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Optimization of glass microelectrode properties by response surface methodology

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Glass microelectrodes filled with electrolyte solutions are standard tools for electrophysiological studies. However, for any given application, there are limitations to the properties of the microelectrode, such as impedance and shank length, that can yield satisfactory results. The trial and error approach in pulling electrodes with the desired properties can be time consuming. The use of a response surface procedure which allows the experimenter to change more than one factor at a time and therefore determine the desired puller condition more efficiently is demonstrated. Also, design improvements for the World Precision Instrument, Model PUL-1, Microelectrode puller, used in this study are suggested.

Introduction

Glass microelectrodes filled with electrolyte solutions are now standard tools for electrophysiological studies of neurons smaller than 20 μm (Brown and Flaming, 1977; Burke, 1979). However, in preliminary electrophysiological studies of SK-N-DZ (human neuroblastoma) and N-18 (C1300 mouse neuroblastoma clone), difficulties were experienced by our research group in obtaining stable impalements with microelectrodes registering impedances below 50 MΩ. Similar observations have been reported by Miyake (1978). Failure to obtain stable impalements both in our study and in that of Miyake (1978) may be attributed to soft cellular membranes and/or poor microelectrode preparations. To solve this problem, one can improve the cellular membrane strength by modifying the tissue culture media and one can establish microelectrode puller conditions for producing very fine tips. The latter will be the focus of this paper. The terms tip and shank are used in this study to refer to different parts of the microelectrode, as defined by Frank and Becker (1964).

The process of establishing puller conditions that yield a desired microelectrode impedance or for that matter any other microelectrode property such as shank length can be time consuming. Recently, a theory of microelectrode tip formation that relates tip size and glass capillary wall thickness has been presented (Brown and Flaming, 1986). Although this theory may be useful in minimizing the required time to produce a desired microelectrode impedance, a trial and error

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approach is still necessary for a given puller. The purpose of this paper is to demonstrate the use of response surface methodology as a tool in establishing overall puller conditions for the production of desired microelectrode properties and also to study the effects of varying operating parameter on puller performance.

Experimental design

Response surface optimization experiments are a special class of statistically designed experiments that provide the means of reaching optimum operating conditions by allowing the experimenter to change more than one factor at a time. The importance of statistical experimental design lies in the fact that multifactor experiments can be used to find the optimum with smaller numbers of experimental runs than would be required by the unifactor approach. Also, information can be obtained about the effects of factor interactions on the response that is not available with the one-factor-at-a-time approach. Chapters on experimental design can be found in most standard texts on statistics. Examples of good references are those by Guttman et al. (1982) and Bayne and Rubin (1986).

Generally an unknown response function:

$$Y = f(X_1, X_2, \ldots X_K) \quad (1)$$

exists that relates Y to K factors. The true function may be complicated and most of the time is unknown. However, a low-order polynomial can be used to approximate the function in the region of interest. The first-order model can be written as:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_K X_K = \beta_0 + \sum_i \beta_i X_i \quad (2)$$

and the second-order model can be written as:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j \quad (3)$$

where β_0 is the y-axis intercept while β_is are the K first-order coefficients and β_{ii}s are the $K(K-1)/2$ cross-product or interaction coefficients for the models written in terms of x_i. Two possible matrices for $K = 2$ or 3 factors (or variables) are the hexagonal and cuboctahedronal designs

TABLE I

DESIGN MATRIX FOR BOROSILICATE GLASS CAPILLARY NO. 6040

Time delay of 2 s ($K = 2$)

Tension PUL-1 scale (N)	Heating current PUL-1 scale (A)	X_1	X_2
8.0 (9.18)	6.0 (21.1)	1.00	0
3.5 (3.45)	8.0 (22.9)	−0.50	0.866
3.5 (3.45)	4.0 (19.6)	−0.50	−0.866
5.0 (5.00)	6.0 (21.1)	0	0
5.0 (5.00)	6.0 (21.1)	0	0
2.0 (2.22)	6.0 (21.1)	−1.00	0
6.5 (7.18)	8.0 (22.9)	0.50	0.866
6.5 (7.18)	4.0 (19.6)	0.50	−0.866
5.0 (5.00)	6.0 (21.1)	0	0
5.0 (5.00)	6.0 (21.1)	0	0

