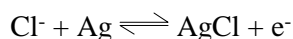

MICROELECTRODES AND MICROPIPETTES

Electrodes, Microelectrodes, Pipettes, Micropipettes and Pipette Solutions¹

Electrodes convert ionic current in solution into electron current in wires; they are made of materials that can participate in a reversible reaction with one of the ions in the solution. The most frequently used electrode material in electrophysiology is a silver (Ag) wire coated with a composite of Ag and silver-chloride (AgCl). For this electrode, chloride ions (Cl⁻) react with the Ag to produce AgCl plus an electron (e⁻), or an electron reacts with AgCl to produce Ag plus Cl⁻. Thus, the current carried by chloride ions in the solution is converted into electrons according to the following reversible reaction:



The electrical potential at one of these electrodes is equal to the standard electrochemical potential for Ag/AgCl plus $RT/F \ln(a_{\text{Cl}^-})$, where R is the gas constant (8.314 V C K⁻¹ mol⁻¹), T is the absolute temperature on the Kelvin scale, F is Faraday's constant (9.648 x 10⁴ C mol⁻¹), and a_{Cl⁻} is the activity (*i.e.*, the effective concentration) of Cl⁻ in solution near the electrode solution interface. The potential difference between a pair of these electrodes should be zero if they are immersed in connected compartments with equal chloride concentrations. These concentrations must be kept constant during the course of an experiment or an offset voltage will occur. An offset may be encountered when a Ag/AgCl electrode is used as a bath electrode and the bath Cl⁻ concentration is changed. To prevent this type of voltage offset, many investigators connect their bath electrode through an agar bridge that maintains a constant Cl⁻ concentration in the immediate vicinity of the Ag/AgCl electrode.

Intracellular and patch microelectrodes contain a AgCl-coated Ag wire placed in a solution-filled glass micropipette. The electrolyte solution provides the fluid connection from the cell to the electrode. Thus, the glass "electrodes" one pulls are not electrodes at all but simple conduits of proper geometry to establish this fluid bridge. In recognition of this, microelectrodes are often referred to as "pipettes" or "micropipettes."

¹Please see footnote (1) in Chapter 3.



The composition of the pipette solution depends on the type of measurement being made. Standard intracellular microelectrodes that are used to penetrate the cell membrane must be filled with a highly concentrated electrolyte solution, usually 2-4 M salt. The high electrolyte concentration reduces the electrode resistance. This minimizes the rectifying current flow, lowers voltage noise and provides a wider recording bandwidth. A solution this concentrated dominates the liquid junction potential formed between the pipette solution and the cell cytoplasm and results in a final junction potential that depends primarily on the relative mobilities of the anions and cations in the filling solution. This is important for the measurement of DC voltages because the cytoplasm anions are, predominantly, low mobility ions (mostly negative charges on large proteins), whereas the cations are small, high-mobility charges. The difference in the mobilities of the cellular anions and cations may result in a positive liquid junction potential. This positive liquid junction potential would lead to the measurement of an artificially depolarized membrane resting potential. The concentrated pipette solution is important because it negates the development of the liquid junction potential, thus preventing an erroneous measurement of the resting potential. However, there is a disadvantage in using the concentrated filling solution since it can enter the cell and produce a hyperosmotic load that would cause the cell to swell and alter its normal anion and cation content. While pipettes with very small tip diameters can minimize or prevent concentrated solution from entering the cell, they do so at the expense of noise, diminishing current passing ability, and limiting recording bandwidth. These limitations result from the high resistance of small-tip pipettes. In practice, the choice of the pipette tip size reflects a compromise between its ability to prevent the concentrated solution from entering the cell and the pipette's resistance.

