control provide the digitally derived persistence necessary for optimal viewing of single-channel currents.

Summary

It should be obvious that there are many ways to construct clamp setups that are either equivalent or sufficient for the experiments planned. The hardware and electronics can be obtained from several manufacturers, as can analysis software. What we have presented here are guidelines primarily meant to point a new experimenter in the right direction and, we hope, to guide more experienced investigators toward techniques that can improve the resolution of their measurements.

[3] Glass Technology for Patch Clamp Electrodes

By JAMES L. RAE and RICHARD A. LEVIS

Introduction

In the simplest sense, a patch clamp electrode is just a fluid bridge of proper geometry to connect a reference electrode to the surface or interior of a cell. The glass envelope which accomplishes this is a passive component of the overall circuit which records currents and applies voltages, yet the properties of the glass electrode can be an important determinant of the quality of the recordings.

Several properties of glasses are important when trying to construct effective electrodes for patch clamping. Thermal properties dictate how easily desired tip shapes can be produced and determine the extent to which the tips can be heat polished. Optical properties determine if the tip can be heat polished to a visually distinct end point. Electrical properties determine the noise the glass produces in a recording situation and determine the size and number of components in the capacity transient following a change of potential across the pipette wall. Noise and capacitance properties are correlated. Good electrical glasses minimize both. Finally, glasses are complex substances composed of many compounds (see Table II). Glass composition may influence how easily a glass seals to membranes but may also yield compounds that can leach into the pipette filling solution to inhibit, activate, or block channel currents.

In this chapter, we expand the present literature concerning patch

clamp electrode technology[1-6] by discussing practical issues about glasses that affect the quality of patch clamp recordings. Those glass properties that optimize single-channel recordings may not optimize whole-cell recordings or may be irrelevant to them and vice versa. It is a reasonable assertion that regardless of the type of patch clamping being done, the use of glasses with good electrical properties is desirable.

Overview of Patch Electrode Fabrication

There are a wide variety of glasses available for patch clamping. Garner Glass (Claremont, CA) has been particularly instrumental in supplying specialty glasses for this purpose. Glass tubing of whatever variety selected for the fabrication of patch electrodes should have walls of substantial thickness (0.2 to 0.3 mm). Thick walls result in decreased electrical noise and increased bluntness at the tip, which prevents penetration of the cell during seal formation. Most investigators use glass tubing with a 1.5 to 2.0 mm outside diameter and a 1.15 to 1.2 mm inside diameter. With an inside diameter this large, it is possible to utilize commercially available 1 mm Ag/AgCl pellets (In-Vivo Metrics, Healdsburg, CA; E. W. Wright, New Haven, CT) which will easily fit into the back of the electrode. For smaller inside diameters, one is constrained to use smaller chlorided wire as the internal reference electrode. These electrodes can be constructed easily from small diameter silver wire which has been rendered free of oxide on its surface by use of fine sandpaper. By immersing this cleaned tip into Clorox bleach for about 20 min, a substantial coating of AgCl can be formed to produce a very good Ag/AgCl internal reference electrode. In either case, any nonchlorided silver wire associated with the electrode should be isolated from the filling solution. This is usually done by surrounding it with a small Teflon tube filled with either Sylgard or epoxy which is subsequently cured to encapsulate the wire.

It is probably a good general idea to clean the glass tubing before using

[1] O. P. Hammill, A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth, *Pflugers Arch.* **391,** 85 (1981).

[2] B. Sakmann and E. Neher, *in* "Single-Channel Recording" (B. Sakmann and E. Neher, eds.), p. 37. Plenum, New York and London, 1983.

[3] D. P. Corey and C. F. Stevens, *in* "Single-Channel Recording" (B. Sakmann and E. Neher, eds.), p. 53. Plenum, New York and London, 1983.

[4] J. L. Rae and R. A. Levis, *Biophys. J.* **45,** 144 (1984).

[5] J. L. Rae and R. A. Levis, *Mol. Physiol.* **6,** 115 (1984).

[6] J. L. Rae, R. A. Levis, and R. S. Eisenberg, *in* "Ion Channels" (T. Narahashi, ed.), p. 283. Plenum, New York and London, 1988.

it to make patch electrodes. In our experience, this is often unnecessary, but at other times we have found it imperative to clean the glass for the best noise performance. Sonicating the glass in 100% ethanol in an ultrasonic cleaner is often effective for this purpose. Another approach suggested in the glass literature[7] is to etch the glass for 10 min with a 1% sodium hydroxide solution at 95°. This is followed by a 2-min cleaning with 5% hydrochloric acid at 50° and then meticulous washing with distilled water. We recommend that all of this be done in an ultrasonic cleaner to assure agitation and movement of the solution inside the glass tubing. Following any cleaning procedure, place the glass in an oven at around 200° for 10 to 30 min to achieve complete drying. Heat treatment of this sort has also proved necessary if low noise recordings are to occur in environments where the humidity is exceptionally high. This is a good idea in high humidity even for glass tubing that either has not been previously cleaned or has been meticulously cleaned at some time in the past.

Patch electrodes require much blunter tips than standard intracellular microelectrodes, and it is usually not possible to pull them adequately on single-stage electrode pullers. Many laboratories have modified standard vertical electrode pullers so that they pull in multiple stages. This modification involves placing stops in the pulling apparatus. These stops can be simple metal or wooden blocks which stop the puller movement after it has experienced a displacement of a few millimeters. One of the puller clamps is then loosened and the glass is moved so that the now hourglasslike tapered region is repositioned near the filament. The glass is then repulled. This stopping and repositioning can occur several times before the pull is allowed to separate the two pieces of electrode glass. With this approach, it is possible to produce quite different tip tapers. Pulling electrodes, however, has become very much easier with the advent of microprocessor-driven microelectrode pullers like the model P-87/PC from Sutter Instruments (Novato, CA). Similar pullers are also made by a number of other manufacturers. With these pullers, it is possible to implement very complicated multistage pulls of electrode glass and to store all of the parameters required in memory. These pullers allow multiple programs to be stored, and consequently it is possible with the push of a button or two to set up the puller to pull optimally almost any kind of glass. The Sutter puller is particularly versatile because it contains a solenoid valve that allows gating of a burst of gas to cool the filament rapidly. This feature gives one a great

[7] L. D. Pye, H. J. Stevens, and W. C. LaCourse, "Introduction to Glass Science," p. 513. Plenum, New York and London, 1972.

deal of control over the final taper of the tips, which is very important for patch clamp recordings.

For the lowest noise recordings, electrodes must be coated with a hydrophobic material to within 100 μm or less of their tip. This prevents bathing solution from creeping up the wall of the electrode and thus limits what would be substantial noise source. A commonly used compound is Sylgard 184 from Dow Corning (Midland, MI). This compound also has exceptional electrical properties (see Table I) and so improves the electrical properties of the glass when it is painted on the glass surface. Sylgard, meticulously mixed until it is frothy with bubbles, can be stored at simple freezer temperatures in small capped centrifuge tubes. The thorough mixing is very important because pockets of the compound not adequately exposed to polymerizer can flow to the electrode tip (even against gravity) and render the tips difficult to seal to cells. When handled in this way, the Sylgard can be stored for several weeks. A tube of this freezer-stored Sylgard, when brought to room temperature for use in painting electrodes, will last for several hours before it begins to polymerize.

The Sylgard is painted on the electrode tip using a small utensil like a piece of capillary tubing pulled to a reasonably fine tip in a flame. This painting can be done using magnifications available with standard dissecting microscopes. It is useful, but not required, to modify the dissecting microscope to work in dark field. This can be done fairly inexpensively by purchasing a fiber optic ring illuminator that can be connected to a standard fiber optic light source. At a location of 3 to 4 inches above the ring illuminator placed on the stage of the microscope, dark-field illumination is achieved, and the walls of the electrode glass show up as bright lines of light. The location of the Sylgard being painted and the tip of the electrode can be very easily discerned with this dark-field illumination. It is important that the Sylgard be directed away from the tip by gravity at all times during the painting procedure. Otherwise, the Sylgard will flow into the tip and make fire polishing and/or sealing impossible. The Sylgard can be cured by holding the tip for 5 to 10 sec in the hot air stream coming from a standard heat gun of the variety used in electronics. Again, the Sylgard must be gravitationally directed away from the tip during the curing process.

