

Abstract Submitted
for the OSF13 Meeting of
The American Physical Society

Utilization of dual-wavelength pH-sensing dyes in the characterization of pH buffers under hydrostatic pressure¹ PAUL URAYAMA, J. AUGUST FOLZ, ZACHARIAH P. CALLAHAN, Miami University — Biologically-compatible pH-buffer solutions are routinely used in biochemical and biophysical studies at ambient pressure. When using buffers under high-pressure however, the existence of a thermodynamic volume change (ΔV) for the buffer's acid-dissociation reaction leads to a pressure-dependent equilibrium constant (pK_a). The result is a pressure-induced shift in pH. Here we use pH-sensing, dual-wavelength probe dyes, SNARF-1 and SNARF-5F, to characterize the pressure-response of three biologically-relevant buffers: MOPS, HEPES, and TRIS. Emission spectra from the dyes, excited at 337-nm wavelength, are used to track the pressure-induced change in pH of the buffer systems, up to 600 atm (61 MPa). From this the ΔV of the buffers' acid-dissociation reaction was determined and found to be in the 0 to +10 ml/mol range. Results presented are important when accounting for pressure-induced pH changes during high-pressure biophysical studies because a large range of biochemical reactions involve H^+ and have a ΔV similar in range to the these buffers.

¹This material is based upon work supported by the National Science Foundation under Grant No. 0957675.

Paul Urayama
Miami University

Date submitted: 13 Sep 2013

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