Patch micropipettes are usually filled with a solution that is iso-osmotic to cell cytoplasm to allow current measurements in the absence of bulk water flow. Because of the much larger diameter of the patch pipette tip (1-2 μm) as compared to the tip diameter of an intracellular microelectrode (0.01-0.1 μm), the lower ionic-strength filling solution results in an electrode whose resistance is as low as a few megohms. The specific choice of the filling solution is dictated by the specific experiment to be done. Care must be taken to minimize junction-potential artifacts. When the pipette tip is immersed in the bath, a junction potential is generated. Its magnitude depends on the concentrations and mobilities of the ions in both the pipette solution and the bath (see discussions of the Henderson equation in electrochemistry textbooks). The junction potential is usually nulled while the pipette tip is immersed in the bath. When the seal forms, the junction potential disappears or changes its magnitude, and, consequently, the nulling voltage is no longer correct. The junction potential also changes upon going whole cell. These effects must be taken into account or offset errors will exist in either single-channel or whole-cell current recordings.

The filling solution in a patch pipette can be changed during the course of an experiment by inserting a fine tube into the back of the pipette through a special post in the electrode holder. Different solutions can be injected into the tube. Since the tube can be placed quite close to the pipette tip, the new solution can reach the vicinity of the pipette tip after a short diffusion delay. The procedure of draining excess solution from the pipette depends on the specific setup. This technique cannot be applied to standard intracellular microelectrodes; due to their small tip size, the tube cannot be placed close enough to the tip to allow changing the tip solution within a reasonable time.



The pipette is mounted in a holder that contains a Ag/AgCl electrode and a proper connector to plug into the headstage of the amplifier. The holder must be constructed of a low-noise, inert material, which is often chosen empirically. Axon Instruments holders are constructed from polycarbonate, which yields low noise with several patch-clamp pipettes. The HL-1-17 (for the CV-type headstages) and HL-2-17 holders (for the HS-type headstages) have an electrode well that can accommodate pipettes with an outside diameter of 1.5-1.7 mm. Pipettes of this size have an internal bore that allows insertion of a 1 mm diameter Ag/AgCl pellet electrode. The HL-1-12 (for the CV-type headstages) and HL-2-12 (for the HS-type headstages) holders have a pipette well that can accommodate pipette sizes of 1.0-1.2 mm diameter. This pipette size requires an electrode made of small-gauge Ag wire coated with AgCl. A simple yet effective coating procedure is to clean the silver wire down to bare metal using fine sand paper and immerse the cleaned wire in Chlorox bleach for 20 -30 min, until the wire is uniformly blackened by the Ag/AgCl coating. Coating of the silver wire must be repeated whenever the AgCl becomes scraped from the wire. Disruption of the coat can be minimized either by heat polishing the back of each pipette before insertion into the holder or by encapsulating the coated portion of the wire in a perforated Teflon sleeve.

The selection of the glass used for making the electrodes is more important for patch pipettes than for intracellular micropipettes. Most intracellular micropipettes are made from Corning #7740 Pyrex glass (Corning, Inc. Corning, NY). However, when used for tiny current measurements as in patch recordings, this glass is too noisy. The best patch pipettes are made from glasses with low loss factors (see Table 4-1). These glasses generate lower noise, and often have lower capacitances and fewer time constants to compensate.

Fabrication of Patch Pipettes

Glass tubing selected for the fabrication of patch pipettes should have walls of substantial thickness (0.2-0.3 mm). A thick wall generates lower electrical noise and increases bluntness at the tip, thereby preventing penetration into the cell during seal formation. Most investigators use glass tubing with a 1.5-2.0 mm outside diameter and a 1.15-1.2 mm inside diameter.

The glass tubing should be cleaned prior to the preparation of the patch pipette. Sonicating the glass in 100% ethanol in an ultrasonic cleaner is an effective cleaning procedure. After cleaning, the glass should be placed in an oven at 200°C for 10-30 min to achieve complete drying. This heat treatment is necessary for low-noise recording in environments with very high humidity.

Patch pipettes require much blunter tips than standard intracellular micropipettes and it is usually impossible to pull them adequately on single-stage electrode pullers. Many laboratories have modified standard vertical pipette pullers to operate as multiple-stage pipette pullers. Pulling pipettes, however, has become remarkably easier with the advent of microprocessor-driven microelectrode pullers like those available from the Sutter Instrument Company. With these pullers, it is possible to implement very complicated multistage pulls of glass pipettes and to store the parameters required for the desired operation in the instrument's memory.