Finally, to promote gigohm seals and to reduce the possibility of tip penetration into the cell during seal formation, electrode tips should be fire polished. In some cells, fire polishing has proved unnecessary, but we have found, as a general rule, that sealing of difficult to seal cells is promoted by fire polishing the electrode tip. Fire polishing can be done either using an upright or an inverted microscope. In fact, many investigators have chosen

to Sylgard coat their pipettes and fire polish them using an inverted microscope with a 40× or so long working distance objective. Another very useful approach is to utilize a standard upright microscope converted to the 210 mm tube length that is standard for metallurgical microscopes and objectives. Several microscope companies, but particularly Nikon (Garden City, NY), make extralong working distance and superlong working distance high-magnification metallurgical objectives. Most noteworthy is the 100× ELWD or 100× SLWD objectives that have 1 and 2 mm working distances, respectively. With these objectives and 15× eyepieces and with the electrode mounted on a slide held in the mechanical stage of the microscope, it is possible to move the electrode tip into the optical field and visualize directly the electrode tip at 1500× magnification (Fig. 1).

At such high magnifications, it is possible to fire polish the tip to a very distinct optical end point under direct visualization. This approach ensures very repeatable electrodes from one electrode to the next. The fire polishing itself is accomplished by connecting to a micromanipulator a rod of inert material to which has been fastened a short loop of platinum iridium wire. The ends of this wire must be soldered to two other pieces of wire that can be connected to a voltage or current source to allow current to be passed through the platinum wire. The platinum loop is generally bent into a very fine hairpin so that it can be brought to within a few microns of the electrode tip under direct observation. Because of early reports that platinum can be sputtered from the wire onto the electrode tip and prevent sealing, the platinum wire is generally coated with a glass like Pyrex (Corning 7740) or Corning 7052 to prevent such sputtering. This is done by overheating the platinum wire and pushing against it a piece of electrode glass that has been pulled into an electrode tip. At high temperatures, the glass melts and flows over the platinum wire and ends up thoroughly coating it and forming a distinct bead of glass. With an arrangement like this, it is possible to fire polish electrode tips very precisely (see Fig. 2).

If the Sylgard has been coated too near the tip, fire polishing causes the tip to droop downward at the juncture where the Sylgard coating ends. If one desires to paint Sylgard extremely close to the tip, it may be necessary to do most of the fire polishing before Sylgard coating and then to fire polish lightly again after Sylgard coating.

Electrode Properties for Single-Channel versus Whole-Cell Recording

Electrodes for patch and whole-cell recordings have some properties in common but other properties which can be very different. First, the noise of the electrode is very much more important in single-channel recordings than in whole-cell recordings. In whole-cell recordings, the dominant noise

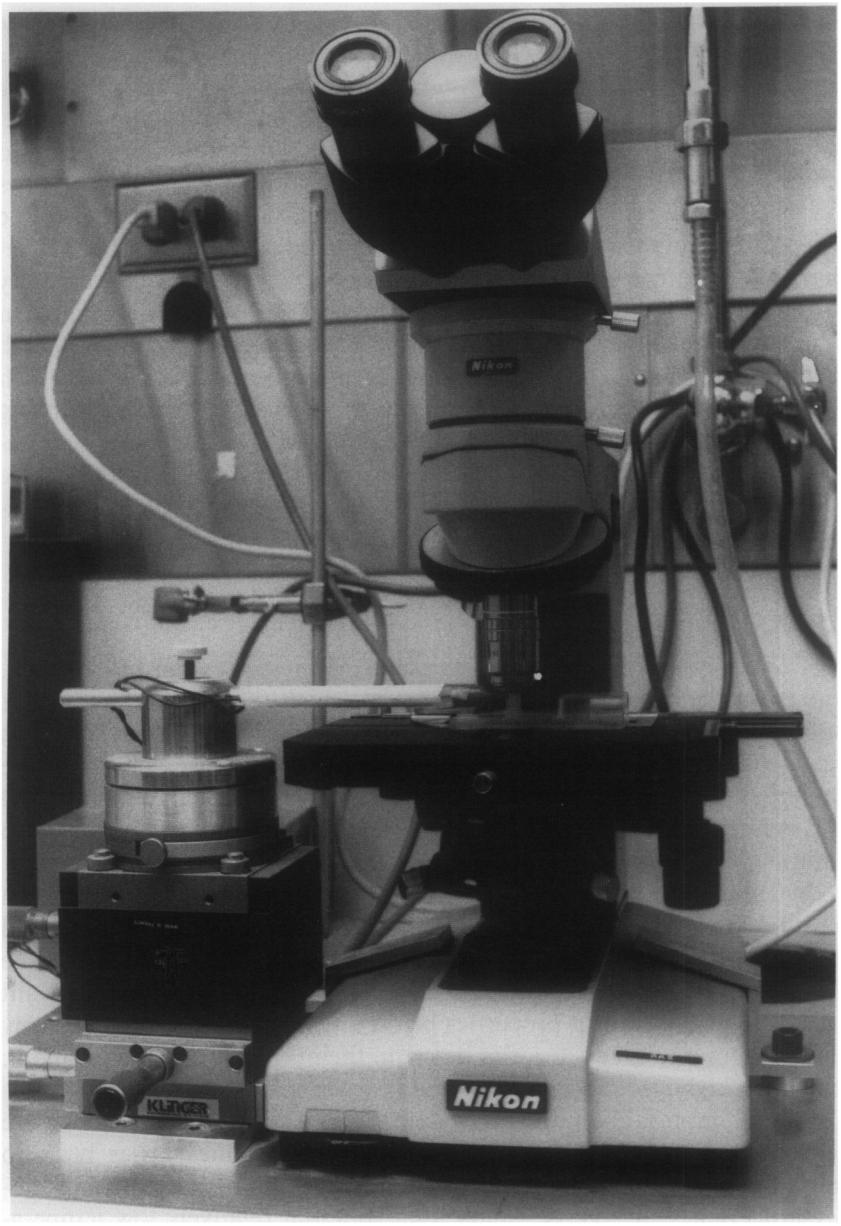


FIG. 1. One possible implementation of a fire polishing setup. The heating filament is attached to the micromanipulator. The electrode rests in a groove cut in a thick acrylic plastic microscope slide and is moved by the mechanical stage of the microscope. The objective is 100× metallurgical and the eyepieces 15×.

source at moderate to high frequencies comes from the resistance of the electrode in series with the capacitance of the entire cell, and so electrode noise is relatively less important. On the other hand, the resistance of a whole-cell electrode should be as low as possible (a few megohms at most) to minimize both dynamic errors associated with series resistance and noise. This is not a requirement for single-channel recording, nor does higher electrode resistance result in much additional noise there until the electrodes become some tens of megohms in resistance. In either single-channel recordings or whole-cell recordings, it is necessary that capacity currents which flow during voltage steps be sufficiently small and simple in time course that they can be corrected by simple circuitry in the patch clamp. In addition, both kinds of electrodes must be made of glasses which do not leach compounds from their walls that can alter the currents being measured from the particular channels of interest. The particular requirements for these two kinds of recording require also that the electrodes be pulled somewhat differently to optimize their use in the particular recording configuration being used.

Types of Glasses and Their Properties

There are several ways in which patch clamp glasses can be classified. One is on the basis of the temperature at which they soften. Another is based on their electrical properties. A third and perhaps more common way is on the basis of their major chemical constituents. Many of these properties are itemized in specification sheets from the manufacturer, and so it is often possible to choose glasses which should be effective for patch clamping just from examining their specifications.

In Table I, we list the properties of a number of glasses that have been used for patch clamping. We also list the properties of quartz and Sylgard because of their relevance to the issues discussed in this chapter. The glasses are listed in increasing order of loss factor times dielectric constant (see section on electrical properties below). We also somewhat arbitrarily classify them into four categories based on their primary chemical content: soda lime, high lead, borosilicate, or aluminosilicate.