TABLE II

DESIGN MATRIX FOR BOROSILICATE GLASS CAPILLARY NO. 6040

(K = 3)

Tension PUL-1 scale (N)	Heating current PUL-1 scale (A)	Delay time (s)	X_1	X_2	X_3
8.0 (7.91)	8.0 (22.1)	1.0	−1	−1	0
10.0 (10.44)	8.0 (22.1)	1.0	1	−1	0
8.0 (7.91)	10.0 (23.6)	1.0	−1	1	0
10.0 (10.44)	10.0 (23.6)	1.0	1	1	0
8.0 (7.91)	9.0 (22.9)	0.5	−1	0	−1
10.0 (10.44)	9.0 (22.9)	0.5	1	0	−1
8.0 (7.91)	9.0 (22.9)	2.0	−1	0	1
10.0 (10.44)	9.0 (22.9)	2.0	1	0	1
9.0 (9.18)	8.0 (22.1)	0.5	0	−1	−1
9.0 (9.18)	10.0 (23.6)	0.5	0	1	−1
9.0 (9.18)	8.0 (22.1)	2.0	0	−1	1
9.0 (9.18)	10.0 (23.6)	2.0	0	1	1
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0
9.0 (9.18)	9.0 (22.9)	0.5	0	0	0

(Guttman et al., 1982). The hexagonal and cuboctahedronal designs require that minimums of 10 and 17 runs/experiment, respectively, be performed to evaluate the second-order coefficients of Eqn. (3). The relative location of experimental points are shown in Tables I and II. The zero level in each design space is the average of the low (−1) and high (+1) levels. The zero level is usually chosen at standard operating conditions or at conditions considered to be optimum. X_1, X_2 and X_3 are the scaled design variables (factors). The step size in X should be large enough to allow effects of factors to show up but small enough so that these effects are approximately linear over each step.

Experimental procedure

Microelectrode puller

All experiments were performed on a horizontal microelectrode puller, Model PUL-1 (World Precision Instruments, Inc., New Haven, CT). This puller is a modified form of the classical version, first reported by Livingston and Dugger (1934). It can accommodate 3 standard U-shaped nickel/chromium heaters (1.5, 3.0 and 6.0 mm wide) between 2 terminals.

Glass capillary tubing

Three capillary glass tubes were used. They were all obtained from A-M Systems Inc., Everett, WA, and had equal lengths of 101.6 mm. The first was a borosilicate type (A-M System catalog no. 6040) and had outer and inner diameter of 2.0 and 1.16 mm, respectively. The last 2 were borosilicate (A-M System catalog no. 6170) and aluminosilicate (A-M System catalog no. 5830) types. They both had the same outer and inner diameters of 1.5 of 1.12 mm, respectively.

Experimental factors

A previous study by Frank and Becker (1964) identified 3 major factors that affect the shape of microelectrodes as follows: (1) the temperature, shape and size of the heater element; (2) the strength and variation of pull with displacement of the movable glass tubing clamp; and (3) kind, diameter and wall thickness of glass tubing used. It was also pointed out that the time course of the drawing process and the velocity at separation are of paramount importance. However, with contemporary pullers, such as PUL-1, these factors cannot be independently adjusted.