For the lowest noise recordings, patch pipettes must be coated with a hydrophobic material to prevent the bathing solution from creeping up the wall of the pipette and, thus, limit a substantial noise source. A commonly used compound is Sylgard #184 from Dow Corning (Midland, MI).



Coating the glass with Sylgard also improves the electrical properties of the glass. After preparing the Sylgard solution per the manufacturer's instructions, it can be stored in the freezer in small, well-capped centrifuge tubes for several weeks. When brought to room temperature for use in painting electrodes, this freezer-stored Sylgard will last for several hours before it begins to polymerize. Sylgard is painted on the pipette tip using a small utensil, such as a piece of capillary tubing pulled over flame to a reasonably fine tip. Sylgard painting can be done using the magnifications available with standard dissecting microscopes. The pipette is coated to within 100 μm or less from its tip. It is important that the Sylgard be directed away from the tip by gravity at all times during the painting procedure. Otherwise, it will flow into the tip and make firepolishing and/or sealing impossible. After painting, Sylgard can be cured by holding the tip for 5 -10 s in the hot air stream coming from a standard heat gun of the type used in electronics fabrication.

To promote gigaohm seals and to reduce the possibility of tip penetration into the cell during seal formation, pipette tips should be firepolished. Firepolishing is accomplished by connecting to a micromanipulator a rod of inert material to which a short loop of platinum iridium wire has been fastened. 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 passing 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 using a compound microscope. Magnifications of 600x -1500x are required for adequate visibility. The platinum wire is usually coated with a glass, such as Pyrex or Corning #7052, to prevent sputtering. This is done by overheating the platinum wire and pushing it against a piece of pipette glass that has been pulled into a pipette tip. At high temperatures, the glass melts, flows over the platinum wire and ends up thoroughly coating it. After positioning the electrode tip near the glass-coated wire, current is passed through the wire to heat it. The amount of current required depends on the softening temperature of the glass from which the pipette is constructed. The hot platinum wire heats the electrode tip and causes sharp corners to round and rough surfaces to smooth. This polishing is best done under direct observation at a magnification of 600x - 1500x .

Pipette Properties for Single-Channel vs. Whole-Cell Recording

Whereas some properties of pipettes used for single-channel and whole-cell recordings are similar, other properties are entirely different. Most importantly, reducing the noise of the pipette is much more crucial in single-channel recording than in whole-cell recording. The dominant noise source in whole-cell recording is the pipette resistance that is in series with the capacitance of the entire cell. Hence, the noise contribution of the pipette is not as important. However, in order to provide sufficient bandwidth for whole-cell current recording and to limit command voltage errors, the resistance of a pipette used in whole-cell recording should not exceed a few megohms. Limiting the pipette resistance is not required in single-channel recording; nor does a higher pipette resistance, up to several tens of megohms, result in a significant increase in the noise (see **Chapter 12** for further discussion of pipette noise). In either single-channel or whole-cell recording, capacity currents following voltage steps must be sufficiently small and simple in time course to allow the researcher to compensate them by simple circuitry in the patch-clamp amplifier. Additionally, whether used for single-channel or whole-cell recording, pipettes must be made of glasses that do not leach compounds from their walls since these compounds might alter the currents measured from a particular channel.



Types of Glasses and Their Properties

Patch-clamp pipette glasses can be classified on the basis of the temperature at which they soften, on the basis of their electrical properties, or on the basis of their major chemical constituents. Many of these properties are itemized in specifications provided by the manufacturers. Therefore, it is often possible to choose glasses that should be effective for patch clamping just by considering their specifications. Table 4-1 lists the properties of a number of glasses that have been used for patch clamping. Glasses are listed in increasing order of loss factors.

