ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK

Buffers, buffer capacity. Oxidoreduction, electrode processes

Practical lesson on medical biochemistry

General Medicine

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Task 1: Calculation of pH of buffers using Henderson-Hasselbalch equation and determination of buffer capacity

1. Test tubes, pipettes, beakers
2. pH meter
3. Solution of NaH_2PO_4 0.1 mol·l^{-1}
4. Solution of Na_2HPO_4 0.1 mol·l^{-1}
5. Solution of NaCl 0.1 mol·l^{-1}
6. Solution of HC1 0.1 mol·l^{-1} 
7. Solution of NaOH 0.1 mol·l^{-1} 

Procedure

1. Use the plastic containers pre-marked 1-4 and glass pipettes to measure the appropriate volumes of all solutions according to table. Mix.

	Container No.			
	1	2	3	4
NaH_2PO_4 c = 0.1 mol·l^{-1}	5 ml	1 ml	9 ml	-
Na_2HPO_4 c = 0.1 mol·l^{-1}	5 ml	9 ml	1 ml	-
NaCl c = 0.1 mol·l^{-1}	-	-	-	10 ml

2. Predict or calculate the theoretical pH values using Henderson-Hasselbach equation.

3. Measure pH of each mixture with pH meter equipped with a glass combined electrode. ***Do not forget to rinse the electrode with distilled water and wipe gently with piece of tissue in between measurements. Also remember that the electrode tip must never get dry!***

4. Compare the predicted pH values with those obtained experimentally.

5. Next, some student groups will add acid (containers marked A), whereas others will add base (containers marked B) instead. You are supposed to share results from this step.

6. Into each mixture prepared according to the table above add either 1 ml of hydrochloric acid (c = 0.1 mol·l^{-1}) – mark the containers 1A-4A; or 1 ml of sodium hydroxide (c = 0.1 mol·l^{-1}) – mark the containers 1B-4B.

7. Measure the pH of all solutions and also calculate the theoretical values using (if possible) Henderson-Hasselbalch equation. Record and compare all results.

Note: *For dissociation of phosphoric acid the following dissociation constants were found:*
To the first degree 6.91×10^{-3}
To the second degree 6.2×10^{-8}
To the third degree 2.13×10^{-13}

Task 2: Relationship of buffer capacity on the molar concentration of buffer

1. Into vessel marked as 'stock buffer' prepare 10 ml of phosphate buffer 1:1, $c = 0.1\ mol \cdot l^{-1}$. Make it in the same way as in Task 1, container No. 1.
2. Into container marked with No. 5 prepare 10 ml of phosphate buffer 1:1, $c = 0.04\ mol \cdot l^{-1}$ in the following way: measure 4 ml of the 'stock buffer' from the previous step, and add 6 ml of distilled water.
3. Measure pH of both mixtures and record the values.
4. Add to both mixtures either 1 ml HCl, $c = 0.1\ mol \cdot l^{-1}$ (mark as 5A), or 1 ml of NaOH, $c = 0.1\ mol \cdot l^{-1}$ (5B). Again choose whether to add acid or base, and share your results with other students. Measure pH after the additions and record the values.
5. Compare the results of 5A with 1A from the previous task, or 5B with 1B, respectively. Explain the differences.

Task 3: Effect of ionic strength on pH of buffer

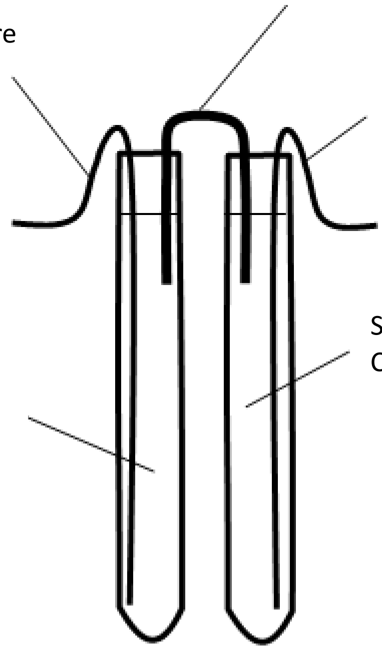
1. Into container marked with No. 6 prepare phosphate buffer 1:1, $c = 0.01\ mol \cdot l^{-1}$ in the following way: measure 1 ml of the 'stock buffer' from Task 2, and add 9 ml of distilled water.
2. Measure pH and record the value.
3. Into container No. 7 prepare phosphate buffer 1:1, $c = 0.001\ mol \cdot l^{-1}$ as follows: measure 1 ml from the container No. 6, and add 9 ml of distilled water.
4. Measure pH and record the value.
5. Compare the results with those obtained for the phosphate buffer 1:1, $c = 0.1\ mol \cdot l^{-1}$ (container No. 1, Task 1), and $c = 0.04\ mol \cdot l^{-1}$ (container No. 5, Task 2).

