YILDIZ TECHNICAL UNIVERSITY

BIOMEDICAL ENGINEERING DEPARTMENT

BME2901-BIOCHEMISTRY COURSE

2020-2021 FALL SEMESTER

EXPERIMENT 1: BASIC BIOCHEMICAL TECHNIQUES

EXPERIMENT DATE:21.10.2020

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STUDENT NAME-SURNAME:

STUDENT NUMBER:

PURPOSE OF THE EXPERIMENT

How can we safely prepare for an experiment? Buffer and pH solutions preparation steps, what should we pay attention to when preparing a solution? Learning answers to these and other questions.

To learn arrangement readiness methods,concentration units,to comprehend ideas of pH and cushion arrangement and to figure out how buffer solutions are prepared.

The main concepts of this experiment to understand solution preparation methods. In addition to comprehend ‘ What is pH and Buffer Solution ‘ and ‘ How we can prepare a Buffer Solution’.

THEORETICAL KNOWLEDGE

Solution is a homogenous mixture of two or more substances . In a solution there can’t be any chemical change. Solubility is the ability of one compound to dissolve in another compound**.[1]** It is expressed in grams of the material dissolved in 100 g of solvent. If there is too much solute in the solution it is named “concentrated solution”, if there is too little solute in the solution it is called “diluted solution”. If the solution contains the maximum solute that it can dissolve , it is called “Saturated Solution” , if the solution contains less solute than its dissolving capacity , it is called “Unsaturated Solution”**[2].**

Dilution means solvent being added to the solution. This process causes the solute concentration to decrease. This changes in concentration can be calculated by using the formula $M1.V1=M2.V2$. 2

Molarity means the number of moles that is contained in per litre of solution. We can formulise it like this : Number of Moles of Solute / Volume of the solution **[3].** It is also called “Molar Concentration”. Molality is the number of moles that is contained in per litre of the solvent. The difference of “Molarity” and “Molality” is that we are calculating the molarity with the volume of the solution and calculating the molality with volume of the solvent. We can formulise molality like this : Number of Moles of Solute / Volume of the Solvent **[4].**

Normality is the gram equivalent weight divided by liters of solution. We can formulise normality like this : Gram Equivalent Weight / Liters of Solution.

In some occasions with very small quantities of matter we have to use special concentration units made for occasions like this. These units are “ppm” and “ppb” . “ppm” means parts per million while “ppb” means parts per billion [**5].**

pH is a way to specify the acidity or basicity of an aqueous solution. Acidic solutions has higher H+ concentrations than basic solutions . pH is defined as the negative log of hydrogen ion concentration so higher H+ concentration means lower pH therefore acids have lower ph values compared to bases. The pH scale starts from 0 and ends at 14. A pH value of 7 means neutral. If the pH value is lower than 7 it's acidic and if it's higher it's basic or alkaline [**6].**

If an acid can dissolve 100% into ions it is called "strong acid" **[7]** .If it can't dissolve 100% , its called "weak acid".When we look at the bases, if the base is 100% ionized in the solution it is called "strong base" ,if it isn't 100% ionized in the solution then it's called "weak base".

A buffer solution is an aqueous solution that is composed of a mixture of a weak acid and its conjugate base. pH value of a buffer solution changes very little when a small amount of strong acid or base is mixed into it. They are used because of their capacity to resist pH changes. They are used as a way to keep the pH close to a constant value in various chemical applications. Buffer capacity, which is indicated by β, is a measurement of the efficiency of a buffer solution's resistance against changes in pH values **[8].**

MATERIALS

Precision balance, Spatula, Distilled water, Weighing bottle, Wash bottle, Graduated cylinder, 100 ml Erlenmeyer flask, 100 ml solution bottle, Magnetic stirrer, Fume hood, KH2PO4, Na2HPO4, NaCl, HCl, KCl, NaOH, pH Meter, Paraffin Wax, Label.

Chemical ingredients: Monosodium Phosphate (NaH2PO4) Disodium Phosphate (Na2HPO4) Sodium Chloride (NaCl) Hydrochloric Acid (HCl) Potassium Chloride (KCl) Sodium Hydroxide (NaOH)

Disposables and Equipments: Precision balance Erlenmeyer flask Distilled Water Graduated Cylinder Magnetic Stirrer Fume Hood Solution Bottle Paraffin Wax

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METHOD

Preparation of 50 ml of KCl (3 M) solution: After calculating the quantity of solute, on a precision balance measure the weight of 11.2 grams KCL, after that moves the KCL to the Erlenmeyer flask. Insert 50 ml of water in a graduated cylinder to measure the amount of it. Using a magnetic stirrer mix the solution until it becomes homogeneous, then move the solution to a solution bottle that is clean and dry.