Glass	Loss Factor	Log ₁₀ Volume Resistivity	Dielectric Constant	Softening Temp. °C	Description
7940	.0038	11.8	3.8	1580	Quartz (fused silica)
1724	.0066	13.8	6.6	926	Aluminosilicate
7070	.25	11.2	4.1	----	Low loss borosilicate
8161	.50	12.0	8.3	604	High lead
Sylgard	.58	13.0	2.9	----	#184 Coating compound
7059	.584	13.1	5.8	844	Barium-borosilicate
7760	.79	9.4	4.5	780	Borosilicate
EG-6	.80	9.6	7.0	625	High lead
0120	.80	10.1	6.7	630	High lead
EG-16	.90	11.3	9.6	580	High lead
7040	1.00	9.6	4.8	700	Kovar seal borosilicate
KG-12	1.00	9.9	6.7	632	High lead
1723	1.00	13.5	6.3	910	Aluminosilicate
0010	1.07	8.9	6.7	625	High lead
7052	1.30	9.2	4.9	710	Kovar seal borosilicate
EN-1	1.30	9.0	5.1	716	Kovar seal borosilicate
7720	1.30	8.8	4.7	755	Tungsten seal borosilicate
7056	1.50	10.2	5.7	720	Kovar seal borosilicate
3320	1.50	8.6	4.9	780	Tungsten seal borosilicate
7050	1.60	8.8	4.9	705	Series seal borosilicate
KG-33	2.20	7.9	4.6	827	Kimax borosilicate
7740	2.60	8.1	5.1	820	Pyrex borosilicate
1720	2.70	11.4	7.2	915	Aluminosilicate
N-51A	3.70	7.2	5.9	785	Borosilicate
R-6	5.10	6.6	7.3	700	Soda lime
0080	6.50	6.4	7.2	695	Soda lime

Table 4-1. Electrical And Thermal Properties of Different Glasses



Note that the electrical properties and the thermal properties of the glasses are not necessarily related to each other. Note also that Sylgard has better electrical properties than most glasses shown in the table. It is, therefore, not surprising that heavy Sylgard coating of pipettes fabricated from many types of glasses improves their electrical properties.

Table 4-2 shows the chemical constituents of many of the same glasses listed in Table 4-1. This table may be useful in deciding which glasses are likely to contain leachable components that might affect channel currents.

Glass	Chemical Constituent													
	SiO ₂	B ₂ O ₃	Al ₂ O ₃	Fe ₂ O ₃	PbO	BaO	CaO	MgO	Na ₂ O	K ₂ O	Li ₂ O	As ₂ O ₃	Sb ₂ O ₃	SO ₃
1724	Not available													
7070	70.7	24.6	1.9	—	—	.2	.8	.8	—	—	.56	—	—	—
8161	38.7	—	0.2	—	51.4	2.0	0.3	.04	.2	6.6	—	.04	.38	—
7059	50.3	13.9	10.4	—	—	25	—	—	.08	—	—	—	—	—
7760	78.4	14.5	1.7	—	—	—	.1	.1	2.7	1.5	—	.18	—	—
EG-6	54.1	—	1.0	3.9	27.1	—	.1	.1	3.4	9.2	—	.2	—	—
0120	55.8	—	—	.03	29.5	—	.25	—	3.6	8.9	—	.4	—	—
EG-16	34.8	—	0.3	—	58.8	—	0.5	0.5	0.1	5.5	—	.2	.3	—
7040	66.1	23.8	2.9	—	—	—	.1	.1	4.1	2.7	—	.1	—	—
KG-12	56.5	—	1.5	—	28.95	—	.1	.1	3.7	8.6	—	.4	.25	—
1723	57.0	4.0	16.0	—	—	6.0	10.0	7.0	—	—	—	—	—	—
0010	61.1	—	—	—	22.5	—	.3	.1	7.2	7.3	—	—	—	—
7052	65.0	18.3	7.4	—	—	2.7	.2	.1	2.4	2.9	—	.6	—	—
EN-1	65.0	18.0	7.6	—	.01	2.7	.1	.1	2.3	3.2	—	.6	—	—
7720	71.4	15.2	2.0	—	6.1	.3	.2	.1	3.7	.3	—	—	.5	—
7056	69.0	17.3	3.9	—	—	—	.12	—	.91	7.5	—	.68	.48	—
3320	75.3	14.3	—	—	—	—	.1	.1	4.0	—	—	—	—	.8
7050	67.6	23.0	3.2	—	—	.1	.1	.1	5.1	.2	—	—	—	—
KG-33	80.4	12.9	2.6	—	.005	—	.05	—	4.0	.05	—	—	—	—
7740	80.4	13.0	2.1	—	—	—	.1	.1	4.1	—	—	—	—	—
1720	62.0	5.3	17.0	—	—	—	8.0	7.0	1.0	—	—	—	—	—
N51-A	72.3	9.9	7.3	—	.02	—	.9	.05	6.5	.7	—	.02	—	—
R-6	67.7	1.5	2.8	—	—	2.0	5.7	3.9	15.6	.6	—	—	—	.2
0080	73.0	.04	—	—	—	.1	4.8	3.2	16.8	.4	—	—	—	.22

