the absorbance obtained is dependent on the repeatability of the robotic operation. The relative standard deviation (RSD) of the dispensed volume of liquid by the master laboratory station was reported as 0.03%, and that of the pipetting volume by the syringe hand was 0.3%.[13] The RSD of the net absorbance from 5 operations with the robotic system was *ca.* 0.7%. This RSD value is slightly larger than that obtained in the preceding paper (RSD = 0.5%).[6] This can probably be imputed to the more complicated experimental procedure. On the basis of this value, when the RSD of the responses at all vertices was less than 1%, the simplex was judged to have reached to the optimum.

The contour plots of response surface obtained from the grid search experiment involving 130 points are illustrated in Fig. 1 (a) to (c). The volumes of HAP and DCC were varied from 0.1 to 2 ml, and the reaction time from 2 to 20 min. The volume of FP was fixed at 0.5 ml and the reaction time at 2, 10, and 20 min in figure (a), (b) and (c), respectively. These figures show the effects of the three factors on the absorbance. The effects of the volumes of the two reagents, HAP and DCC, are not independent. In addition, the volumes of reagents required to obtain maximum absorbance slightly decrease as the reaction time increases. The optimum region is spread over a broad range in the variable space of three dimensions

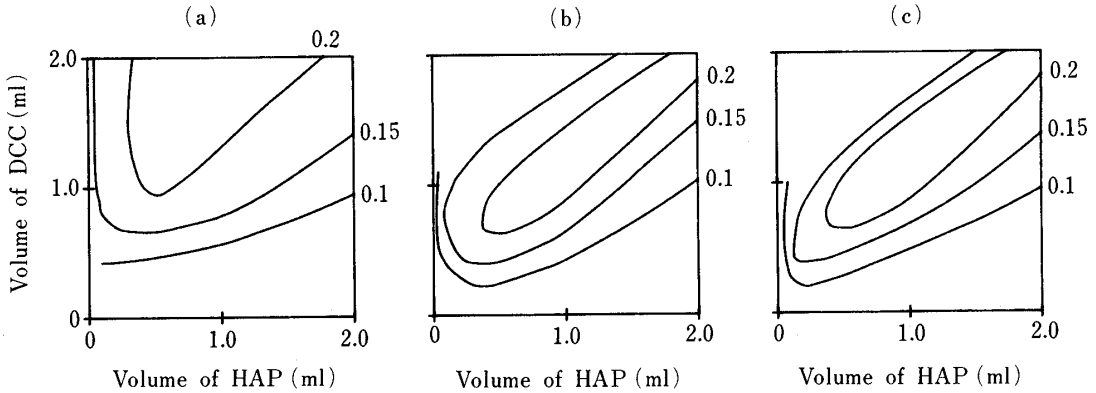

Fig. 1. Contour Plot of Response Surface

Reaction time was (a) 2 min, (b) 10 min and (c) 20 min.

TABLE I. Conditions of the Initial Simplex and Resultant Optimum

	Initial simplex			Resultant optimum			
	Parameter			Parameter			Absorbance
	1[a]	2[b]	3[c]	1	2	3	
a	0.1	0.1	0				
	1.5	0.1	0				
	0.1	1.5	0				
	0.1	0.1	20	1.032	1.157	12.22	0.219
b	0.1	0.1	0				
	1	0.1	0				
	0.1	1	0				
	0.1	0.1	10	0.847	0.968	8.31	0.214
c	0.1	0.1	0				
	0.5	0.1	0				
	0.1	0.5	0				
	0.1	0.1	5	0.605	1.111	1.80	0.210
d	0,8	0.8	12				
	1.2	0.8	12				
	0.8	1.2	12				
	0.8	0.8	20	0.62	0.993	15.87	0.208
e	1.0	0.1	10				
	1.5	0.1	10				
	1.0	0.6	10				
	1.0	0.1	20	0.632	0.695	10.05	0.210
f	0.1	1.0	10				
	0.1	1.5	10				
	0.6	1.0	10				
	0.1	1.0	20	0.535	1.098	2.65	0.208
g	2.0	2.0	25				
	2.0	0.1	25				
	0.1	2.0	25				
	2.0	2.0	0	1.052	1.584	7.29	0.210

a) Volume of HAP (ml). *b*) Volume of DCC (ml). *c*) Reaction time (min).

along the axis of reaction time. The width along the other two axes, the volumes of the two reagents, is rather small in contrast.

The combination of these three parameters was optimized by the automated system using the SMS2 method. In this experiment the volume of the FP was fixed at 0.5 ml. The simplex has 4 vertices with 3 coordinates. Table I lists the optimum conditions obtained from different initial simplices. The location and the size of the initial simplex are dispersed over a wide range in the variable space. The size of initial simplex of (a) and (g) is larger than others. The initial values of parameters of (a) are smaller than those of (g). The volume of HAP (parameter 1) in the initial simplex of (e) is much larger than the volume of DCC (parameter 2). The two parameters are reversed in (f). The initial simplex of (d) is located in the middle of the bounded variable space, while the other initial simplices are on the side. In spite of the difference in the initial conditions, the values of net absorbance with the final optimum conditions are the same.