Several important points can be noted from Table I. The first is that the electrical properties and the thermal properties of the glasses bear no obligatory relationship. It has been a misconception among some biophysicists that "soft" glasses, those that soften at relatively low temperatures, are poor glasses electrically, whereas "hard" glasses, those that soften at relatively high temperatures, are good glasses electrically. A comparison of 8161, a very soft glass, and 7760, a medium hard glass, quickly dispels that notion. Both glasses have very low loss factors yet soften at substan-

TABLE I
ELECTRICAL AND THERMAL PROPERTIES OF GLASSES

Glass	Loss factor (LF)	Log vol. r.	Dielectric constant (DC)	LF × DC	Softening temperature (°C)	Description
7940	0.0038	11.8	3.8	0.01444	1580	Quartz (fused silica)
1724	0.0066[a]	13.8	6.6	0.04365	926	Aluminosilicate
7070	0.25	11.2	4.1	1.025	—	Low loss borosilicate
Sylgard	0.58	13.0	2.9	1.682	—	#184 Coating compound
7059	0.584	13.1	5.8	3.387	844	Barium borosilicate
7760	0.79	9.4	4.5	3.555	780	Borosilicate
8161	0.50	12.0	8.3	4.15	604	High lead
7040	1.00	9.6	4.8	4.8	700	Kovar seal borosilicate
0120	0.80	10.1	6.7	5.36	630	High lead
EG-6	0.80	9.6	7.0	5.6	625	High lead
7720	1.30	8.8	4.7	6.11	755	Tungsten seal borosilicate
1723	1.00	13.5	6.3	6.3	910	Aluminosilicate
7052	1.30	9.2	4.9	6.37	710	Kovar seal borosilicate
EN-1	1.30	9.0	5.1	6.63	716	Kovar seal borosilicate
KG-12	1.00	9.9	6.7	6.7	632	High lead
0010	1.07	8.9	6.7	7.169	625	High lead
3320	1.50	8.6	4.9	7.35	780	Tungsten seal borosilicate
7050	1.60	8.8	4.9	7.84	705	Series seal borosilicate
7056	1.50	10.2	5.7	8.55	720	Kovar seal borosilicate
EG-16	0.90	11.3	9.6	8.64	580	High lead
KG-33	2.20	7.9	4.6	10.12	827	Kimax borosilicate
7740	2.60	8.1	5.1	13.26	820	Pyrex borosilicate
1720	2.70	11.4	7.2	19.44	915	Aluminosilicate
N-51A	3.70	7.2	5.9	21.83	785	Borosilicate
R-6	5.10	6.6	7.3	37.23	700	Soda lime
0080	6.50	6.4	7.2	46.8	695	Soda lime

[a] We question the loss factor given for 1724. It seems to be too low.

tially different temperatures. An even more dramatic comparison is that of KG-12, a high lead glass, with 1723, an aluminosilicate glass. They have the same low loss factor and yet soften at temperatures which differ by almost 300°. The high lead glasses which soften at the lowest temperatures of any glasses included in Table I, have, as a group, the lowest loss factors.

A second significant point is that Sylgard, a coating compound commonly used to paint patch electrodes, has better electrical properties than most glasses shown in Table I. It is therefore not surprising that placing a heavy Sylgard coating on pipettes fabricated from many glasses improves their electrical properties. The wall of the electrode ends up having properties intermediate between those of its glass and those of Sylgard. It is also

TABLE II
COMPOSITION OF GLASSES

Glass	SiO_2	B_2O_3	Al_2O_3	Fe_2O_3	PbO	BaO	CaO	MgO	Na_2O	K_2O	Li_2O	As_2O_3	Sb_2O_3	SO_3
1724	NA[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7070	70.7	24.6	1.9	—	—	0.2	0.8	0.8	—	—	0.56	—	—	—
8161	38.7	—	0.2	—	51.4	2.0	0.3	0.04	0.2	6.6	—	0.04	0.38	—
7059	50.3	13.9	10.4	—	—	25	—	—	0.08	—	—	—	—	—
7760	78.4	14.5	1.7	—	—	—	0.1	0.1	2.7	1.5	—	0.18	—	—
EG-6	54.1	—	1.0	3.9	27.1	—	0.1	0.1	3.4	9.2	—	0.2	—	—
0120	55.8	—	—	0.03	29.5	—	0.25	—	3.6	8.9	—	0.4	—	—
EG-16	34.8	—	0.3	—	58.8	—	0.05	0.05	0.1	5.5	—	0.2	0.3	—
7040	66.1	23.8	2.9	—	—	—	0.1	0.1	4.1	2.7	—	0.1	—	—
KG-12	56.5	—	1.5	—	28.95	—	0.1	0.1	3.7	8.6	—	0.4	0.25	—
1723	57.0	4.0	16.0	—	—	6.0	10.0	7.0	—	—	—	—	—	—
0010	61.1	—	—	—	22.5	—	0.3	0.1	7.2	7.3	—	—	—	—
7052	65.0	18.3	7.4	—	—	2.7	0.2	0.1	2.4	2.9	0.6	—	—	—
EN-1	65.0	18.0	7.6	—	0.01	2.7	0.1	0.1	2.3	3.2	0.6	—	—	—
7720	71.4	15.2	2.0	—	6.1	0.3	0.2	0.1	3.7	0.3	—	—	0.5	—
7056	69.0	17.3	3.9	—	—	—	0.12	—	0.91	7.5	0.68	0.48	—	—
3320	75.3	14.3	—	—	—	—	0.1	0.1	4.0	—	—	—	0.8	—
7050	67.6	23.0	3.2	—	—	0.1	0.1	0.1	5.1	0.2	—	—	—	—
KG-33	80.4	12.9	2.6	—	0.005	—	0.05	—	4.0	0.05	—	—	—	—
7740	80.4	13.0	2.1	—	—	—	0.1	0.1	4.1	—	—	—	—	—
1720	62.0	5.3	17.0	—	—	—	8.0	7.0	1.0	—	—	—	—	—
N51-A	72.3	9.9	7.3	—	0.02	—	0.9	0.05	6.5	0.7	—	0.02	—	—
R-6	67.7	1.5	2.8	—	—	2.0	5.7	3.9	15.6	0.6	—	—	—	0.2
0080	73.0	0.04	—	—	—	0.1	4.8	3.2	16.8	0.4	—	—	—	0.22

[a] NA, not available.

expected and can be shown experimentally that poor electrical glasses are helped more by Sylgard coating than are good glasses. A third notable point is that fused silica (quartz) has substantially better electrical properties than any glass that has been used to date and so offers a potential way to further reduce patch clamp noise when a reliable method is available to fabricate patch clamp electrodes from it. Corning glasses 1724 and 7059 would also appear to be very good glasses electrically, but we are unaware of reports of their use to date for low noise patch clamp recordings. Corning 7070, low loss electrical, appears to be an excellent glass electrically, but to date no way has been found to pull electrodes from it. The glass changes its properties when it is heated.

Table II shows the chemical constituents of many of the same glasses shown in Table I. We do not presently have a way to predict which of these glasses will be useful for patch clamping simply based on these constituents, but Table II may be useful in deciding which glasses have a high

probability of containing leachable components that might affect channel currents.

A number of interesting observations can be made about Table II. A small number of the glasses contain antimony compounds. Corning 3320 is most notable in this regard, but substantial content is also found in 8161, a highly utilized patch clamp glass. The majority of the high lead glasses and 7760, a borosilicate glass, contain arsenic compounds. These particular glasses are noteworthy because of their low noise properties. Yet other glasses contain barium compounds. This is true of 8161, 1723, and 7052, three glasses that have found considerable use for patch clamping. Corning 7059, a barium borosilicate glass of extremely good electrical properties, contains very much more barium than other kinds of glass. Notice also that high lead glasses do not contain the boron compounds found at high concentrations in most other glasses. These glasses make up for this lack by having exceptionally high contents of lead compounds. Corning 8161 and EG-16 are most notable in this regard, each having PbO_2 amounting to more than 50% of the total composition. The high lead glasses not only have elevated lead compounds but also reduced levels of SiO_2.

The kind of information shown in Table II is not made available by companies that manufacture these glasses but rather comes from direct assay of the glasses. The variability in the composition of the glasses from one batch to another is unknown, and in several cases the percentages shown here do not add up to 100%. Therefore, Table II cannot be considered to be a highly accurate assessment of the composition of these glasses but is presented only as a guideline. It is the best information that we could obtain, but we are sure that there are many other trace compounds that exist in these glasses that do not show up in Table II. It seems unlikely that one will ever know all of these trace compounds since, in general, the material composition is considered proprietary information by the glass industry.

