Further measurements of liquid junction potentials, and their implications, using a refined freshly-cut 3M KCl reference salt-bridge methodology

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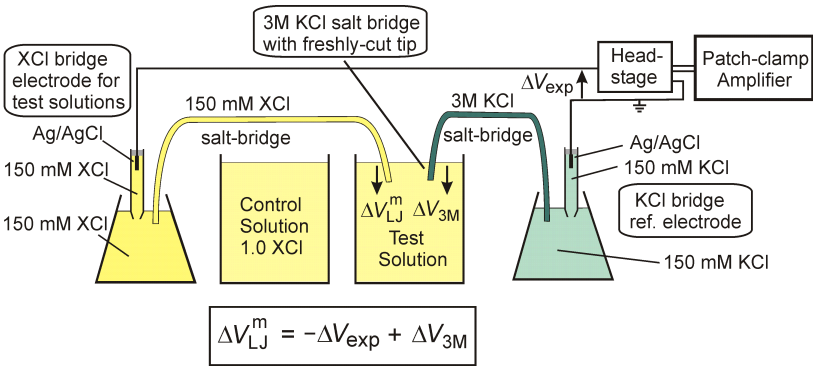
In electrophysiological experiments, accurate potential measurements require corrections for liquid junction potentials (LJPs). Under certain conditions, as in patch clamp experiments and dilution potential measurements, their magnitude can often be ~5-10 mV or more. In most cases, where the ion mobilities are known, LJP corrections can be simply calculated. To check such calculated corrections or if the ion mobilities are not known accurately, it is necessary to measure shifts in LJPs (ΔV_{LJ}^{m}; see Figure) experimentally. We have already reported a refined simple and accurate method for doing this using a freshly-cut 3M KCl-agar salt-bridge (in polyethylene tubing) as a reference electrode (Barry *et al.*, 2011), provided corrections are applied to the experimental value (ΔV_{exp}; Figure) for the small, well-defined and easily calculable shifts in LJPs at that electrode (ΔV_{3M}; Figure). We measured LJPs for a range of dilution and biionic measurements in LiCl, NaCl, KCl and CsCl physiological solutions containing 10 mM glucose and 10 mM HEPES titrated to pH 7.4 with XOH (where X is Li, Na, K or Cs), and with the diluted solutions (*e.g.* 145 to 75 and 145 to 37.5 mM XCl) kept isoosmotic with the control solution with added sucrose. This matched the case in physiological ion selectivity measurements. We showed that there was excellent agreement generally within ±0.1 mV between measured values and those predicted using the generalised Henderson equation (*e.g.* Barry & Lynch, 1991) when calculated using ion activities, the values being calculated using the Windows version *JPCalcW* of the *JPCalc* program (Barry, 1994). We also validated the approach with a *de novo* theoretical analysis of salt (*e.g.* NaCl) diffusion to and from the 3M KCl salt bridge.



We have since extended the measurements for dilution and biionic sodium physiological solutions of two non-chloride halides (I^- and F^-, with extreme high and low ion mobility values, respectively). We have shown that there was generally good agreement between measured and predicted LJPs within ±0.1 mV for the dilutions and ±0.2 mV for the biionics.

We have now also measured dilution LJPs for a very large concentration range of simple NaCl solutions (with no added glucose, pH buffer nor sucrose). As expected, even in the absence of osmotically balanced solutions, there is still excellent agreement between measured and predicted LJPs, particularly when ion activities were used. Calculation rounding errors of ±0.1 mV were expected for comparison of measured (corrected) and predicted values. From a control solution of 150 mM, for shifts to 300 and 75 mM, the measured LJP ΔV_{LJ}^{m} values were respectively +3.5 and −3.5 mV and were within 0.1 mV of the predicted values. For 150-500, 150-37.5 and 150-25 mM shifts, the ΔV_{LJ}^{m} values were +6.1, −7.0 and −9.0 mV, respectively and were all within 0.2 mV of the predicted values. From a control solution of 50 mM to 500 mM, the measured shift ΔV_{LJ}^{m} = +11.6 mV and predicted value was 0.3 mV lower. It was found that the use of activities tends to slightly underestimate the magnitude of the predicted LJP by about 0.2 mV for a 4:1 NaCl gradient and 0.3 mV for a 10:1 NaCl gradient, but that the use of concentrations overestimates the LJP by 0.4 mV for a 4:1 gradient and 0.6 mV for a 6:1 NaCl gradient. This indicates that it is preferable to use activities for more accurate dilution potential calculations (though the use of concentrations still gives reasonable estimates). These consistent differences suggest that the effective concentration under steady-state diffusion conditions is still affected by the interionic attractive forces between anions and cations, but perhaps not to the full extent that it would be in the case of activities under equilibrium conditions.

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* available on-line at: http://www.uow.edu.au/~adamt/BPC/BioPhysChem2011/BioPhysChem2011.html