Implementation of Virtual Laboratory Platform to Study Human Buffer Solutions in the Era of COVID-19

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Abstract

The emergence of Covid-19 disrupts the normal mode of educational activities leading to institutions to device other means of content delivery methods. The challenge was more significant teaching laboratory or other hands on courses. Virtual buffer laboratory experiments were implemented to simulate the pH of the major human buffer systems. The virtual lab was chosen for this educational activity because the experiments were performed at no financial cost, extra programing or downloading an app was not required, and it was easy to perform the experiments at preferred locations. Prior the surge of Covid-19, students were taught using wet lab experiments because it gives the students a chance for a full experience of preparing and performing experiments while interacting with peers and co-workers. It is well known that the virtual lab has no time constraints and repeated results are obtained without any human and equipment errors. The goal of this paper is to introduce the implementation of the virtual laboratory platform to study the human buffer solutions in the era of Covid-19. The experiment is designed to teach the fundamental laboratory techniques while emphasizing the practical application of buffer preparation experiments to first year college students. Buffer solutions were successfully prepared using phosphoric acids, ethanoic acid-ammonia with buffer capacity testing for each resulting solution. The pH of the buffer solutions were chosen to match the digestive body fluid in the human body. A comparison was made with the three buffer solutions prepared to that of the human body where the human blood has a pH of 7.4 ± 0.5, phosphoric acid with a pH of 6.8, and the amino acid buffer pH of 5.00-9.00. The virtual experiment is successfully implemented and hopefully will give chemistry instructors methods to design and implement similar lab experiments to teach students virtually.

Introduction

The emergence of Covid-19 disrupts the normal mode of educational activities leading to institutions to device other means of content delivery methods. The challenge was more significant teaching laboratory or other hands on courses. Chemistry experiments have evolved from wet or bench experiments of acid-base titrations, to buffer solution kits that can be bought and performed at any location, to the present virtual computer or cloud-based technical experiments. Downloaded applications require a large computer laboratory space with individual workstations equipped with expensive licensed software, several probes and other miscellaneous equipment that become obsolete with time.

Virtual laboratory experiments currently available offer the latest student accessible technology that are improved periodically by educational sponsors. During this Covid-19 pandemic lockdown rules, virtual lab experiments were available for students and scientist to access advanced stage programs, obtain results, graph the data, and prepare laboratory reports for online submission. Users only need an internet connection and a computer to access virtual labs and were able to work at their own pace. The only potential downside is physical observation, sound, and direct instructional interaction is missing. Switching to the virtual lab in the era of Covid-19 has other advantages like speed, elasticity, procure processing time, cost-saving chemical, equipment cost and no internal or external dependence on others is needed. Users can work from preferred locations and at their own pace.

This experiment is designed on preparation of a buffer solution using virtual laboratory platform. A buffer solution is
composed of a weak acid and its conjugate base, that serves as a neutralizing cushion when a small concentration of acids or bases are added to the solution as seen by small fluctuations in the pH of the solution. Buffers have a working range of 1 pH and each solution is tested for their fluctuation in their working pH. Buffer equation is derived from the acid equilibrium constant. For an acid dissociation constant:

\[ \text{HA} + \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^- \]

for this reaction, the acid dissociation constant is

\[ K_a = \frac{[\text{H}_3\text{O}^+][\text{A}^-]}{[\text{HA}]} \]

\[-\log K_a = -\log[\text{H}_3\text{O}^+] - \log([\text{A}^-]/[\text{HA}]).\]

Rearranging this equation gives the Henderson Hasselbalch equation:

\[ \text{pH} = pK_a + \log([\text{A}^-]/[\text{HA}]). \]

\( K_a \) is only affected by temperature, and changes in volume and pressure, concentration changes will be compensated by Le Chatelier’s principle so that equilibrium rules are obeyed. The assumption is based on the Henderson Hasselbalch equation, that if the acid concentration is equal to the conjugate base then pH = pK_a and the buffer is at its peak performance. For bases:

\[ \text{pOH} = pK_b + \log([\text{HB}^-]/[\text{B}]). \]