Thermal Properties

It is clear from experience that glasses which soften at lower temperatures offer several advantages in fabricating patch clamp electrodes. This is particularly true of the high lead glasses like 8161, EG-6, 0120, EG-16, KG-12, and 0010. Soda lime glasses such as R-6 and 0080 also offer many of these thermal advantages, but we do not recommend them because of their poor electrical properties. First, low softening temperature glasses are easy on microelectrode pullers. Because of the low filament current required to pull these glasses, filaments rarely change their properties with

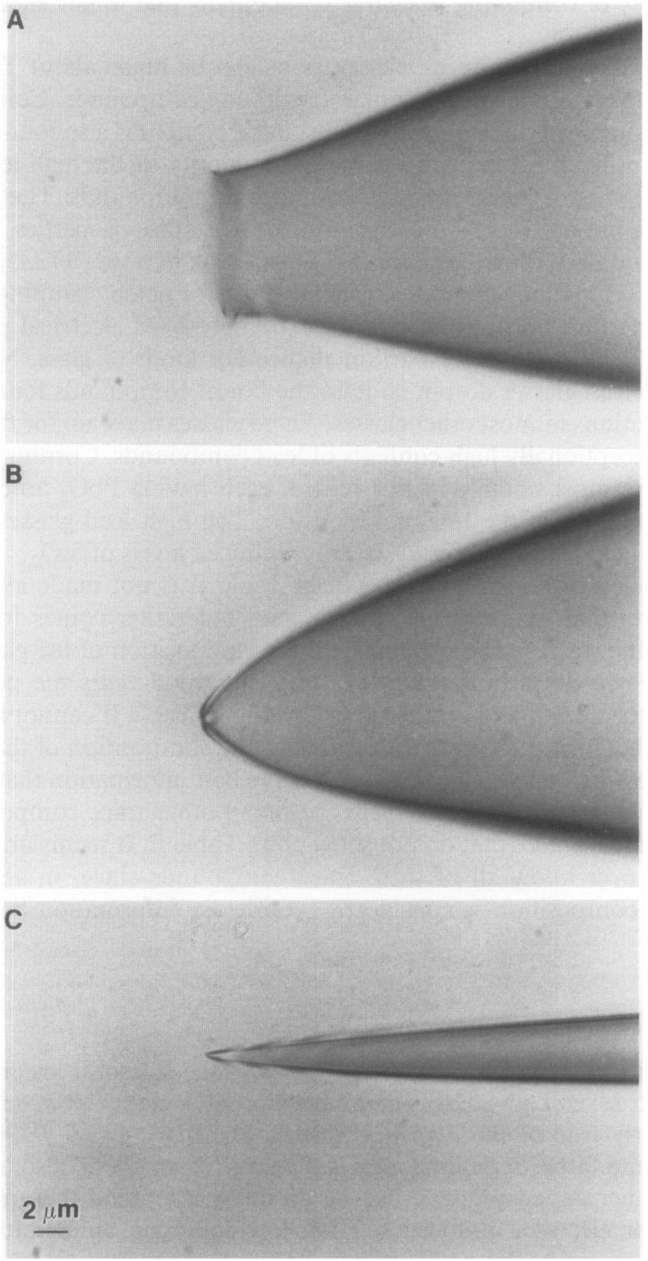


FIG. 2. Versatility of Corning 8161 (or other high lead glasses) in fabricating patch pipette tips. (A) Tip just after pulling. (B) Tip from (A) after fire polishing. (C) An 8161 tip pulled and fire polished for small tip diameter.

extended use and do not require replacement even after a year or two of continued operation. Second, they allow the fabrication of extremely blunt tips much more readily than glasses with higher softening temperatures. We illustrate this point in Fig. 2 wherein we show high magnification photographs of electrode tips pulled from Corning 8161 before and after fire polishing. This is one of the high lead glasses which as a group soften in the 580°–632° range. This is more than 200° lower than the softening temperature of 7740 (Pyrex), the glass most commonly used for intracellular microelectrodes. Corning 8161 also offers a fire polishing ability not provided by higher softening temperature glasses.

Figure 2A shows a very blunt tip immediately after pulling; Fig. 2B is the same tip after fire polishing. Such blunt tips, which are formed exceedingly easily with low softening temperature glasses, offer several advantages. They provide the lowest access resistance for whole-cell recordings. Also, their blunt taper makes them less likely to penetrate when they are pressed against the cell during seal formation. The cells can be indented to a larger extent than with sharper electrodes, and this often helps in seal formation. These high lead glasses are so amenable to fire polishing that it is possible to pull electrodes at such low temperature that the resulting tips are broken and jagged with diameters in excess of 50 μm and yet are easily fire polished into usable patch electrodes. The resulting tips are exceedingly blunt but have proved sealable to cells even when their final resistance is less than 0.5 MΩ.

Blunt tips are very important for perforated patch recordings (see [8], this volume). Such tips draw in large omega-shaped pieces of membrane when suction is applied. This large membrane area maximizes the number of parallel amphotericin or nystatin channels than can be inserted and thus minimizes the final access resistance achievable.

On the other hand, these same glasses can be pulled at slightly higher temperature to yield tips that are very sharp with resistances exceeding 20 MΩ (Fig. 2C). Such electrodes can be useful, for example, for trying to minimize the number of channels in a membrane patch by reducing the size of the patch. Therefore, these low softening temperature glasses are extremely versatile with respect to achievable tip geometries.

Borosilicate glasses soften at temperatures in the 700°–850° range. Those at the low end of the softening range (see Table I) are quite easily pulled and fire polished although they are clearly not in the same class with the high lead glasses in this regard. Fire polishing of these glasses is much more dependent on the shape of the tip after pulling than with the high lead glasses. In general, most pullers fabricate sharper tipped pipettes from hard glasses than from soft. In fact, there were several reports in the early patch clamp literature that whole-cell electrodes could not be made from

these glasses. With the advent of multistage, computerized electrode pullers, that restriction no longer holds. One can routinely make both patch and whole-cell pipettes from almost any glass. Corning 7070, 7760, 7040, 7052, EN-1, 7720, 7056, 3320, 7050, KG-33 (Kimax), N51-A, and 7740 (Pyrex) are examples of glasses in this borosilicate, intermediate softening temperature category. This class of glasses contains those most often used for patch clamp recordings.

Aluminosilicate glasses, which are very hard, high softening temperature glasses, were found in early work to produce low noise single-channel recordings and so were highly recommended. That low noise came at a high price, however. Glasses in this class soften at temperatures above 900° and so pulling them is quite hard on puller coils and filaments. The coils change their properties with time, and so they must be replaced or readjusted frequently. In addition, these glasses have had the undesired property of being very thin at the tip after pulling. This, along with their high softening temperature, has made them much more difficult to fire polish than softer glasses. The thin wall at the tip may in part offset the inherently good electrical and noise properties of this glass.

Noise Properties

To date, there has been no convincing theoretical analysis of a patch electrode as a noise source in current recordings. At present, we know of no way with simple equations to predict the noise that will come from a particular glass when it is used for patch clamping (but see [2] in this volume for a useful approximation). It is clear both theoretically and in practice that the glass is not a major noise contributor in whole-cell recordings. There, at moderate to high frequencies, the major noise comes from the series resistance associated with the electrode and the whole-cell capacitance (see [2], this volume). Therefore, our discussion of noise here is aimed primarily at single-channel recordings.

Figure 3 shows the power spectral density of noise measured from a particularly low noise resistive patch clamp headstage and the noise that comes from several patch clamp electrodes pulled from different glasses, Sylgard-coated to within 100 μm of the tip and sealed to Sylgard in the bottom of a chamber containing about a 2 mm depth of solution. Several important points are evident in Fig. 3. First, the noise of the headstage approaches a limiting value at low frequency but then rises with increasing frequency. Although the three electrode glasses in Fig. 3 show similar behavior, all have higher noise levels at low frequencies than does the headstage alone, and the noise increments even more steeply, in comparison to the headstage, as frequency increases. An extremely good electrical glass like Corning 1723 rises less steeply with frequency than does Corning

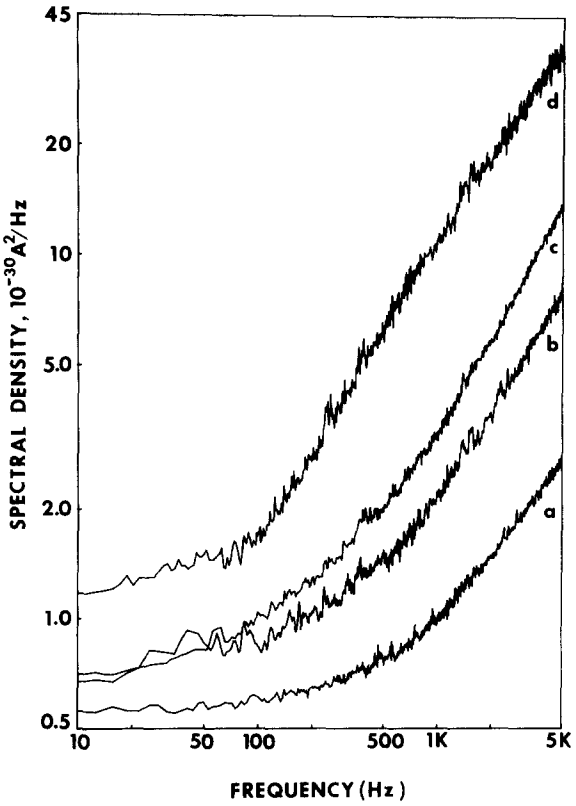


FIG. 3. Power spectral density of headstage (a) and headstage and electrode (b–d) noise. For (b–d), electrodes are coated with Sylgard 184 coating and sealed to Sylgard at the bottom of a 2 mm deep chamber filled with normal saline. b, Corning 1723 (aluminosilicate), c, Corning 7052 (borosilicate), d, Kimble R-6 (soda lime). [From J. L. Rae and R. A. Levis, *Biophys. J.* **45**, 144 (1984).]

