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PROFESSOR: Many chemistry and biochemistry procedures will require you to perform a reaction at a specific pH. To accomplish this task, you must understand how to both make a buffer and how to use a pH meter. This video will demonstrate two methods of buffer preparation as well as proper use of the pH meter.

A buffer is an ionic compound that resists changes to its pH when strong acids or bases are added to it. The amount of strong acid or strong base that can be neutralized by a particular buffer is dictated by the buffer's capacity, which is determined by the concentration of weak acid and weak base in the buffer. A higher concentration of buffering reagents translates into greater buffering capacity.

In the first part of this video, we will walk through two methods of buffer preparation. In the first example, you will need to prepare 1 liter of a 50 millimolar buffer for a protein that is stable at pH 7.3. The first step is choosing an appropriate buffer system. This buffer system should produce a stable pH in the range needed and should have a capacity large enough to accommodate your solutions.

The weak acid used for the system must have a pKa that is plus or minus 1 unit of the desired pH-- the closer the better. From this abbreviated list of weak acids, the phosphate ion, with a pKa of 6.82, is clearly the best choice for a buffer with a desired pH of 7.3. Therefore, the weak acid used for this system is H₂PO₄⁻, and the conjugate base is HPO₄²⁻, which correspond to the salts sodium phosphate monobasic and sodium phosphate dibasic.

After determining an appropriate buffer system, the Henderson-Hasselbalch equation is used to calculate the ratio of acid to conjugate base needed at your desired pH. If your desired pH of 7.3 and the pKa of the phosphate ion are substituted into this equation, then the ratio of conjugate base to acid is calculated to be 1.6. Therefore, your buffer must contain 1.6 times the amount of sodium phosphate dibasic to sodium phosphate monobasic.

After determining the appropriate ratio of conjugate base to acid, the exact amount of each reagent is calculated in the following way. Because you are planning to make 1 liter of a 50 millimolar phosphate buffer at pH 7.3, 50 millimolar is equal to the concentration of acid plus the concentration of conjugate base. In addition, you already know that the ratio of base to acid is 1.6, so the concentration of base is equal to 1.6 times the concentration of acid.

By substituting Equation 2 into Equation 1, you can see that the concentration of acid plus 1.6 times the concentration of acid is equal to 50 millimolar. Through manipulation of this equation, the concentration of acid needed for this particular buffer is 19.2 millimolar. And the concentration of base is 30.8 millimolar, which corresponds to 2.30 and 4.37 grams respectively for a 1-liter solution. To make the buffer, simply dissolve 2.30 grams of sodium phosphate monobasic and 4.37 grams of sodium phosphate dibasic in 1 liter of distilled water.

An alternative method of buffer preparation is simple, accurate, and requires much less math. To demonstrate this second method, we will prepare 1 liter of a 1.0 millimolar Tris buffer at pH 7.60. The first step involves the determination of the amount of weak acid, or in this example, weak base, needed in the system. To make 1 liter of a 1.0 millimolar Tris buffer, 1.0 millimoles or 121 milligrams of Tris are required.

Add approximately 800 milliliters of distilled water as well as a stir bar to the beaker. Then weigh 121 milligrams of Tris and transfer it to a 1-liter beaker. Ensure that all of the material has been transferred by rinsing the weigh boat and transferring the rinses into the beaker. While stirring, monitor the pH of this solution and add a sodium hydroxide solution dropwise until the pH is exactly 7.60.

The concentration of the sodium hydroxide solution doesn't really matter. However, a solution that is too dilute may require an excessive volume to be added to the buffer, while a solution that is too concentrated may cause you to overshoot the desired pH. If you do happen to exceed the desired pH, then add a hydrochloric acid solution to bring the pH back down.

Transfer this solution to a 1-liter volumetric flask. Wash the beaker with distilled water several times, and add these washings to the volumetric flask. Dilute the buffer to the mark on the volumetric flask and mix the solution by inverting the flask several times.

For the second method of buffer preparation, you needed a pH meter. The final part of this video will discuss the proper use of this equipment. A pH meter allows the determination of hydrogen ion activity by a potentiometric measurement, using a glass electrode coaxially joined to a silver/silver-chloride reference electrode. When the probe is immersed in solution, the reference electrode makes contact with the sample, which completes the electrical contact between the reference electrode, sample, and pH electrode. If the unknown solution has different pH from the solution in the probe, an electrical potential results, which is registered on the meter.

This video will demonstrate the use of an Orion SA520 pH meter. The important components of a pH meter include the display, the probe, and the electrode tip. The electrode tip contains a fragile glass bulb. Be extremely careful not to hit the probe on the side of a beaker and when using stir bars.

To operate the pH meter, first make sure that the electrode is connected to the meter. Then calibrate the pH meter. The pH meter must be recalibrated every time you turn it on and after three to four hours of use. We will perform a two-point calibration. This method involves calibrating the pH meter with two different buffers of known pH.

Turn on the instrument and select the pH mode as 0.1 or 0.01. Then press the ISO button and verify that the pH reads 7.00. Rinse off the probe with distilled water into a waste beaker and gently blot it with a chem wipe. Place the probe into the first buffer and press the CAL button. The number 1 should appear on the display.

The display will alternate between 1 and the pH value of the solution. Wait until the display stabilizes and press Enter. The number 2 should then appear on the display. Rinse the probe with distilled water, blot it with a chem wipe, and place it into the second buffer. If your solution has an acidic pH, then use the 4.01 buffer. If it has a basic pH, use the 10.01 buffer.

The display will alternate between 2 and the pH value of the solution. Wait until the display stabilizes and press Enter. The letters pH will be displayed, indicating that the pH meter is now ready to take measurements. Before taking a measurement, make sure that your sample buffer is at the same temperature you'll be using in your experiments because pH is temperature dependent. Rinse and blot the probe and place it into the buffer. Wait for the display to stabilize and record the pH.

Correct buffer preparation and pH meter technique are essential to ensure that your proteins and other sensitive chemicals live long functional lives.

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