The buffer capacity of human blood is 7.4 + 0.5 and it is maintained by bicarbonate, amino acids and haemoglobin. Buffer solutions were prepared using weak acid and their conjugate bases in \( \text{H}_3\text{PO}_4/\text{Na}_3\text{PO}_4 \) (pH = 2.1), \( \text{HAc}/\text{NaAc} \) (pH = 4.75), \( \text{H}_2\text{CO}_3/\text{NaHCO}_3 \) (pH = 7.4), \( \text{NH}_3/\text{NH}_4\text{Cl} \) (pH = 9.25). Other important examples in the human body is the digestive system that starts with saliva in the mouth with pH = 6.5-7.5, gastric acid in the stomach with pH = 1.5-4.0, and small and large intestine with a pH of 4.0-7.0. The buffer in this study was selected to simulate the pH of the human body ranging from pH 2.1 to 9.25.

**Method**

A method was developed for students to prepare a buffer solution that meets the specific buffer pH using the available solutions utilizing a virtual lab. The virtual lab has different size-glassware as well ready-made solutions prepared for mixing. The stock room has 100 mL of 1.0 M of each acid and 100 mL of 1.00 M of the conjugate bases. In addition, 100 mL of 1.00 M HCl and 100 mL of 1.00 M NaOH was available for testing. The pK_a is matched with the pH for maximum buffer performance. The pH for conjugate acid/base pair is as follows: \( \text{H}_3\text{PO}_4/\text{Na}_3\text{PO}_4 \) (pH = 2.1), \( \text{HAc}/\text{NaAc} \) (pH = 4.75), \( \text{H}_2\text{CO}_3/\text{NaHCO}_3 \) (pH = 7.4), \( \text{NH}_3/\text{NH}_4\text{Cl} \) (pH = 9.25). Once the buffer solution is prepared, the buffer capacity of the solution is tested. Equal quantities of the weak acid and its conjugate base are used for \( \text{HAc}/\text{NaAc} \) to make pH of \( \text{H}_3\text{PO}_4/\text{Na}_3\text{PO}_4 \) 100 mL of 1 M \( \text{H}_3\text{PO}_4 \) and 19 mL of 1 M \( \text{Na}_3\text{PO}_4 \) with a 119 mL of total solution (Figure 1) [1].

**Figure 1:** Buffer capacity of \( \text{H}_2\text{CO}_3/\text{NaHCO}_3 \).

**Result**

For each of the solutions in the experiment the following information is displayed in the virtual lab. The table lists pH, molarity, and the concentrations of available ions in the solutions (Table 1).
Buffer limitations

Buffer solution of 100 mL of 0.1 M NaAc was mixed with 100 mL of 0.1 M HAc. The pH range for the effectiveness of the buffer is one pH on each side of the peak. Figure 2 shows the pH range of the HAc/NaAc buffer solution. The buffer’s range of operation is between 3 lower limit and pH 6 upper limit. 0.1 M of HCl was added 10 mL at a time for the acid limitations. 0.1 M of NaOH was added 10 mL at a time and the pH limit was about pH 3.

Analysis

A good buffer solution is made up of equal concentrations of a weak acid to conjugate base so that the pH will be equal to the pKₐ and the buffer will be at optimum buffer capacity. In addition, the concentration 1 M of each component is selected as a starting material so that the solution is strong enough to withstand buffer fluctuations. The preparation of pH 7.4 carbonic acid/bicarbonate combination required 10 mL of 1 M H₂CO₃ and 113 mL of 1 M HCO₃⁻. The 11 to 1 ratio of the buffer solution indicates that in order to maintain pH of 7.4, an abundance of bicarbonate is need for each hydronium ion. This result agrees with the abundance of bicarbonate ion present in the human blood. The following reaction shows how the pH is maintained in human blood.

