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t Design and preparation of buffers effective at different pHs Abstract These experiments aimed to determine the optimum pH ranges various buffers are effective and provide opportunity for the use of the Henderson-Hasselbalch equation to prepare a buffer of a specific pH. Three different buffer systems were initially investigated; volumes of weak acid and weak bases of specified concentration were prepared and titrated against strong acid or strong base solutions with pH readings taken at frequent intervals to determine pH ranges over which the systems are most effective. The Henderson-Hasselbalch equation was used to calculate an appropriate ratio of acid: base volumes in order to prepare a buffer solution with a given pH value. The main results conveyed the optimum pH range for each buffer system, where significant changes of pH value are resisted with additions of small amounts of acid or base. Introduction Our body cells have natural ability to resist excessive pH changes. Metabolic activity produces acid and to a lesser extent, base and they hydrogen ions associated can alter the overall charge, configuration and function of various proteins. The majority of acid results from carbohydrate and fat metabolism, producing CO_2, which combines with H_2O to form H_2CO_3, carbonic acid, dissociating to form H^+ and HCO_3^- ions. Most bases come from metabolism of anionic amino acids and oxidation of organic anions, producing HCO_3^- ions. The pH changes associated are resisted by various physiological buffering systems. So preparation of a buffer system at a particular pH, as well as finding the effective pH range for different buffers, is of use as artificial buffers are therefore necessary to mimic this ability when studying biochemical processes in vitro. The Henderson-Hasselbalch equation defines the relationship between values of pH and pK and the molar ratio of conjugate acid and conjugate base

concentrations. pH = pK + log([A-]/[HA]) If pK and desired pH is known, molar ratio of acid: base can be calculated. The equation was rearranged to find the ratio necessary to produce a buffer of a given pH. The aims of the experiment were: * Determination of pH ranges over which the buffering systems acetic acid/acetate, Tris(base)/Tris (acid) and glycine (acid)/glycine (base) respectively are effective and plotting the associated values on a titration curve. * Use and rearrangement of the Henderson-Hasselbalch equation to prepare a buffer of specific pH. * Use and calibration of a pH meter to measure pH. Method The pH meter was calibrated using solutions of pH appropriate to the titration. Volumes of glycine and Tris (0. 1M) were made up and acetic acid was titrated against NaOH, Tris against HCl and glycine against NaOH. After each aliquot was ran in from the burette, pH values of the resulting solutions were measured and recorded. Hydrochloric acid was titrated against sodium hydroxide with pH measured in the same way, to give a comparison curve. The ratio of acetic acid: sodium acetate was calculated through rearrangement of the Henderson-Hasselbalch equation and a buffer solution of pH 5. 2 was made through mixing the appropriate volumes of each. Results Aliquots of 0. 2M NaOH at intervals of 1ml were ran into a 0. 1M solution of acetic acid. The pH was measured, using a pH meter, after the addition of each aliquot. Fig 1. shows the resulting change in pH after each 1ml addition of NaOH. Raw data is presented in Table 1 in the Appendix. A steady increase in pH is seen with the addition of up to 10ml of NaOH. The pH ranges from 2. 90 — 5. 82. The acetic acid/acetate buffer system is successfully resisting excessive changes in pH with the addition of alkali, this is the range over which the buffer system is most effective. A sharp change in pH is seen after 11ml of NaOH is

added, increasing to 11. 20. The pK value is 4. 56, after addition of 5ml of NaOH. Aliquots of 0. 2M HCl at intervals of 1ml were ran into a 0. 1M solution of Tris. The solution of Tris of 0. 1M concentration was prepared using 1. 21g of Tris and 100ml of water . The pH was measured, using a pH meter, after the addition of each aliquot. Fig 2. shows the resulting change in pH after each 1ml addition of HCl. Raw data is presented in Table 2 in the Appendix. A steady decline in pH is displayed with the addition of up to 6ml of HCl. The pH ranges from 7. 19 — 10. 51. The Tris (base)/Tris (acid) buffer system is successfully resisting excessive changes in pH with the addition of acid, this is the range over which the buffer system is most effective. A sharp change is pH is seen after 7ml of HCl is added, decreasing to 2. 50. The pK value is 8. 35, after addition of 3ml of HCl. Aliquots of 0. 2M NaOH at intervals of 1ml were ran into a 0. 1M solution of glycine. The solution of Tris of 0. 1M concentration was prepared using 0. 75g of glycine and 100ml of water. The pH was measured, using a pH meter, after the addition of each aliquot. Fig 3. shows the resulting change in pH after each 1ml addition of NaOH. Raw data is presented in Table 3 in the Appendix. A steady increase in pH is displayed between 1 — 11ml of added NaOH. The pH ranges from 9. 18 — 12. 58. The glycine (acid)/glycine (base) buffer system is successfully resisting excessive changes in pH with the addition of alkali, this is the range over which the buffer system is most effective. The pK value is 10. 43, after addition of 5ml NaOH. Aliquots of 0. 2M NaOH at intervals of 1ml were ran into a 0. 2M solution of HCl, to provide a comparison titration curve. Fig 4. shows the resulting change in pH after each 1ml addition of NaOH. Raw data is presented in Table 4 in the Appendix. This buffer system can resist changes in pH with the addition of a much larger volume of alkali. Up to 18ml of NaOH