Table 4-2. Chemical Compositions of Different Glasses

Thermal Properties of Glasses

Glasses that soften at lower temperatures offer several advantages over glasses that soften at higher temperatures when fabricating patch-clamp pipettes. First, they do not impose a burden on the heating filaments of microelectrode pullers. Since low-filament current is required to pull these glasses, filaments rarely change their properties with extended use and do not require replacement even after one or two years of continued operation. Second, pipettes with extremely blunt tips can be more readily fabricated from these glasses than from glasses with high softening temperatures. Blunt tips provide the lowest access resistance (R_a) for whole-cell recording. Furthermore, the blunt-tapered pipettes are less likely to penetrate into the cell when pressed against the cell membrane during seal formation. High-lead glasses pull at the lowest



temperatures and are remarkably amenable to firepolishing. It is possible to pull pipettes at such a low temperature that their tips are broken and jagged, forming tip openings with diameters in excess of 50 μm , and yet the pipettes are easily firepolished into usable patch pipettes. Although the resulting tips are exceedingly blunt, these pipettes are effective in forming a tight seal with the cell even when the final pipette resistance is $< 0.5 \text{ M}\Omega$.

Blunt tips are very beneficial for perforated-patch recording because they are capable of drawing in large omega-shaped pieces of membrane when suction is applied. The large surface area of the omega-shaped membrane maximizes the number of parallel channels created by amphotericin B or nystatin, thus minimizing the final R_a that is achievable (see **Chapter 5**).

Most of the pipettes used for patch-clamp recording are fabricated from borosilicate glasses. These glasses soften at temperatures in the 700-850°C range (see Table 4-1). Although those at the low end of the range are quite easily pulled and firepolished, they are clearly not as good as the high-lead glasses in this regard. Compared to high-lead glasses, the success of firepolishing borosilicate glasses depends more on the shape of the tip obtained after pulling. However, with the advent of multi-stage, computerized pipette pullers, one can routinely make both patch and whole-cell pipettes from almost any glass.

Aluminosilicate glasses are very hard glasses with high softening temperatures. They produce low-noise single-channel recordings. However, their low noise comes at a high price. Glasses in this class soften at temperatures above 900°C and, therefore, pulling them wears out the coils and filaments of pipette pullers. Consequently, the coils change their properties with time and must be replaced or readjusted frequently. Moreover, pipettes fabricated from aluminosilicate glasses produce undesirably thin-walled tips after pulling. This, along with their high softening temperature, makes them much more difficult to firepolish than softer glasses.

