A new simple method for the experimental measurement of liquid junction potentials

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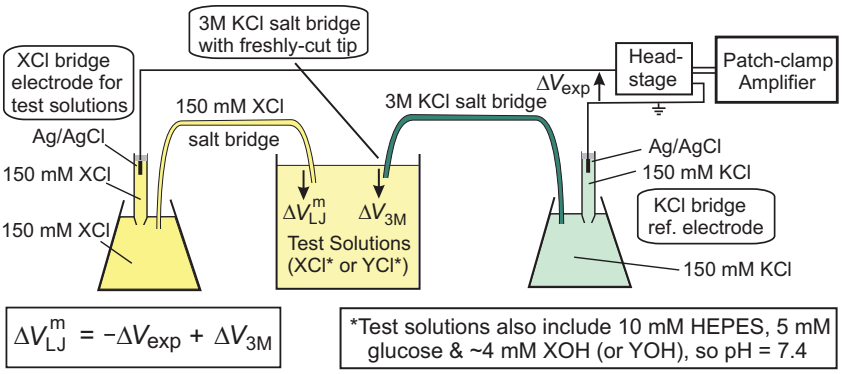
In electrophysiological experiments, particularly with patch clamp recordings, accurate potential measurements require corrections for liquid junction potentials (LJPs). Changes in LJPs can be fairly large especially under the dilution conditions used to determine relative ion permeabilities. In most cases, where the ion mobilities are known, these corrections can be calculated. To validate such calculated corrections, or to determine them if the ion mobilities are not known accurately, it is necessary to measure LJPs experimentally. 3M KCl electrodes have been widely used in the past with sharp microelectrodes to minimise LJPs, because K^+ and Cl^- ions have very similar, but not equal, mobilities and at such a concentration tend to overwhelm the LJP contribution of other ions, so that the LJPs tend to be somewhat independent of solution composition. Unfortunately, 3M KCl also produces major history-dependent problems in agar salt bridges or in the absence of a free-flowing junction (see Barry & Diamond, 1970; Neher, 1992), because the tip of the salt bridge or electrode takes up the composition of the previous solution in which it has been placed and no longer behaves as a 3M KCl junction. However, we have now shown that this problem with 3M KCl agar salt bridges can be overcome if the last 5 mm (at least) of the salt bridge (encased in polyethylene tubing) are cut off just before the salt bridge is placed into a test solution of different composition (or concentration), to thus ensure a fresh 3M KCl agar-solution in contact with the test solution. The measurements still need to be corrected for the small, but non-trivial, well-defined calculable shifts in reference potentials at the fresh 3M KCl agar-solution interface (ΔV_{3M}). The experimental setup is shown in the adjacent figure (modified from Fig. 3 of Barry *et al.*, 2010). X and Y represent different cations, for example, for LiCl dilution potentials, X would represent Li and there would be no Y needed. In this situation, the test solutions would represent ~1.0, ~0.5 and ~0.25 dilutions of the XCl salt, whereas in biionic measurements for two different salts at the same concentration, for example, X may represent K and Y represent Na, for a change from KCl to NaCl.



Using this technique, we have recently measured LJPs in diluted solutions of LiCl and NaCl (with and without 4 mM $CaCl_2$) and found them to agree within the experimental error (0.1 to 0.2 mV) to those LJPs calculated with the Henderson equation (using ion activities, rather than ion concentrations, and using the Windows version of the liquid junction potential program JPCalc; Barry, 1994), reported in Sugiharto *et al.* (2010) and Barry *et al.* (2010). We have also now measured dilution potentials for KCl salts and biionic potentials for KCl:NaCl, with excellent agreement between the corrected experimental measurements of the biionic LJP [4.5 ± 0.1 mV (n=14), after a ΔV_{3M} correction of 0.7 mV] and the theoretically predicted LJP value of 4.4 mV (using ion activities). Excellent agreement was also true for measured and predicted KCl dilution potentials, though here the LJP correction was small, equal and opposite to the ΔV_{3M} value (0.3 mV and 0.7 ± 0.1 mV for the 0.5 and 0.25 dilutions respectively), so that the uncorrected ΔV_{LJ}^{m} value was ~0.0 mV in each case (n=5). This is due to the test and 3M KCl solutions being primarily KCl solutions, unlike the case where the 3M KCl overwhelms the LJP contributions of salts with very different anion and cation mobilities.

We have thus developed a straight-forward and reliable method to measure LJPs, which we have now validated with a number of salt solutions with well documented ionic properties.

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Sugiharto S, Carland JE, Lewis TM, Moorhouse AJ, Barry PH. (2010) *Pflügers Archiv* **460:** 131-152.