Task 4: Electrochemical cell

1. Analog voltmeter HD-075 1V
2. Copper wire and zinc-coated steel wire (each about 15 cm long)
3. Aqueous solution of $CuSO_4$ 1 $mol \cdot l^{-1}$
4. Aqueous solution of $ZnSO_4$ 1 $mol \cdot l^{-1}$
5. Saturated solution of NaCl
6. Cables with connectors and crocodile clips, absorbent paper
7. Emery paper

Procedure

1. Assemble Daniell's electrochemical cell:

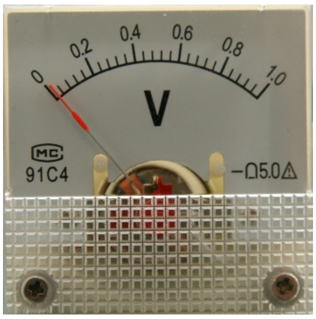


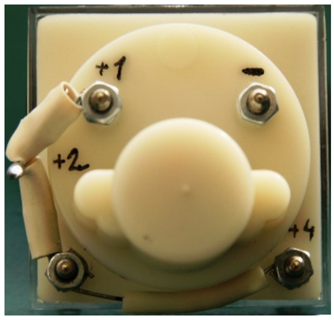
   a. Into adjacent positions of a tube rack place two long glass test tubes, one almost filled with solution of copper sulfate (1 mol/l), the other one with solution of zinc sulfate (1 mol/l).

   b. Connect the electrolytes in both test tubes with a salt bridge: take a firm and well absorbing paper (such as a piece of paper towel), roll it into a rod and wet it thoroughly with saturated solution of sodium chloride. Arrange the paper roll so that one its end reaches into the copper sulfate solution and the other end into the zinc sulfate solution.

   c. Put a copper electrode (copper wire) into the tube with copper sulfate, and a zinc electrode (zinc-coated steel wire) into the tube with zinc sulfate. The wires must not be in contact with salt bridge. The electrodes must be perfectly clean; they can be cleaned with emery paper if necessary (but take care not to damage the zinc layer of the zinc electrode).

2. Use the cables with crocodile clips to attach the voltmeter. Connect the copper electrode to the position "+1" , the zinc electrode to position "–".

A


B


The front (A) and rear (B) view on the voltmeter HD-075 1 V. The connectors on the rear side are designed for attachment of crocodile clips and offer several ranges of measurement. The one marked as "–" is for the negative electrode, whereas the other contacts are for the positive electrode, range "+1 V", "+2 V", "+3 V" and "+4 V").

3. Read the voltage of the assembled electrochemical cell. Calculate what would be the voltage, if the cell worked with 100% efficiency.
4. Remove both electrodes from the solutions, wash with distilled water and dry carefully. Then immerse them to the electrolytes in the opposite way, i.e. the copper wire is placed in the solution of zinc sulfate and the zinc electrode in the solution of copper sulfate. Measure the voltage of this assembly. Observe whether the electrodes undergo any changes in this arrangement.

Note: Standard redox potentials:
Cu^{2+} / Cu^{0} +0.34 V Zn^{2+} / Zn^{0} –0.76 V

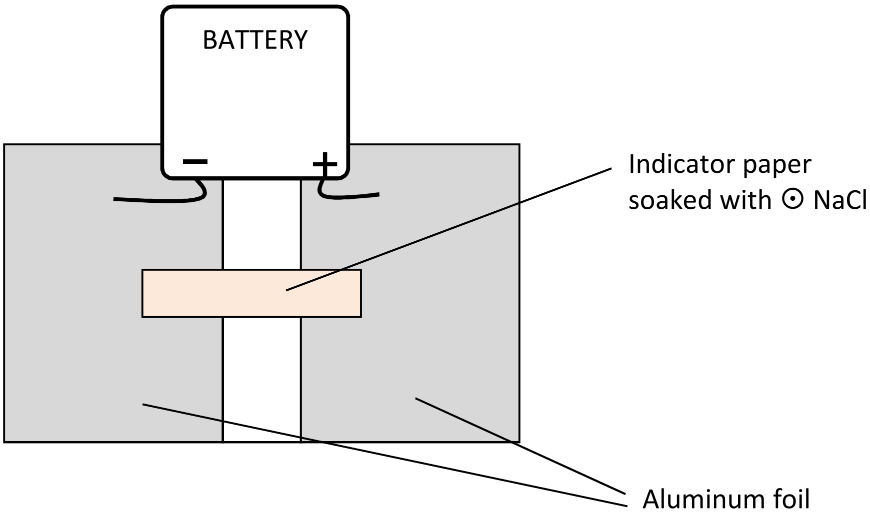
Task 5: Electrolysis

1. Glass plate, aluminum foil
2. Indicator paper
3. Saturated solution of sodium chloride
4. Battery 9 V

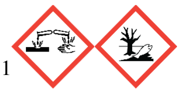
Procedure

Wrap the glass plate with two strips of aluminum foil so that two metallic surfaces are produced with a gap of about 1 cm in between them. Across the gap place about 3 cm long strip of universal indicator paper and wet it with saturated solution of sodium chloride.

When the sodium chloride solution has soaked into the indicator paper, remove any eventual excess of the electrolyte with a piece of tissue. Then connect the plate to a 9 V battery so that one metallic surface is in contact with the positive pole whereas the other one with the negative pole. Observe the color changes of the indicator paper.



Task 6: Electrochemical series of metals

1. Copper wire (about 5 cm)
2. Zinc-coated steel wire (about 5 cm)
3. Silver wire (about 5 cm)
4. Aqueous solution of $CuSO_4$ 1 mol·l^{-1} 
5. Aqueous solution of $ZnSO_4$ 1 mol·l^{-1} 
6. Aqueous solution of $AgNO_3$ 2 mol·l^{-1} 
7. Petri dishes

Procedure

In this experiment you are supposed to observe what happens to the surface of zinc-coated, copper or silver wires after immersion into solutions of various metal ions (Cu^{2+}, Zn^{2+} and Ag^{+}).

Use about 5 cm long piece of wire. Always clean before use: it is best done by immersion to HCl solution from your basic set of chemicals. Then immerse one end of the wire to the selected solution in Petri dish for about 30 s. Stir the solution with the wire during the whole period. Observe whether any visible changes occur on the immersed end of the wire. Try successively all combinations of the wires and metal ion solutions.