7052, and both of these glasses are very much better in this regard than Kimble R-6 glass. Therefore, substantially greater noise comes from the electrode plus the headstage than comes from the headstage alone. This is true of all frequencies shown but particularly at high frequencies.

It is expected and borne out by experiments that the noise coming from a patch electrode is related to the product of the loss factor and the dielectric constant of the glass. The loss factor is a parameter used by manufacturers to describe the dielectric properties of a glass. It is technically defined as the tangent of the loss angle at a frequency of 1 MHz.[8]

[8] R. H. Doremus, "Glass Science," p. 190. Wiley, New York, 1973.

Simply stated, if the glass wall could be modeled as a perfect capacitor, a sinusoidal voltage applied across it would produce a sinusoidal current through it that was 90° out of phase with the voltage. If on the other hand the glass wall were a lossy capacitor, the phase angle would differ from 90°. The difference between the actual angle and the 90° angle for a perfect capacitor is what is defined as the loss angle. The loss angle should and does depend on frequency. The 1-MHz frequency is often used by manufacturers in their specification sheets. It is less in the few kilohertz range important for patch clamping, but unfortunately the data describing the loss angle as a function of frequency are not available for most glasses. One usually has only the value at 1 MHz to use in predicting which glasses might be most useful for low noise single-channel recordings.

Figure 4 demonstrates the dependence of noise in a patch electrode on the loss factor of the glass and its dielectric constant. In these experiments, patch electrodes were fabricated from various glasses, coated with Sylgard to within 100 μm of the tip, filled with solution, and sealed to Sylgard lining the bottom of a fluid-filled chamber about 2 mm in depth. The root mean square (rms) noise was measured with the pipette in air just above the bath and then again with the electrode sealed to Sylgard. Many commercial patch clamp amplifiers contain root mean square meters to allow experimenters to assess noise at desired points in their experiments. The 10-kHz noise on Fig. 4 was measured through an 8-pole Bessel filter and comes from the root mean square subtraction of the noise in air from the noise sealed to Sylgard:

$$\mathrm{rms}_{\mathrm{total}} = (\mathrm{rms}^2_{\mathrm{Sylgard}} - \mathrm{rms}^2_{\mathrm{air}})^{1/2}$$

The noise is plotted against loss factor times capacitance rather than loss factor times dielectric constant so that each glass can be normalized for its wall thickness (see [2], this volume). The major finding is that the glasses with the lowest loss factor–dielectric constant product show the lowest noise. Realize, however, that the noise shown here is not exclusively glass noise. It also includes the thermal noise of the Sylgard seal, but this is expected to be quite small because of the exceedingly high resistance of the Sylgard–glass seal.

Several other important points are apparent. First, the noise decreases as the wall thickness increases (compare 7052 with 7052TW, thin walled). Therefore, there is a noise advantage that comes from the use of thick walled pipettes. Second, the shape of the curve at low loss factor–dielectric constant is undetermined since no data are available for glasses with loss factor–dielectric constant products lower than 7760. The data show little tendency to flatten so it seems likely that significant improvement in this component of patch clamp noise could occur with the use of, for example,

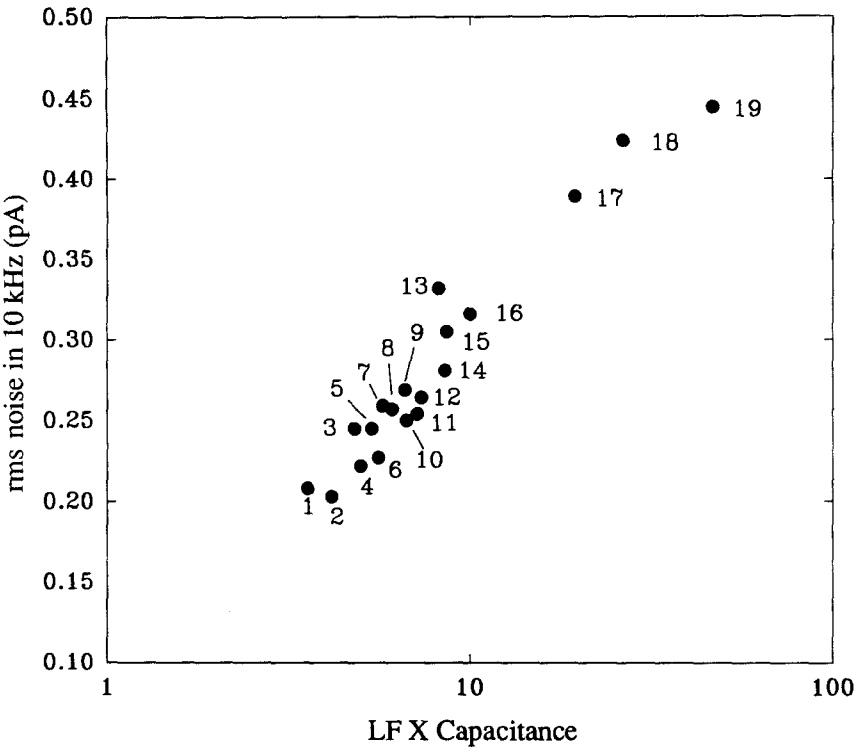


FIG. 4. Glass noise versus loss factor times capacitance. Each electrode was coated with Sylgard 184 coating and sealed to Sylgard at the bottom of a 2 mm deep chamber filled with normal saline. The root mean square noise shown is the root mean square difference between the noise when the electrode is sealed to Sylgard and the noise with the tip in air just above the surface of the bath. The capacitance was obtained by multiplying the dielectric constant by 0.225/wall thickness. [Adapted from J. L. Rae, R. A. Levis, and R. S. Eisenberg, *in* "Ion Channels" (T. Narahashi, ed.), Vol. 1, p. 288. Plenum, New York, 1988.] The glasses shown are as follows: (1) 7760, (2) 8161, (3) 7040, (4) 1723, (5) 0120, (6) EG-6, (7) 7052, (8) 7720, (9) EN-1, (10) KG-12, (11) 0010, (12) 3320, (13) 7052TW, (14) 7056, (15) EG-16, (16) 7740, (17) 1720, (18) R-6, (19) 0080.

quartz (fused silica) electrodes. Corning 7070 (low loss electrical) or Corning 1724 (aluminosilicate) also offer the potential for improvements if one were to work out proper techniques to fabricate patch electrodes from them.

It is clear from Fig. 4 that the noise does not scale with volume resistivity. One notable example is Corning 1720, an aluminosilicate glass with very high volume resistivity but quite bad noise properties. It is also clear that noise is not a direct function of dielectric constant alone. Corning 8161 has a very high dielectric constant and yet produces very low

noise. Plots of noise against either volume resistivity or dielectric constant would not produce a monotonic relationship as found for noise against loss factor times dielectric constant.

In summary, the high lead glasses (particularly 8161) which have low loss factors at present offer the best noise performance for single-channel patch clamping. However, Corning 7760 and 1723 have produced quite comparable results. Unfortunately, 1723 is no longer available, but perhaps 1724 will prove to be effective in the future. Corning 7760, which in our opinion is the best all-around electrode glass available, is also scarce and expensive at the present time and can only find extensive use in patch clamping if future demand warrants the effort for commercial concerns to make it available.

Capacity Compensation

Another important electrical property associated with the dielectric characteristics of the pipette wall is the shape of the capacity transient resulting from the application of a step voltage command to a sealed pipette. Obviously a large portion of this transient arises from stray capacitance at the headstage input. However, a significant portion comes from the pipette itself. This is particularly true of relatively slow components of the transient.