In preliminary pull experiments, it was found that the 1.5- and 3.0-mm heater elements could not provide enough heat to soften any of the 3 glass capillaries. Hence, only temperature effects of the 6.0-mm heater element were considered (first factor in this study). Since PUL-1 can only accomodate U-shaped heaters, it was not possible to include heater shape as a factor. PUL-1 is designed such that the initial tension in the pull can be varied, independent of all other machine variables. Therefore, strength of pull (second factor in this study) was studied by varying the initial tension. However, it was not possible to monitor the variation of pull tension with displacement. Also PUL-1 can be operated manually or automatically. In the manual mode, a push button operates the heater for as long as the button is depressed. In the automatic mode when the button is depressed momentarily, heating is initiated and the heater will remain on until the glass reaches its softening temperature. At this point a motion sensor detects the yield of the glass and triggers the heater off via a time delay circuit that has 4 selectable delay times (D) of 0.25, 0.5, 1.0 and 2.0 s. This feature was perhaps incorporated in PUL-1 to impart some flexibility in velocity of separation. In this study, the last 3 delay times (third factor in this study) were considered as the first was found to be too short for breakage of the 3 glass capillary types.

Although 3 differing glass capillaries were used, no effort was made to test 'kind, diameter and wall thickness of glass tubing' (factor 3 in the Frank and Becker (1964) study) for purposes of limiting the number of experiments. Also, further preliminary experiments suggested that it was impossible to get satisfactory pulls with the 3 delay times over a wide range of heater temperature and initial tension. Therefore, it was decided to conduct experiments, with 2 factors (initial tension and heater temperature, $K = 2$) at a fixed delay time for each glass capillary and to run an

additional experiment in a narrower region of the puller conditions with 3 factors (initial tension, heater temperature and delay time, $K = 3$).

The heater temperature was characterized by the heating current (C: in ampere, A) at 3 or 5 levels. The strength of pull was characterized by initial tension (T: newtons, N) at 3 levels. The velocity of separation was characterized by delay time (D: in seconds) at 3 levels. To convert from machine setting to A and N, calibration curves were generated. The current was established before and after each experiment due to drifts caused by the oxidation of the heater element after going through many heating cycles. Typical design matrices are shown in Tables I and II. The actual values of the independent variables and the corresponding scaled variables are shown in each case. Scaling of each independent variable was done such that the magnitude of the minimum value and maximum value correspond to scaled values of -1 and 1, respectively, or -0.866 and 0.866. Scaling is important to eliminate the influence the independent variables might exert on the statistics for the significancy of the variable. However, for unbalanced designs, there are statistical packages such as the one used in this study that are immune to the effects of lack of balance in experimental design. It was not possible to achieve balance for D due to limitations imposed by the PUL-1 design.

Dependent variables

The dependent variables were microelectrode impedance (R: MΩ) and shank length (L: mm). Shank length is important in electrophysiological work where recording is done on cells that are deep in tissue. For each trial, in each design, 4 microelectrodes were generated and their dependent properties were averaged.

Impedance measurement

Standard electrophysiological recording equipment was used. Each microelectrode was filled with 3M KCl just before being used. This was necessary to minimize variation in impedance measurements due to KCl attack. The well known bridge null technique was used to measure microelectrode impedances. The bridge on a high input impedance Degan 8700 preamplifier (Degan Corporation, Minneapolis, MN), was used with current set at 1 nA. A 10-mV stimulus pulse from the Degan S-900 stimulus wave form generator (Degan Corporation, Minneapolis, MN), lasting for 100 ms/s was used to drive the bridge.

Data analysis

The general linear model (GLM) procedure available in the SAS statistical software package was used in estimating the intercept and coefficients in Eq. (3). GLM uses the method of least squares to fit general linear models and can handle unbalanced designs. An IBM P/S 2, model 55SX personal computer was used. The statistical theory behind GLM will not be repeated here. Interested readers are referred to the SAS/STAT User Guide for Personal Computers (SAS Institute Inc., Cary, NC).

