

BIOC 463a

Purpose of Experiment: To experimentally determine the pKa of phosphate buffer by mixing together specific ratios of Weak Acid and Conjugate base forms, measuring the pH of the buffer, then from a titration curve determine the pKa. Finally, compare the experimentally determined value with the literature value.

Flowchart Example for Expt. 1.1: Determination of pKa of Phosphate Buffer

Note: refer to Table 1.1 in protocol for Expt. 1.

Prepare 200 mL of 0.1 M monobasic (KH_2PO_4) or dibasic (K_2HPO_4) phosphate.

KH_2PO_4 calculation:

$$(0.2 \text{ L})(0.1 \text{ mol/L})(136 \text{ g/mol}) = 2.72 \text{ g}$$

K_2HPO_4 calculation:

$$(0.2 \text{ L})(0.1 \text{ mol/L})(174.2 \text{ g/mol}) = 3.48 \text{ g}$$

Weigh out salt and dissolve in ~150 mL H_2O . When completely dissolved, bring final volume to 200 mL.



Prepare buffers:

pH range = 5.2 to 8.5.

Total volume = 10 mL.

Use H-H eqn. To calculate Vol (KH_2PO_4) and Vol (K_2HPO_4).

Fill in values in Table 1.1 of Expt 1.1 protocol.



Calibrate pH electrode/meter with pH 4 and 10 standards.



Pour buffers into small beakers and measure pH.

Record value in Table 1.1.



Plot: pH(calculated) vs. Fraction (HPO_4^{2-}) as solid line; pH(observed) vs. Fraction (HPO_4^{2-}) as individual data points.

Determine regions of greatest deviation of pH(observed) from pH(calculated).