was ran into the HCl, and the pH only varied from 0. 92 — 2. 20. A sharp increase is seen after the addition of 20ml NaOH but subsequent additions up to 24ml did not alter the pH value significantly, suggesting that this buffer system is effective over pH ranges 0. 92 — 2. 20 and also 11. 07 — 12. 41. The pK value is 1. 11, after addition of 9ml NaOH. In order to prepare the pH 5. 2 acetic acid/acetate buffer, a ratio of 3: 1 of [sodium acetate] and [acetic acid] was used. This was calculated using the Henderson-Hasselbalch equation. If pK and desired pH is known, molar ratio of acid: base can be calculated. The equation was rearranged to find the ratio necessary to produce a buffer of a given pH. Details of the calculation are given as Figure in the appendix, as Figure 5 in the Appendix. 100ml 0. 1M sodium acetate solution was prepared using 0. 82g sodium acetate and 100ml of water. 75ml of the sodium acetate solution and 25ml of acetic acid were then mixed to prepare 100ml of 0. 1M acetic acid/acetate buffer solution. The pH was found to be 5. 05. Discussion The experiment aimed to determine pH ranges over which each of the tested buffer systems are effective and to use the interrelationship between pH and pK as defined by the Henderson-Hasselbalch equation to prepare a buffer at a specific pH. The acetic acid/acetate buffer was found to be effective within the pH range 2. 90 — 5. 82. The Tris (base)/Tris (acid) buffer was found to be effective within the pH range 7. 19 — 10. 51. The glycine (acid)/glycine (base) buffer system was found to be effective within the pH range 9. 18 — 12. 58. The pK value is the pH where the weak acid is half neutralised. The experiment showed that for acetic acid pK = 4. 56 in comparison to the recognised value of 4. 72, for Tris pK = 8. 35 in comparison to the recognised value of 8. 00 and for glycine pK = 10. 43 in comparison to the recognised value of 9. 6. There is little

variance present in these values, suggesting that the trends found are valid. The pK values for each buffer system lies towards the middle of the pH range over which the system is most effective. This is because the maximum buffering capacity is found when pH = pKa, and buffer range is considered to be at a pH = pKa ± 1. It is clear from the respective titration curves that HCl is a more effective buffer, resisting changes in pH through addition of a larger volume of NaOH, 18ml in comparison to 6ml. This is due to the more complete dissociation of HCl in aqueous solution, as it is a stronger acid. The pH of the acetic acid/acetate buffer prepared using the Henderson-Hasselbalch equation to find an appropriate ratio was 5. 05, which is close to the desired pH of 5. 2. Metabolic activity results in acid and base product and the pH changes associated are resisted by various physiological buffering systems. Therefore it is important to be able to prepare a buffer system at a particular pH, as well as finding the effective pH range for different buffers, as artificial buffers are necessary when studying biochemical processes in vitro to mimic the cell's natural ability. Appendix Volume NaOH (ml) | pH | 0 | 2. 9 | 1 | 3. 6 | 2 | 3. 15 | 3 | 4. 19 | 4 | 4. 39 | 5 | 4. 56 | 6 | 4. 72 | 7 | 4. 91 | 8 | 5. 09 | 9 | 5. 36 | 10 | 5. 82 | 11 | 11. 2 | Table 1. volume of NaOH (ml) added to acetic acid and resulting pH change. Volume HCl (ml) | pH | 0 | 10. 51 | 1 | 9. 02 | 2 | 8. 64 | 3 | 8. 35 | 4 | 8. 09 | 5 | 7. 73 | 6 | 7. 19 | 7 | 2. 5 | 8 | 2. 02 | Table 2. volume of HCl (ml) added to Tris and resulting pH change. Volume NaOH (ml) | pH | 0 | 5. 05 | 1 | 9. 18 | 2 | 9. 59 | 3 | 9. 87 | 4 | 10. 11 | 5 | 10. 43 | 6 | 10. 83 | 7 | 11. 53 | 8 | 12. 2 | 9 | 12. 33 | 10 | 12. 5 | 11 | 12. 58 | Table 3. volume of NaOH (ml) added to glycine and resulting pH change. Volume of NaOH (ml) | pH | 0 | 0. 98 | 1 | 0. 96 | 2 | 0. 92 | 3 | 0. 92 | 4 | 0. 93 | 5 | 0. 97 | 6 | 0. 99 | 7 | 1. 02 | 8 | 1. 06 | 9 | 1. 11 | 10 | 1. 15 | 11 | 1. 21 |

12 | 1. 29 | 13 | 1. 38 | 14 | 1. 45 | 15 | 1. 53 | 16 | 1. 67 | 17 | 1. 87 | 18 | 2. 2 | 19 | 6. 33 | 20 | 11. 07 | 21 | 12 | 22 | 12. 18 | 23 | 12. 31 | 24 | 12. 41 |

Table 4. volume of NaOH (ml) added to HCl and resulting pH change. pH = pK + log([acetate]:[acetic acid]) 5. 2 = 4. 72 + log([acetate]:[acetic acid]) 0. 48 = log10([acetate]:[acetic acid]) 100. 048 = 3. 02 = [acetate]:[acetic acid] = 3: 1 ratio. Figure 5. calculation of appropriate ratio of sodium acetate and acetic acid used to prepare buffer of specific pH.