Results and discussion

In general, for a parameter to be considered important, the significance probability (P) must be ≤ 0.05. The first trial involved fitting the model with all the parameters and the second trial was conducted with only those parameters where $P \leq 0.05$. The results are summarized in Table III. Generally high R^2 values show that the model is a good fit to the experimental data and that scatter within the data is not unreasonable. The F test on the complete model is a joint test of whether any parameters, with the exception of the intercept, are non-zero. Large values suggest that some of the parameters are non-zero. Thus in this study, models were considered reliable if $P \leq 0.05$, $R^2 \geq 0.7$ and the F value was high.

The fact that for all models where $K = 2$, significant parameters were not found to be the same may be attributed to differences in glass type. For the purpose of demonstrating how to obtain puller conditions for a desired microelectrode property, this minor discrepancy is not of great concern. What is important is to get a model that accurately fits the data as indicted by the values of R^2, F and P.

Fig. 1 shows a typical comparison of actual impedances versus values predicted by a model. The 95% confidence limits on the means are also included. Results for Models 1, 3 and 5 (Table III) are presented in Figs. 2A, B and C, respectively. It is evident that with this approach the limits of the puller can be established with very few experiments. At a fixed C, for borosilicate glass and a D of 2 s, R increases linearly with increasing T. The difference between the aluminosilicate glass (Fig. 2C) response, when compared to that for borosilicate (Figs. 2A and B) may be attributed to the higher melting temperature nature of aluminosilicates. Therefore, higher C is needed to obtain successful pulls. It should

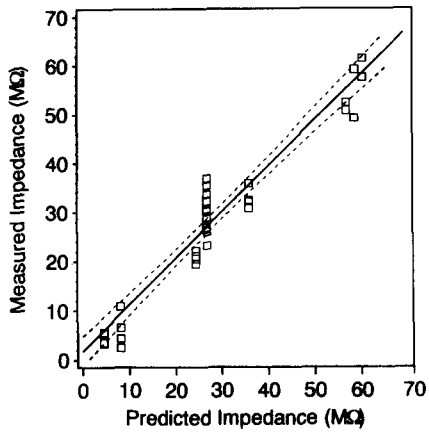

Fig. 1. Actual versus predicted impedances (Model no. 1 from Table III).

TABLE III

SUMMARY OF RESULTS

Glass capillary type	Model no.	Variable		P	F Value	R^2	Model reliability [a]	Significant parameters and corresponding coefficient estimates
		Independent	Dependent					
6040	1	C and T ($K = 2$)	R	0.0001	98.88	0.8918	+	Intercept (98.3690) C (−14.0503) C^2 (0.4155)
6170	2	C and T ($K = 2$)	L [b]	–	–	–	–	CT (0.3788) –
	3	C and T ($K = 2$)	R	0.0001	156.21	0.9287	+	Interecept (−154.8473) C (14.6008) T (2.6883) C^2 (−0.3431)
	4	C and T ($K = 2$)	L	0.0001	57.45	0.8272	+	Intercept (−0.4559) C_2 (0.7070) T^2 (−0.2524) CT (0.1623)
5830	5	C and T ($K = 2$)	R	0.0001	50.68	0.7326	+	Intercept (−21.3382) T^2 (−0.7159) CT (0.5004)
	6	C and T ($K = 2$)	L	0.9243	0.01	0.0003	–	–
6040	7	C, T, and D ($K = 3$)	R	0.0001	33.29	0.8234	+	Intercept (10055.4) C (−909.9240) T (−6.7287) D (318.507) C^2 (20.5817) D^2 (−52.49) TD (16.0261) CD (−13.4241)
	8	C, T, and D ($K = 3$)	L	0.0028	31.79	0.3083	–	–

[a] + = reliable, − = unreliable.

[b] L not measured.