Preparation of 200 ml of 3M NaOH solution: Similar to what we did earlier after checking the calibration of the precision balance we put the weighing plate on the precision balance and reset the balance so that the reading is zero. After that, we weigh the calculated amount of NaOH (which is 24 g. All hand calculations are shown in the RESULTS part) and transfer it to the Erlenmeyer flask, then we use a graduated cylinder to measure exactly 200 ml of distilled water and add it to the Erlenmeyer flask that contains NaOH. Finally, we use a magnetic stirrer to stir the solution until it is homogeneous and then we transfer it to a clean dry solution bottle, and we label it.

Preparation of 200 ml of 1 M HCl (37%) solution: First, turn the fume hood on, then open the main bottle of HCl, transfer a small amount to a clean and dry solution bottle, and label the solution by writing the name of the component and the date of the experiment. Using paraffin wax cover the cap of the main bottle. Then using a graduated cylinder measure the amount of 0.0166L HCl and add the acid to the measured distilled water in another solution bottle. Again label the prepared HCl acid solution, and mix till the magnetic stirrer gets homogenous.

PH meter calibration: First, turn the PH meter on and remove the filling capsule and the protective, then wash the electrode with deionized water and dry it. After pressing the CAL button, place the electrode in the solution of ph 7.01 buffer. After it reads the CFM on the screen, press the CFM button to save the record. Again Wash the electrode with deionized water and dry it.
Do the same steps with solutions of PH 4.01 buffer and PH 10.01 buffer respectively, and make sure to wash the electrode before measuring PH values. After finishing the calibration replace the protective and filling capsule on the electrode.

Preparation of 250 ml 0.01M pH 7.4 Phosphate Buffered Saline (PBS): To prepare PBS start with weighing 2 grams NaCl, 0.05 grams KCl, 0.36 grams Na2OH4 and 0,061 grams KH2PO4 by a precision balance. Then add 200ml of distilled water to a solution bottle and add the measured amounts of NaCl, KCl, Na2OH4, and KH2PO4 respectively. After mixing them till they become homogeneous add distilled water until volume is 250ml. Use NaOH and HCl to adjust the solution to the desired pH. To measure the PH value of the PBS turn the PH meter on, and remove the filling capsule and tye protective and rinse the electrode with distilled water. Measure the PH value as 7.4.

RESULT

Calculations for 50ml of 3M KCl Solution:

Molecular weight of KCl = 39 (K) + 35,5 (Cl) = 74,5 g

3M = 3n/v(L) = n mole/0.05 L => n = 0.15

0.15 mole KCl = 11.175 g

Calculations for 200ml of 3M NaOH Solution:

Molecular weight of NaOH = 21 (Na) + 16 (O) + 1 (H) = 40 g

3M = 3n/v(L) = n mole/0.2 L => n = 0.6

0.6 mole NaOH = 24 g

Calculations for 200ml of 1M HCl Solution from %37 Stock Solution:

M1.V1=M2.V2

?.?=1M.0.2L

Calculation of M1:

M= (Percentage).d.10/ molecular weight

Molecular weight of HCl = 1 (H) + 35,5 (Cl) = 36,5 g

M1= 37.(1.19).10/36.5=12,0630137

M1.V1=M2.V2

12,0630137.V1=1M.0.2L

V1= 0,165796 L ≈ 16.580 ml

Calculations for 250ml 0.01M pH 7.4 Phosphate Buffered Saline (PBS):

There is a standart protocol (Cold Spring Harbor Protocol) to prepare PBS [5]. So concentrations will be like this.

NaCl -> 8g/L

KCl -> 0.2g/L

Na2HPO4 -> 1.44g/L

KH2PO4 -> 0.245g/L

We are preparing 250ml solution. So;

NaCl -> 8g/4 = 2g

KCl -> 0.2g/4 = 50mg

Na2HPO4 -> 1.44g/4 = 360mg

KH2PO4 -> 0.245g/4 = 61mg

Calculating the amount of KCL:

We know that the molarity should be 3 M and the volume of the solvent (water) is 50 ml, thus:

$$Molarity = \frac{Amount\ of\ solute\ in\ moles}{Volume\ of\ solvent\ in\ Litres} \rightarrow 3\ M = \frac{x\ mol\ KCL}{50\ ml\ water}$$

Also, the molecular mass of KCL is 74.5513 g/mol.

The mass of KCL:

$$m_{kcl} = \frac{3\ \cancel{mol\ KCL}}{1\ \cancel{L\ water}} \times \frac{74.5513\ \boldsymbol{g\ KCL}}{1\ \cancel{mol\ KCL}} \times \frac{1\ \cancel{L\ water}}{1000\ \cancel{ml\ Water}} \times 50\ \cancel{ml\ Water}\ = 11.183\ \mathrm{g\ KCL}$$

Finally, the amount of KCL we need to weigh is 11.183 g.