Noise Properties of Glasses

While there is no simple way to predict the noise that will be generated from a particular glass when it is used for single-channel patch clamping, it has been observed that the noise of a patch pipette is related to its loss factor. The loss factor is a parameter used by glass manufacturers to describe the dielectric properties of a glass. Figure 4-1 illustrates the dependence of the noise of a patch pipette on the loss factor of the glass. Patch pipettes fabricated from various glasses, coated with Sylgard to within 100 μm of the tip and filled with solution were sealed to Sylgard lining the bottom of a fluid-filled chamber. The rms noise was measured using an rms meter, with the pipette in air just above the bath and then again with the pipette sealed to Sylgard. The 10 kHz rms noise in this figure was calculated by subtracting the noise measured in air from the noise measured when the pipette was sealed to Sylgard.



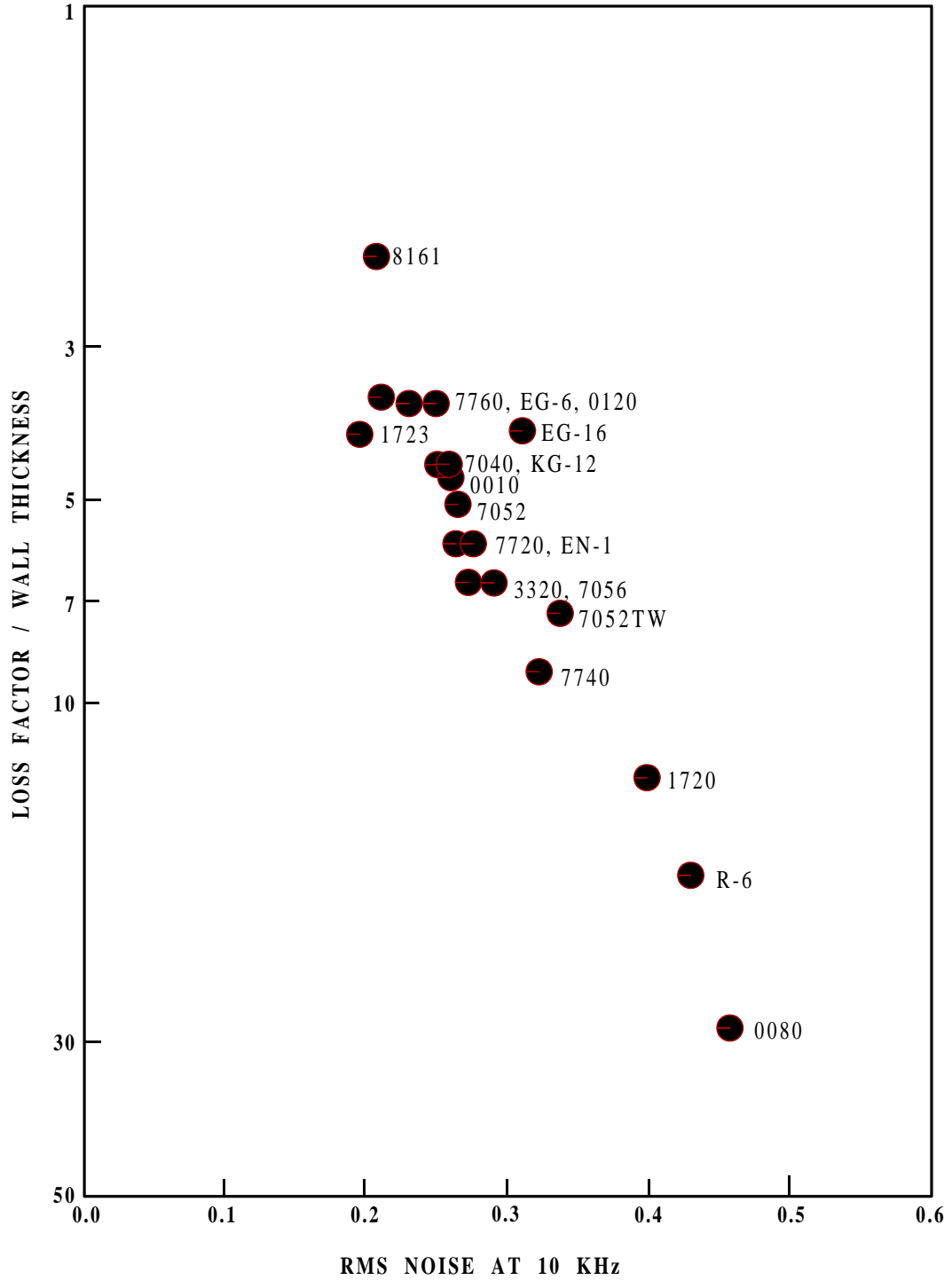


Figure 4-1. Noise Properties of Glasses

The dependence of the rms noise of a patch pipette on the loss factor of the glass.