If the glass of the pipette wall were an ideal capacitor, the capacity transient associated with a step of voltage applied to the pipette would be a simple rapid spike with a shape that would essentially reflect the time derivative of the command voltage waveform (as modified, of course, by the bandwidth of the headstage electronics). It is well known, however, that the capacity transient associated with tight-seal patch clamping has slower components that can be of significant amplitude and with durations of many milliseconds. When studying voltage-gated ionic channels, it is traditional to observe single-channel currents immediately following step changes in the command potential. The presence of the capacity transient in the current measured at the headstage output can obscure or distort single-channel currents for some time following a voltage step, especially when there is a relatively large, slow tail of capacity current. Commercial patch clamps provide capacity compensation with one or two time constants to eliminate a major portion of the capacity current from the headstage output. Provided that the residual transient in the current output is of sufficiently small amplitude and is essentially constant, it is possible in the fortunate situation that a few blank records (i.e., records which contain no channel openings) are obtained to average several blanks and then subtract this average from each record containing channel openings. This proce-

dure is frequently quite satisfactory for removing capacity current from channel records. However, if the channels do not cooperate by periodically not opening in response to a step change in command voltage, the problem can be more difficult to deal with.

The slow component of the capacity transient of all glass types we have tested is *not* well described by a single exponential, and therefore electronic compensation even from commercial patch voltage clamps which provide a slow component of capacity compensation (and some manufacturers do not) is not really adequate to eliminate the transient. The best solution is to choose a glass for pipette fabrication that shows a minimum amount of slow component in its response to step changes in potential. Because this slow component arises from the lossy dielectric characteristics of the glass wall of the pipette, it is to be expected that glasses with low loss factors (e.g., Corning 8161, 7760) will display smaller slow components than glasses with relatively high loss factors (e.g., soda lime). We have verified this expectation experimentally. Fortunately such low loss factor glasses also display less noise and are therefore the natural selection for the fabrication of pipettes for high quality patch clamp measurements.

To study the capacity transients arising from patch pipettes, we pulled pipettes from several different types of glass. Pipettes were normally coated with Sylgard 184 to approximately 100 μm from the tip. The pipettes were then sealed to Sylgard at the bottom of a chamber that was always filled to the same depth (about 2 mm) with Ringer's solution. Typical pipette resistance prior to sealing was about 3 MΩ. Because we were interested in slow components of the capacity transient, the fast component was electronically canceled as completely as possible, and the residual transient was studied at times greater than 50–100 μsec following the start of a step voltage command. Command steps had an amplitude of 200 mV to maximize the measured signal; pulse durations varying from 2.5 to 500 msec were used. We studied pipettes fabricated from Corning 7052, 7040, 7760, 8161, and 0010 and Fisher Blue Dot (soda lime).

Although there was some variability among pipettes fabricated from the same glass, we found, as expected, that glasses with low loss factors showed significantly less slow component in their capacity transients than glasses with relatively high loss factors. Not only were the slow components of low loss factor glasses such as 8161 and 7760 smaller in amplitude than those of glasses like Fisher Blue Dot, but the duration of the slow component was also very much less. However, in no case was it found that the slow component of any glass was well described by a single exponential decay. In fact, even for the best glasses it was found that the decay more closely approximated a logarithmic function of time than an exponential, as might be anticipated for a lossy dielectric. Figure 5 shows some typical results.

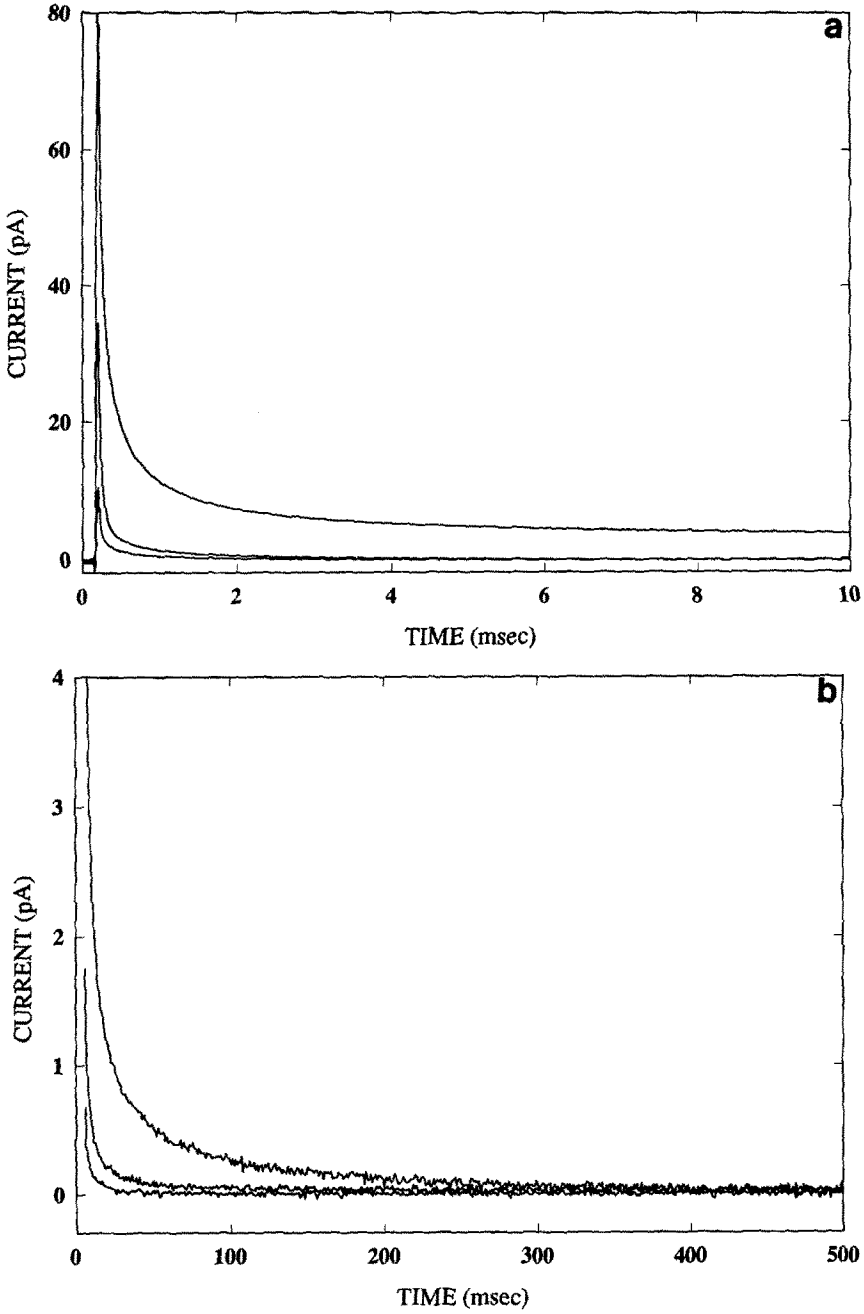
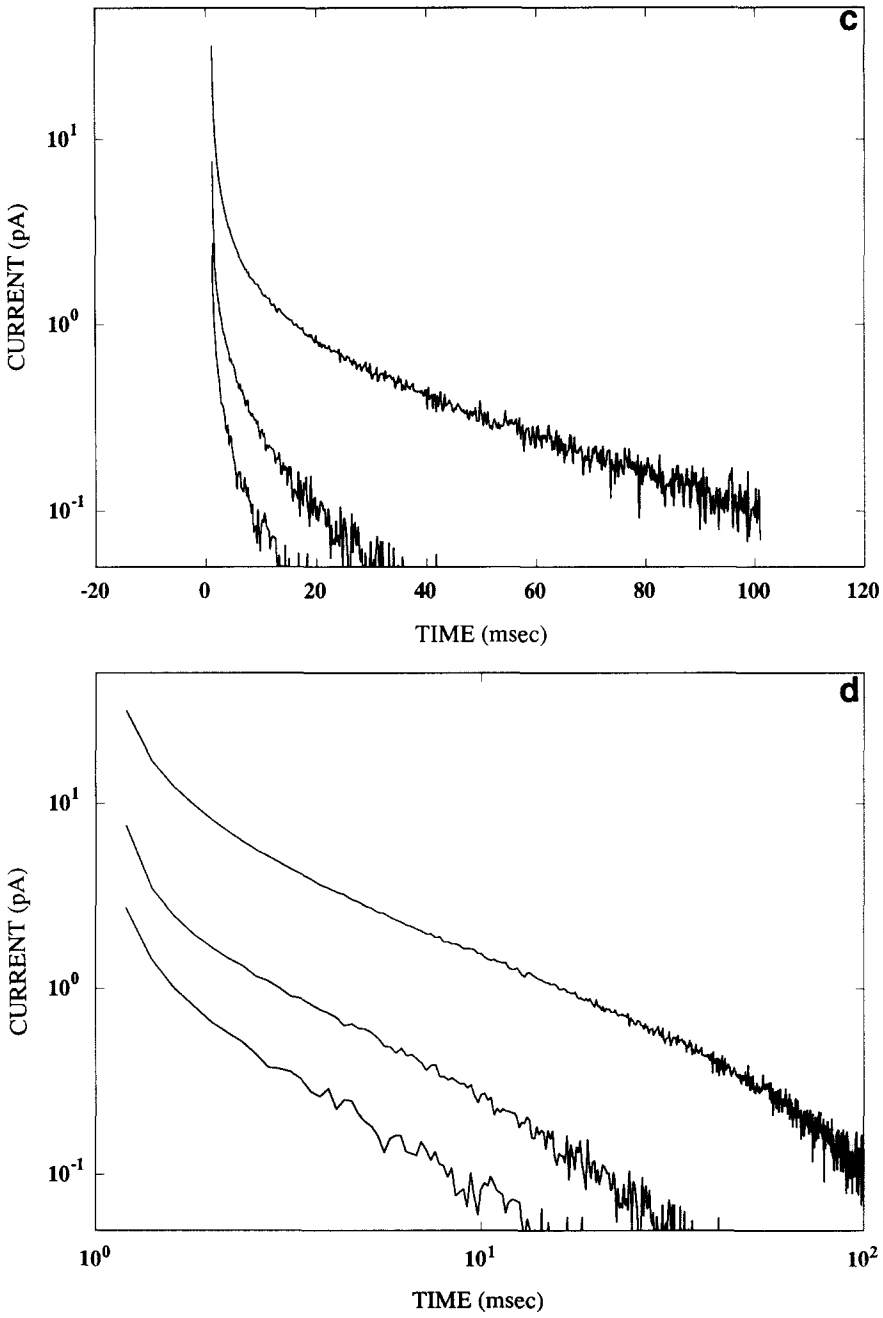