Calculating the amount of NaOH:

We know that the molarity should be 3 M and the volume of the solvent (water) is 200 ml, thus:

$$Molarity = \frac{Amount\ of\ solute\ in\ moles}{Volume\ of\ solvent\ in\ Litres} \rightarrow 3\ M = \frac{x\ mol\ NaOH}{200\ ml\ water}$$

Also, the molecular mass of KCL is 40 g/mol.

The mass of NaOH:

$$m_{NaOH} = \frac{3\ \cancel{mol\ NaOH}}{1\ \cancel{L\ water}} \times \frac{40\ \boldsymbol{g\ NaOH}}{1\ \cancel{mol\ NaOH}} \times \frac{1\ \cancel{L\ water}}{1000\ \cancel{ml\ Water}} \times 200\ \cancel{ml\ Water}\ = 24\ \mathrm{g\ NaOH}$$

Finally, the amount of NaOH that we should weigh is 24 g.

Calculating the amount of HCl (37%) solution:

M_i: molarity of initial material

M_f: molarity of final material

V_i: volume of initial material

V_f: volume of final material

$$M_i \times V_i \;=\; M_f \times V_f$$

$$M_i = ? \quad , \quad M_f = 3M \quad , \quad V_i = ? \quad , \quad V_f = 0.2\ L$$

Molar mass of HCL: 36.5 g/mol.

Density of HCL: 1.19 g/ml.

Our stock solution consists 37% HCL by volume so the molarity of the stock solution:

$$M_i = \frac{370\ \text{ml}\cancel{\text{H}}\text{CL}}{1000\ \boldsymbol{ml\ Solution}} \times \frac{1.19\ \text{g}\cancel{\text{H}}\cancel{\text{C}}\text{L}}{1\ \text{ml}\cancel{\text{H}}\text{CL}} \times \frac{1\ \boldsymbol{mol\ HCL}}{36.5\ \text{g}\cancel{\text{H}}\cancel{\text{C}}\text{L}} = 12.06\ mol\ HCL/L \sim 12\ M$$

Calculating the amounts of NaCL, KCL, Na_2HPO_4 and KH_2PO_4:

Fortunately, we do not have to calculate the amounts of salts, because -as many researchers do- we can check standard procedures for the required amounts, we can give the desired volume and an algorithm would return the required amounts of salt for 0.01M pH 7.4 Phosphate Buffered Saline (PBS)[iii]

To prepare 0.25 L of **PBS (Phosphate Buffered Saline) (1X, pH 7.4)**:

Change the value in the textbox above to scale the recipe volume

Table 1. Required components

Component	**Amount**	**Concentration**
NaCl (mw: 58.4 g/mol)	2 g	0.137 M
KCl (mw: 74.551 g/mol)	50 mg	0.0027 M
Na_2HPO_4 (mw: 141.96 g/mol)	360 mg	0.01 M
KH_2PO_4 (mw: 136.086 g/mol)	61 mg	0.0018 M

As we can see above, the required amount for NaCL, KCL, Na_2HPO_4 and KH_2PO_4 are: 2 g, 0.05 g, 0.360 g, and 0.061 g, respectively.

DISCUSSION

Initially, we calculate amount of KCl and prepare 3M KCl solution. We added water to the base while preparing it, but always dilute base solutions by adding base to water and not water to base. Adding water to concentrated bases may cause violent boiling of the solution and splashing.so it would be more correct to add base to the water [9]. Then we prepare hydrochloric acid through fume hood because it is dangerous and strong acid.It is better to do so because the steam from the acid can harm the environment.Then we should label the bottles as we do in the experiment. we may be confused by being unable to distinguish between materials that are very similar in appearance and may lead to dangerous consequences.And also, we cover the cap of bottle with paraffin wax as well against any risk of contamination and breathing. After that, when calibrating the pH meter, we must clean the electrode thoroughly before inserting it into each tube as we do in video. It will also be an advantage for us to know how to use the pH meter and what the keys like CFM CALL do.by using pH meter we calculate ph of 3 calibration solutions which have respectively 7.01,4 and 10 ph. While preparing the PBS solution, the ideal amounts for 1 liter are 8g NaCl 200mg KCl 1.44 g Na_2HPO_4 and 245 mg KH_2PO_4[6], but since we will prepare a 250 ml solution, we took one fourth of the entire material. As a result, the pH was found to be 7.4, and if we wanted to increase it we could use more base solution(NaOH) and conversely, if we wanted to reduce it we could increase the amount of hydrogen chloride and we used sodium hydroxide and hydrogen chloride which is strong acid to regulate the pH of the buffer. NaOH and HCl are chemicals that are frequently used in buffer solutions because they have a simple reaction with each other. Adjust pH of buffer with NaOH and HCl because generally HCl use the adjustment of pH and NaOH basic adjustment. Because these are react as a simple salt

$NaOH + HCl \rightarrow NaCl + H_2O$

ANSWER OF HOMEWORK QUESTION

1.

2.

This part will be evalauted if any question exist in the lab manual.

REFERENCES

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