As seen in Figure 4-1, glasses with the lowest loss factor exhibit the lowest noise. Furthermore, noise decreases as the wall thickness increases (compare 7052 with 7052TW, thin walled). Even greater improvement in noise can be achieved using quartz (fused silica) pipettes. The noise of pipettes fabricated from Corning 7070 (low loss electrical) or Corning 1724 (aluminosilicate) glasses could also be improved if proper techniques of fabricating patch pipettes from these glasses would be developed. The noise values shown in Figure 4-1 are higher than would be obtained with the Axopatch 200 in single-channel recording mode since its capacitive headstage produces lower noise than is produced by standard resistive headstages. It has been observed that as the noise of the headstage electronics decreases, the noise of other components, such as holder and glass, is reduced as well.

Leachable Components

Glasses are complicated substances made of many different compounds as shown in Table 4-2. While glasses have major constituents that lead to their classification as soda lime, aluminosilicate, borosilicate, etc., they have many trace compounds whose location in the glass is unknown. Glasses may have components on their surfaces that can leach into an aqueous environment with which they are in contact. Leachable components could be particularly problematic in single-channel and whole-cell recording due to the close proximity of the channels to the glass. The literature contains reports of undesirable effects of the leaching of constituents from the glass on membrane currents.

Further Reading

Cota, G., Armstrong, C. M. *Potassium channel "inactivation" induced by soft-glass patch pipettes.* **Methods in Enzymology.** Plenum Press. New York and London, 1983.

Sakmann, B., Neher, E. *Geometric parameters of pipettes and membrane patches.* **Single-Channel Recording.** Sakmann, B., Neher, E., Eds. pp. 37-51. Plenum Press. New York and London, 1983.

Cota, G., Armstrong, C. M. *Potassium channel "inactivation" induced by soft-glass patch pipettes.* *Biophys. J.* 53, 107-109, 1988.

Corey, D. P., Stevens, C. F. *Science and technology of patch-recording electrodes.* **Single-Channel Recording.** Sakmann, B., Neher, E. Eds. pp. 53-68. Plenum Press, New York and London, 1983.

Hammill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. *Improved patch-clamp techniques for high resolution current-recording from cells and cell-free membrane patches.* *Pflügers Arch.* 391, 85-100, 1981.

Rae, J. L., Levis, R. A. *Patch-clamp recordings from the epithelium of the lens obtained using glasses selected for low noise and improved sealing properties.* *Biophys. J.* 45, 144-146, 1984.



Rae, J. L., Levis, R. A. *Patch voltage clamp of lens epithelial cells: theory and practice.* Molec. Physiol. 6, 115-62, 1984.

Rae, J. L., Levis, R. A., Eisenberg, R. S. **Ion Channels.** Narahashi, T. Ed. pp. 283-327. Plenum Press, New York and London, 1988.

Ogden, D. C., Stanfield, P. R. **Microelectrode Techniques: The Plymouth Workshop Handbook.** Standen, N. B., Gray, P. T. A., Whitaker, M. J. Eds. pp. 63-81. The Company of Biologists Limited, Cambridge, 1987.

Furman, R. E., Tanaka, J. C. *Patch electrode glass composition effects on ion channel currents.* Biophys J. 53, 287-292, 1988.

Rojas, L., Zuazaga, C. *Influence of the patch pipette glass on single acetylcholine channels recorded from Xenopus myocytes.* Neurosci. Lett. 88: 39-44, 1988.