On the other hand, the number of vertices required to reach the optimum conditions varies from 6 to 16 with the initial conditions. Figure 2 shows the examples. The net absorbance is plotted against the number of the vertex. The first 4 vertices are included in the initial simplex. All vertices in the initial simplex shown in (a) give net absorbance of less than 0.1. These initial conditions are far from the optimum, but the conditions are improved

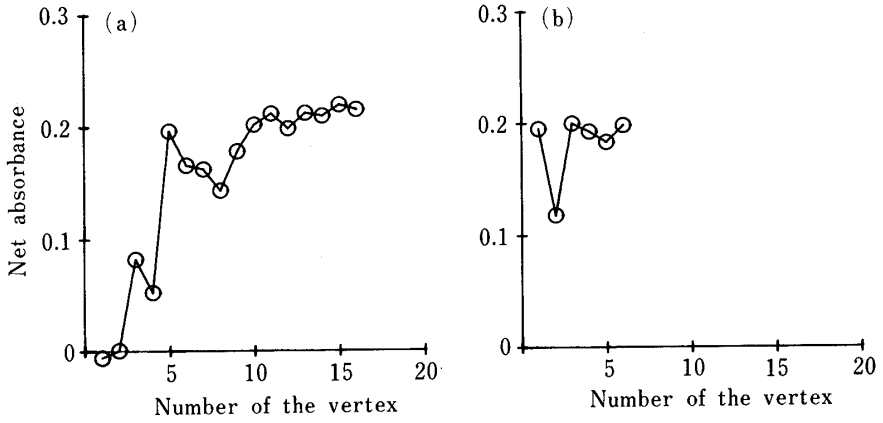

Fig. 2. Net Absorbance *versus* Number of the Vertex for Optimization of 3 Parameters

Initial conditions were (a) a and (b) d shown in Table I.

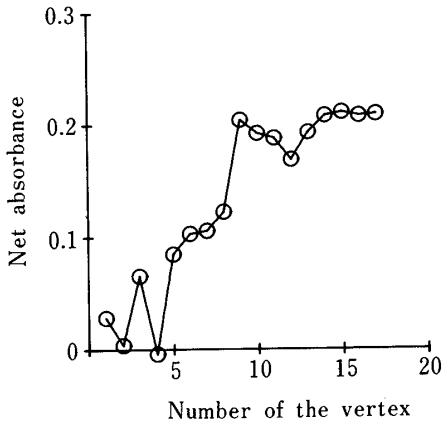

Fig. 3. Net Absorbance *versus* Number of the Vertex for Optimization of 4 Parameters

successively and the simplex attains the optimum after 28 experiments (involving 12 reflection points). The number of vertices involved in (b) is small compared to the others. Three of four vertices of the initial simplex are located near to the optimum region, and the simplex attains the optimum after only two movements.

Figure 3 shows the result of SMS optimization for the reaction conditions including 4 parameters. Three of them are the same as those in the above experiment and the fourth is the volume of FP solution, that is held at 0.5 ml in the above case. In this experiment, this system finds the optimum after only 12 simplex movements.

Discussion

The results clearly show that this automated system is feasible for optimization of the reaction condition involving 3 or 4 parameters. The number of experiments performed until the optimum condition are obtained is at most 28 for the three-parameter design, and 29 for four-parameter design. The numbers of experiments are greatly reduced compared with those necessary in grid search design experiments. The number of experiments and consumption of time and reagents for the grid search design increase with the number of parameters. Moreover, most of these experiments offer little information about the optimum conditions. It is impractical to perform so many experiments for optimization even if they are done automatically with a robot. The superiority of the simplex method is obvious, especially in the case of optimization of many parameters.

Since the simplex method is an iterative algorithm, the experimental conditions vary in an unpredictable way each time. This experimental design is suitable for an automated system, because it can be easily designed to be regulated based on the result of the preceding experiment, that is, feedback regulation. Additionally, the robotic system is able to perform the experiments accurately even with irregularly varying conditions. Therefore, the simplex optimization is more effective when it is adopted in an automated system.

In addition to the superior efficiency, the optimum conditions selected by the simplex method are less dependent on the initial conditions. Let us assume that one attempts to optimize this reaction with the generally used method, where only one variable is varied while the others are fixed: If the optimization is started from an inappropriate initial condition, the optimum condition may be missed, because two parameters, the concentrations of the two reagents, are interacting as shown in Fig. 1.

Although they give the same absorbance, the conditions obtained from different initial simplices are rather different from each other (see Table I). This is attributed to the fact that the optimum region of this reaction system is quite large, as shown in Results, and the variation of the response in this region is very gentle. This defect may be improved by setting a stricter criterion of conversion, (set at 1% in this experiment). If it is set at 0.5%, for instance, the selected optimal conditions would move nearer to the true optimum. This criterion must be chosen taking account of the precision of the experiments. Since the RSD of the absorbance is *ca.* 0.7% (see Results), too small a value for the criterion of the conversion would result in numerous experiments owing mainly to the experimental error.

It is difficult to select the optimum in a response surface that varies gently, and knowledge of the variation of the response around the optimum is indispensible. The simplex algorithm lacks a global view over the variable space, because it discards the information of less-optimum points, and a more sophisticated algorithm such as curve fitting to the polynominals should be adapted to the robotic system to optimize the reaction conditions.

Conclusion

The feasibility of the described optimization system was confirmed. This system gives the optimum combination of three or four parameters which interact with each other, with a fairly small number of experiments. Additionally, the performance of the optimization process is completely unattended. This system will be of great help in many analytical laboratories.

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