FIG. 5. Slow capacity transients from representative patch electrodes constructed from three different glasses. In each plot, 8161 is the lowest trace, 7052 is the middle trace, and Fisher Blue Dot is the uppermost trace. Electrodes were coated with Sylgard 184 coating and sealed to Sylgard at the bottom of a 2 mm deep chamber filled with normal saline. The fast capacity transient was negated using circuitry inherent to the patch clamp, and so the traces



shown are residual transients. (a) Fast time base recording; (b) slow time base recording, linear scale; (c) slow time base recording, linear–log scale; (d) slow time base recording, log–log scale.

Figure 5a shows, on a fast time scale, that different glasses have very different amplitudes and durations of their slow components. Figure 5b–d illustrates the nonexponential nature of the slow component of the capacity transient for several representative glasses. It is obvious that a single exponential component of electronic capacity compensation is not adequate for even the best glasses. In fact for all glasses that we studied, a minimum of three exponential components was required to achieve reasonable (although not perfect) fits to the data. Clearly with existing commercial patch clamps which have at most one exponential component for electronic compensation of these slow transients, the magnitude and duration of the residual (uncompensated) transient will be a function of the quality of the glass.

Of the glasses tested, 8161 showed the least slow component of its capacity transient. Corning 7760 typically had a slightly larger slow component, 7052 and 7040 displayed somewhat larger and longer lasting slow components, and Fisher Blue Dot (soda lime) glass showed by far the largest and longest lasting slow component in its capacity transient. It is instructive to compare the results of a good 8161 pipette and the worst of the four soda lime pipettes that we tested. For a 200-mV pulse, the slow component for 8161 had an amplitude of about 9 pA 100 μsec after the start of the step; it declined to less than 1 pA after about 1.4 msec and fell to 0.5, 0.2, and less than 0.1 pA after about 3, 8, and 17 msec, respectively. For the same size command step, the slow component of the soda lime pipette transient amplitude was about 50 pA 1 msec after the start of the pulse, was still somewhat larger than 10 pA in amplitude after 10 msec, and was about 4 pA after 50 msec. Even after 200 msec, the transient was still about 1.2 pA above its final value. The amount of capacitance, C_{slow}, associated with the slow component for each glass can be estimated by integrating the slow component of the transient to determine the amount of charge and then using the relationship $C = Q/V$ to determine the capacitance. For the pipettes described above this capacitance is only about 0.05 pF for 8161 but is about 1.2 pF for the soda lime pipette.

It should also be noted that in the absence of Sylgard coating, the slow component of the capacity transient of all glasses studied increased. The increase was smaller for low loss glasses than for soda lime glass. However, in the case of low loss factor glasses, applying an exceptionally heavy coat of Sylgard and painting very close to the tip did not yield significant improvement relative to results obtained with "normal" Sylgard coating. On the other hand, applying a heavy Sylgard coating does reduce the magnitude of the slow component of the capacity transient for high loss factor glasses such as Fisher Blue Dot, and such a coating is recommended if one must use glasses of this variety.

Finally, we observed (but did not systematically study) that for soda lime glass the magnitude of the slow component was not constant for repeated pulses delivered at rates of once every 1 to 3. Over a series of such pulses, the magnitude of the slow component was often seen to decline with repeated pulses, reaching a steady level only after some 20–30 pulses had been delivered. As expected, this effect was decreased if the repetition frequency was decreased. No such phenomena were observed for low loss factor glasses. This effect could prove to be troublesome for some types of patch clamp measurements. We did not examine the linearity of the slow component of the capacity transient over a significant range of voltages.

From these results, it is reasonable to conclude that the use of Sylgard-coated, low loss factor glasses is desirable for high quality patch clamp recording. This is particularly true for pulsed single-channel recordings.

Sealability

It is not clear what happens physically when a gigohm seal forms between a membrane and a glass. Any fluid pathway that remains between the two is highly restricted and greatly curtails the movement of even small ions like Na^+ and K^+. It largely excludes the movement of larger molecules. When patch clamping was a new technique, there was hope that there existed some optimal glass that would promote this sealing to cells. We initially reported that Corning 7052 was a glass with exceptional sealing properties[4] and continue to believe that it is a solid choice for use in patch and whole-cell clamping when all of its properties are considered. Subsequent work, however, has shown that essentially any glass is capable of making a seal with cell membranes, and there is not much solid evidence that one glass seals better than another. Such evidence would require that tips of electrodes fabricated from glasses being tested be of similar shape and wall thickness and fire polished to the same end point, etc., to rule out factors other than glass composition in the promotion of seals. In short, we have not done careful experiments aimed at quantifying sealability nor are we aware of any such experiments in the literature. There is much anecdotal information, but to our knowledge there are no controlled experiments published on this aspect of patch clamping. Even if such experiments were to be done, there is no reason to believe that what is found for one cell type would necessarily be applicable to others.

One of us (J.L.R.) has experience in sealing about 40 different cell types. During the course of that experience, there were many specific examples where one glass seemed to seal a particular cell better than other types. One notable example was isolated bile duct epithelium wherein we failed to obtain a single seal with 7052 in over 20 attempts but never failed

to obtain seals with 8161 in 12 attempts. Since our early publication of 7052 results, some investigators have reported that after switching to 7052 their sealing frequency remarkably improved. Others have found no effect, and yet others have obtained worse results. It seems clear that there is no final answer available concerning sealability of glasses. It is a complex issue that depends on tip geometry, details of suction application, etc., but probably more on the quality of the cells or what might be coating them than on the glass. We have found that seals form readily with many different kinds of cells we have used when electrodes are constructed from 7040, 7050, 8161, 7760, or 7052. More than one seal with the same pipette has been achieved with 7040, 7050, and 7052 but not with most other glasses. It has always been true that we could optimize sealability by changing the tip geometry and extent of fire polishing of any particular glass. It is possible that any glass might be made quite sealable by such an effort. Therefore, we are cautious about conclusions concerning sealability. Clearly, there has not been enough detailed work on each glass for one to make general rules about sealability.

Leachable Component

Glasses are complicated substances made of many different compounds. Table II contains a listing of the major constituents found in many glasses that have been used for patch clamping. Although glasses have major constituents that lead to their classification as soda lime, aluminosilicate, borosilicate, etc., they have many trace compounds as well. The location of these compounds in the glass is itself a complicated phenomenon. It is difficult to predict which of the particular compounds may be at the surface of the glass. It is clear, however, that glasses can have components at their surface which can leach into an aqueous environment in which they are in contact.[9] There are also many reports in the literature of atomic absorption measurements of cell constituents that have been contaminated by Na^+ or Ca^+ that leached into the solutions from glass containers.