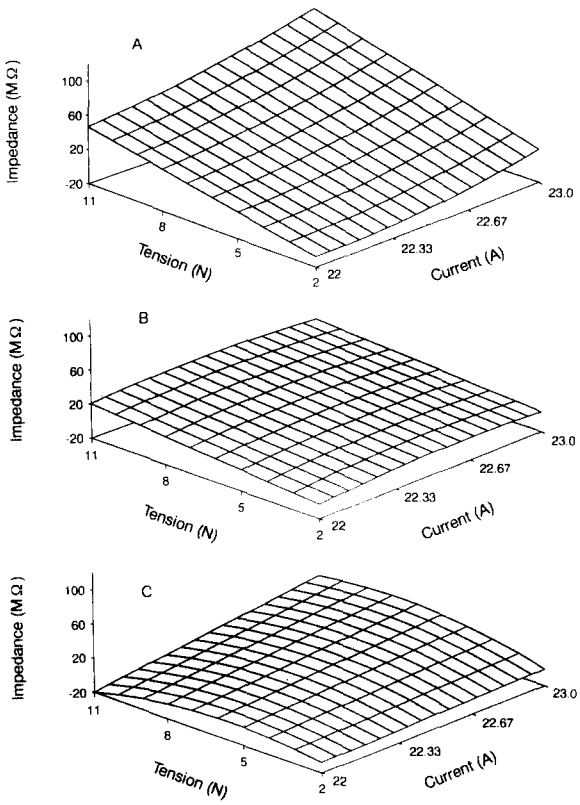

Fig. 2. Impedance response surface. A: capillary glass no. 6040 – Model 1 from Table III. B: capillary glass no. 6170 – Model 3 from Table III. C: capillary glass no 5830 – Model 5 from Table III. For borosilicate glass capillary (A and B), maximum impedance was obtained at the current and tension limits of puller. Differences in behavior between borosilicate and aluminosilicate (C) glass capillaries were attributed to the higher energy requirement of aluminosilicates.

be pointed out that the aluminosilicate model predictions of negative Rs at low Cs are practically meaningless since in this puller region, no successful pulls will be obtained, however the variation of R with respect to T in this region serves to confirm the speculated high temperature behavior of aluminosilicates. It is expected that if the range of C were to be extended beyond the PUL-1 limit of 23 A, a response surface similar to those shown in Figs. 2A and B would be obtained.

Availability of the aforementioned impedance response surfaces provides insights for reliably producing desired pulls. As an example, suppose one wanted to generate a microelectrode using glass no. 6040, with $R \geq 65$ MΩ. By inspection of Fig. 2A it is evident that one has to operate in a very narrow region. This region can easily be established using model 1 as follows:

$$R \leq 98.3690 - 14.0503\ C + 0.4155\ C^2 + 0.378\ CT$$

$$65 \leq 98.3690 - 14.0503\ C + 0.4155\ C^2 + 0.3788\ CT$$

$$-33.369 \leq -14.0503\ C + 0.4155\ C^2 + 0.3788\ CT \quad (4)$$

The above inequality can be solved for given values of T to yield the results presented in Table IV. To verify these results, several capillary glasses were pulled at a T setting of 8 (9.18 N) and a C setting of 7 (22.0 A), yielding an average valve of 67.1 MΩ with a 95% confidence range of ± 4.7 MΩ, thus confirming the utility of this procedure. For beveling purposes where very high impedances are needed, approximate puller conditions can be determined by inspecting the response surface. If a maximum exists, the model can be differentiated with respect to the appropriate variable, equated to zero and a solution obtained.

Fig. 3A, B and C present the effects of D on the shape of R response surfaces. At low D values, maximum Rs were observed at the PUL-1 limiting C. Obviously, the lower D setting does not provide heat long enough after yielding. Therefore the microelectrode tips harden and break before the glass capillary tube diameter is significantly narrowed. High C, however, compensates for this effect to a degree. As D increases, there is a transition from high to low C, at which maximum R values are obtainable. However on average the higher the D the higher the R values. Clearly, the greater R values result

