Key for Designing Buffer Practice

The compound 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, which also is known as HEPES, is used to prepare buffers for biochemical and systems. The sodium salt of HEPES, $C_8H_{17}N_2NaO_4S$, is a weak acid with a pK_a value of 7.48. When present in samples that are being assayed for proteins using the Bradford method, HEPES must be present at a total analytical concentration less than 100 mM. You have been asked to prepare a HEPES buffer for your research group; your recipe for its preparation is subject to the following three constraints:

(1) a total volume of 0.250 L
(2) a pH of 7.07
(3) a total analytical concentration of HEPES of 65.0 mM

How many grams of the sodium salt of HEPES and how many mL of 0.60 M NaOH will you need to include in your recipe? The id number for your recipe, which is placed on each new preparation of your buffer, is #118.

Your recipe is due on Friday.

Answer. First, we must determine the moles of HEPES and the moles of its conjugate weak base; for simplicity, we represent HEPES as HA and represent its conjugate weak base as A^-. From the buffer's pH, we know that

$$7.07 = 7.48 + \log\frac{\text{mol } A^-}{\text{mol HA}}$$

and from the total analytical concentration and the total volume, we know that

$$\text{mol } A^- + \text{mol HA} = \left(65.0 \times 10^{-3} \text{ M}\right) \times (0.250 \text{ L}) = 0.0163 \text{ mol}$$

This gives us two equations and two unknowns, which means we have sufficient information to calculate the moles of HA and of A^-. Solving the first equation for the moles of A^- in terms of the moles of HA gives

$$\text{mol } A^- = \text{mol HA} \times 10^{(\text{pH}-\text{p}K_a)} = \text{mol HA} \times 10^{(7.07-7.48)} = \text{mol HA} \times 0.389$$

Next, we substitute this back into the equation for the combined moles of HA and A^-

$$\text{mol } A^- + \text{mol HA} = \text{mol HA} \times 0.389 + \text{mol HA} = \text{mol HA} \times 1.389 = 0.0163 \text{ mol}$$

Solving gives the moles of HA as 0.0117 and the moles of A^- as 0.0046. To prepare the buffer, we measure out a mass of HEPES that is equivalent to the combined moles of HA and of A^-

$$\text{g HEPES} = 0.0163 \text{ mol} \times 260.29 \text{ g/mol} = 4.230 \text{ g}$$

and dissolve using an amount of water equivalent to about half of the desired total volume. To complete the buffer, we convert 0.0046 moles of HA to A^- by adding

$$0.0046 \text{ mol } A^- \times \frac{\text{mol NaOH}}{\text{mol } A^-} \times \frac{1\text{L}}{0.60 \text{ mol NaOH}} \times \frac{1000 \text{ mL}}{\text{L}} = 7.59 \text{ mL}$$

and then diluting to the desired final volume of 0.250 L.