\[
\text{H}_2\text{O}(l) + \text{CO}_2(g) \rightarrow \text{HCO}_3^-(aq) + \text{H}^+(aq)
\]

\[
\text{H}^+(aq) + \text{HCO}_3^-(aq) \rightarrow \text{H}_2\text{CO}_3(aq)
\]

Figure 1 shows the addition of 1 M HCl to the buffer solution. Another buffer solution is prepared, and the buffer capacity is tested by adding increments of 1 M NaOH. The above equation will be effective if an abundance of bicarbonate ion is present while CO₂ gas can be released easily to maintain equilibrium of the pH. When needed the CO₂ gas is breathed out or more bicarbonate is consumed to maintain the pH of 7.4.

Protein is a good buffer that regulates the intercellular pH; the two active amino acids in this case are histidine and cysteine. These amino acids serve as a zwitterion molecule having both the amino group and carboxylate ion on the same molecule. To test the limitation of amino acids, two buffer solutions were used in this experiment namely NH₃/NH₄⁺ and HAc/NaAc. Acetic acid is used because it is the most available and forms simple dimer of all the existing carboxylic acids. Equal amount of 1 M HAc and 1 M of NaAc is used in figure 2 to prepare the buffer.

Figure 2 shows the plot of the pH of the solution as 1 M HCl is added and then 1 M of NOH is added in the second half of the graph. The graph shows that the buffer capacity is in the range of pH = 5.00 ± 1.00. HAc is a monoprotic acid. The fluctuation in the pH is seen when 100 mL of NaOH is added to the buffer solution which can be explained by the protonation of the acid. The equivalent point for the neutralization of the buffer is at pH = 8.5 when 100 mL of 1 M NaOH is added to the buffer solution. On the acid side when 100 mL of HCl is added the pH shows another variation which can also be explained by the water ionization in the solution:

\[
\text{NaCH}_3\text{COO} \rightarrow \text{Na}^+ + \text{CH}_3\text{COO}^- + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{H}^+
\]

The other part of the protein is the amine group and the reaction is as follow:

\[
\text{NH}_4^+(aq) + \text{OH}^-(aq) \rightarrow \text{NH}_3(aq) + \text{H}_2\text{O}(l) \quad \text{NH}_3(aq) + \text{H}^+(aq) \rightarrow \text{NH}_4^+(aq) + \text{H}_2\text{O}(l).
\]
Figure 2: Buffer capacity of HAc/Ac solution.

Figure 3 shows the buffer capacity testing results. Acid (hydronium ion) added to the ammonia molecules in the buffer mixture reacts with the hydronium ions to form ammonium ions and reduces the hydronium ion concentration almost to its original value. The buffer capacity of NH₃/NH₄Cl is pH 7.4 ± 1.0. Both buffer solutions have buffer capacities that mimic the zwitterion properties of the protein that carries buffering the solution.

Summary

In this study the three most important buffer solutions in the human body were studied using chemicals and virtual lab as a laboratory workstation. The online virtual lab was chosen as a platform because of closure of school secondary to Covid-19 pandemic. The virtual lab platform chosen in this experiment gave limitless privileges like removing human and experimental errors, providing ease of operation, eliminating chemical and other laboratory expenses, chemical waste removal and spillage, and time and location constraints. The three buffers mirrored the pH of blood and other liquids in the body at stable pH ranges so that different molecules in the body can demonstrate the maintenance of physiological bodily functions. The bicarbonate buffer prepared in this experiment had a buffer capacity of 7.4 ± 0.5 which aligns with the critical buffer of the blood. In addition, the main purpose of a buffer, to regulate the hydrogen ion concentration, is shown by carbonic acid/bicarbonate buffer that compensated for the lack or excess hydrogen ion in the solution. The amino acid buffer simulated by the ammonia and acetic acid buffer solutions and the buffer capacity agreed with the pH range of proteins. Based on this experiment, virtual lab exercise was successfully implemented in preparing buffer solutions that have similar pH as that of the three most important physiological buffering systems in the human body.
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References

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