TABLE IV

PULLER CONDITIONS FOR PRODUCING $R \geq 65$ MΩ WITH CAPILLARY GLASS NO. 6040

Tension (N)	Current (A) ≥
7	24.1
8	23.0
9	22.0
10	20.8
11	19.7

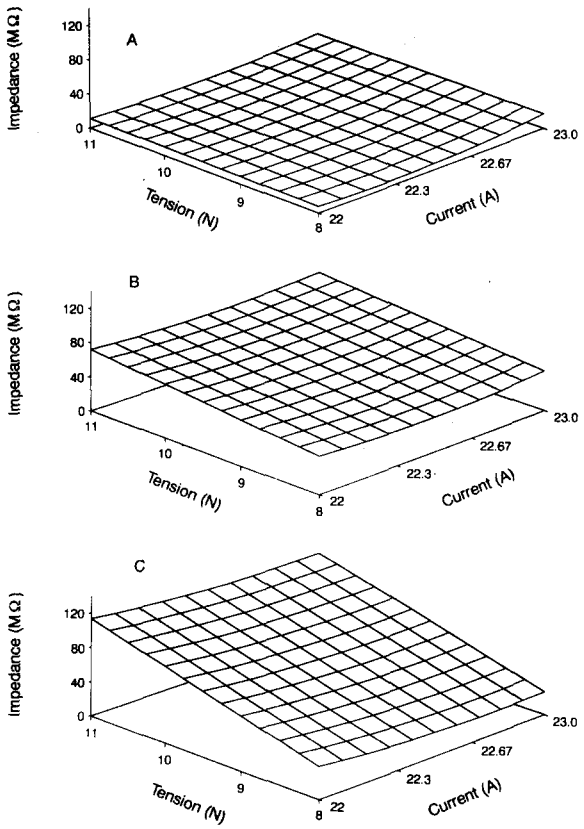

Fig. 3. Impedance response surface for capillary glass no. 6040 – Model 7 from Table III. A: D = 0.5 s; B: D = 1.0 s; C: D = 2.0 s. Higher D values resulted in higher impedances. This was attributed to prolonged heat transfer through the initial phases of the capillary glass lengthening.

because of prolonged heat transfer through the initial phases of capillary lengthening. This provides longer pulls before breakage as well as higher velocities of separation. Therefore the capabilities of PUL-1 can be extended by equipping it with longer D values beyond the current limit of 2 s. At high D values, R becomes less dependent on C, as shown in Fig. 3C. Unlike the R response surface, a maximum shank length (L) was found at intermediate T values as shown in Fig. 4.

The response surface methodology has been successfully used in establishing overall puller conditions for producing desired microelectrode properties. This methodology is also expected to prove useful for investigating the behavior of glass pipette pullers in general. Based on the experience gained with PUL-1 and the results obtained in the study, it is apparent that the range of impedances obtainable can be increased by equipping PUL-1 with longer delay times beyond the 2-s period now available. In operating PUL-1, it was also found that accurate T settings were difficult to make. Therefore, equipping the puller with a tensiometer as well as fine adjustment for this parameter is expected to improve the machine utility. It should be pointed out that more expensive modern pullers have more features in comparison to PUL-1. However, with more features the trial and error approach becomes even more time consuming.

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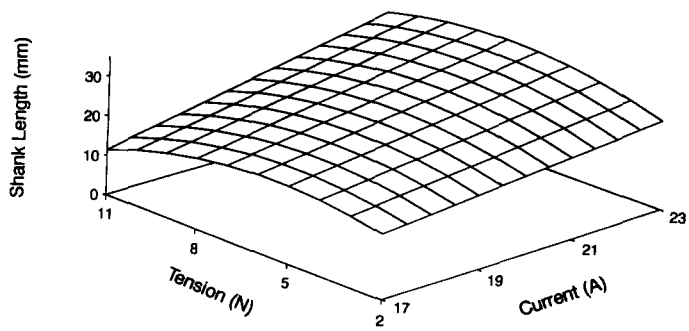

Fig. 4. Shank length response surface for capillary glass no. 6040 (Model 4 from Table III) at $D = 2$ s. At a given current setting, maximum shank length was obtained at intermediate tension and increased with increasing current.

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