Leachable components could be particularly problematic in patch clamp and whole-cell recordings owing to the close proximity of the channels to the glass. Several glass constituents such as Ba^{2+}, Rb^+, and As^{2+} are known blockers of ionic channels which could alter the recording of chan-

[9] R. H. Doremus, "Glass Science," p. 229. Wiley, New York, 1973.

nel currents were they to reach a sufficiently high concentration in the solution in the immediate proximity of the channels. Glasses contain many such compounds that might have these kinds of effects.

The literature now contains several reports that these undesirable effects do occur in patch and whole-cell recordings. Cota and Armstrong[10] reported that Corning 8161 blocked K^+ currents in single pituitary cells. Furman and Tanaka,[11] in the most complete study to date, reported that several glass types caused blockade of or otherwise altered currents in photoreceptor cells when used to construct electrodes for excised patch recordings. In that particular study, they found that Corning 0010, a high lead glass, was best for their currents but that several other high lead glasses caused blockade. The use of Corning 7052 resulted in larger inward currents at negative voltages than obtained with glasses thought to be inert to these particular currents. Rojas and Zuazaga[12] reported substantial kinetic differences in nicotinic acetylcholine receptors when a "hard" pipette glass was used for recording rather than a "soft" glass. In our own unpublished experiments, we found that 8161 produced a flickery blockade of lens inward rectifier single-channel currents at large negative voltages. Others have found that 8161 activates chloride channels in tracheal epithelium. Yet another investigator has found that solutions perfused onto neurons through a 7052 pipette prevent the activation of some receptor types by their agonists. Although many of these findings have not been formally published, there is enough published information to merit that one seriously consider the possibility that the glass used for the electrode may modify the currents in some way. It therefore seems imperative that one record currents with several different kinds of pipette glass to investigate this possibility.

Low Noise Recording Techniques

Modern patch clamps, particularly those implemented with integrating technology (see [2] in this volume) are capable of very low noise, particularly below 10 kHz of bandwidth. To utilize this performance, the user must pay close attention to other sources of noise. The total root mean square noise of a patch clamp recording is the square root of the sum of the

[10] G. Cota and C. Armstrong, *Biophys. J.* **53,** 107 (1988).
[11] R. E. Furman and J. C. Tanaka, *Biophys. J.* **53,** 287 (1988).
[12] L. Rojas and C. Zuazaga, *Neurosci. Lett.* **88,** 39 (1988).

individual squared root mean square noise sources. This means that any particular noise source that is large will dominate the total noise. Therefore, all potential contributory noise sources must be minimized. Specifically, the headstage, the electrode glass, the holder, and the seal contribute significantly even under circumstances where extraneous noise pickup from the environment is negligible. It is, of course, necessary that the entire preparation be properly shielded and hum from power supply mains, etc., be made negligible. Here, we suggest some approaches to low noise recording of single channels. Whereas these same approaches are a good idea for whole-cell recording, they are less important there since in whole cell recording the dominant noise source, at bandwidths above a few hundred hertz, comes from the access resistance in series with the whole-cell capacitance.

The noise from electrode glass itself arises from the lossy characteristics of its walls. Therefore, it is expected that glasses with the lowest inherent loss factors will have the lowest noise, and it is expected that the thicker the wall the lower the noise will be.

Even if one uses electrically superior glasses, low noise will not result unless the outer surface of the glass is coated with a hydrophobic substance like Dow Corning Sylgard 184 coating to prevent bathing solution from creeping up the outer wall of the electrode glass. A thin film of solution produces a distributed resistance which interacts with the glass capacitance to produce a noise source which rises with frequency. It becomes the dominant noise source and so must be eliminated. The Sylgard also decreases the capacitance of the electrode wall and so reduces the lossiness of the wall as well. It has been shown experimentally that Sylgard coating will improve the noise of any glass but will not turn a poor electrical glass into a good one. Low loss glasses coated with Sylgard give significantly less noise than poor glasses coated with Sylgard. The Sylgard should be painted as close to the tip as is practically possible, but the majority of the noise improvement is achieved if one paints to within 50–100 μm from the tip.

Holders must be made of low noise materials. Polycarbonate has been found experimentally to produce the lowest noise in limited tests of several likely materials, but it was only slightly better than polyethylene, polypropylene, and Teflon. When constructed from one of these materials, holders contribute only a small fraction of the total noise. We cannot, however, exclude the possibility that the holder material has some further effect on the noise associated with the holder–electrode combination. Holders should avoid metal and shielding which are noise sources. Holders do become a significant noise source if they get fluid in them. Therefore, great care must be taken in filling electrodes with solution. They should be filled

only far enough from the tip so that the end of the internal reference electrode is immersed. Any solution that gets near the back of the electrode should be dried with dry air or nitrogen to keep it from getting into the holder. Holders that become contaminated with solution should be disassembled and sonicated in ethanol or pure deionized water and allowed to dry thoroughly before being used again. It is also a good idea to clean the holders periodically this way even if no solution has been observed in them.

The noise of the holder and electrode can be checked before each attempt at a seal. When the holder and filled electrode has been inserted in the headstage connector and the electrode tip is positioned just above the bathing solution, the root mean square current noise seen on the meter of most commercially available patch clamp amplifiers should not be much above 0.1 pA in a 5-kHz bandwidth when using an integrating patch clamp and 0.2 pA for a standard resistive feedback headstage.

The seal will usually be the dominant noise source if it is only a few gigohms (at least up to bandwidths of several kilohertz). Seal resistances in excess of 20 GΩ must be obtained if exceptionally low noise single-channel recordings are to be routinely possible. The quality of the seal can also be tested each time by looking again at the root mean square noise meter. The noise also depends on the depth of the electrode tip below the surface of the bathing solution since the effective electrode capacitance increases as the depth of immersion increases. The voltage noise of the headstage interacts with the electrode capacitance to produce a noise source which rises with frequency. With integrator technology and with excised membrane patches lifted to just under the surface of the bathing solution, it has been possible for the authors to produce background noise as low as 0.13 pA rms in a 5-kHz band in a membrane patch with channels from several preparations using, for example, 7052 or 7760 glasses. A background noise of 0.15–0.17 pA rms was routinely possible.

One last potential noise source to consider is the noise in the signal generator which provides the command. In most patch clamps, this noise is reduced by heavily attenuating the external command, but it is possible, particularly if the command signal comes from a digital–analog (DA) converter, for this noise source to be significant.

Summary

Based on all of the properties of glass described here, it is obvious that no one glass can be recommended for all purposes and for all cells. Borosilicate glasses like 7760, 7052, and 7040 are good general purpose glasses for

both single-channel and whole-cell recordings. They are good initial choices but, of course, must be checked for each cell type for problems associated with leaching of blockers, etc., from the glass. Corning 8161 is the best glass studied to date with respect to electrical and thermal properties but must be checked carefully for leachable components. If perforated-patch whole-cell recordings are to be used, 8161, KG-12, or some other high lead, low melting point glass are probably the best choices.

[4] Ion Channel Selectivity, Permeation, and Block

By TED BEGENISICH

Introduction

One of the first steps in an effort to understand how cells perform their observed function is to determine what ion channels are in the membrane of the cell of interest and the properties of those channels. The channels can be classified by the ion that is most permeant and by what other ions can also permeate the channel. Further information can be obtained from a proper analysis of blockage of the channel by impermeant ions. The procedures described here assume the ion permeation pathway is a water-filled pore and that ions diffuse through the pore without associated large movements of the protein channel as might occur in "carrier"-mediated transport. As we have learned more of the details of these two general types of mechanisms, the distinction between them has diminished. However, single-channel currents that represent more that 10^5 ions/sec (0.02 pA) clearly fall into the pore category.[1,2]

Most of the permeation properties of pores can be determined through the use of macroscopic currents obtained with the whole-cell variant of the patch clamp technique. In most cases, use of these macroscopic currents is preferred but there are situations where single-channel currents more easily reveal specific permeation properties.[3] Unless specifically stated, the pro-

[1] B. Hille, "Ionic Channels of Excitable Membranes." Sinauer Associates, Sunderland, Massachusetts, 1984.
[2] B. P. Bean, this volume [11].
[3] E. Moczydlowski, this volume [54].