ÚSTAV LÉKAŘSKÉ BIOCHEMIE A LABORATORNÍ DIAGNOSTIKY 1. LF UK

Buffers, buffer capacity. Electrode processes

Practical lesson on medical biochemistry
General Medicine

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2024/25

Contents

Task 1: Construction of titration curves

Equipment requirements

1. Solution $c(CH_3COOH)$ = 0.1 mol/l
2. Solution $c(NaOH)$ = 0.1 mol/l

Procedure

1. Pipette 20.0 ml of weak acid into a pure plastic container and measure the pH using a pH-meter.

2. While mixing, add step by step the required amount of sodium hydroxide as directed in the table below. Measure the pH of the solution after each addition of sodium hydroxide and good mixing.

3. Record the obtained values in the table on the report sheet.

Note: *Do not wash the pH electrode between the measurements but avoid leaving the electrode outside the solution for a long time –* ***remember that the electrode must not get dry!***

	20 ml **CH_3COOH** c = 0.1 mol/l		
	NaOH c = 0.1 mol/l		pH
	Addition in ml	Σ ml	
1	0	0	
2	1	1	
3	2	3	
4	3	6	
5	4	10	
6	5	15	
7	3	18	
8	1	19	
9	1	20	
10	1	21	
11	1	22	
12	3	25	
13	5	30	
14	10	40	

Note: *In the graph try to indicate also how the course of titration curve would look like for the strong acid, such as HCl.*

Objectives

1. Write the ionic reaction, which is the essence of the determination of titration.
2. Mark the obtained values on a graph: on the x-axis, plot the quantity of added hydroxide in ml, and on the y-axis, plot the measured values of pH.
3. Calculate a theoretical pH value for $c(CH_3COOH) = 0.1$ mol/l ($K_a = 1.75 \times 10^{-5}$).
4. Find and indicate the pK_a of acetic acid in your graph and compare this value with the one provided above.
5. Determine the pH of the equivalence point for both acids.

Task 2: Calculation of pH of buffers using Henderson-Hasselbalch equation and determination of buffer capacity

Equipment requirements

1. pH meter
2. Solution $c(HCl) = 0.1$ mol/l 
3. Solution $c(Na_2HPO_4) = 0.1$ mol/l
4. Solution $c(NaCl) = 0.1$ mol/l
5. Solution $c(NaH_2PO_4) = 0.1$ mol/l
6. Solution $c(NaOH) = 0.1$ mol/l 
7. Test tubes, pipettes, beakers

Procedure

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

	Container No.			
	1	**2**	**3**	**4**
NaH_2PO_4 c = 0.1 mol/l	5 ml	1 ml	9 ml	-
Na_2HPO_4 c = 0.1 mol/l	5 ml	9 ml	1 ml	-
NaCl c = 0.1 mol/l	-	-	-	10 ml

2. Predict or calculate the theoretical pH values using the Henderson-Hasselbach equation.
3. Measure the pH of each mixture with a pH meter equipped with a glass combined electrode.
   Note: *Do not forget to rinse the electrode with distilled water and wipe it gently with a piece of tissue 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 groups of students will add acid (containers marked A), while others will add base (containers marked B) instead. You are supposed to share the results from this step.
6. To each mixture prepared according to the table above, add either 1 ml of hydrochloric acid (c = 0.1 mol/l) – mark the containers 1A-4A; or 1 ml of sodium hydroxide (c = 0.1 mol/l) – mark the containers 1B-4B.
7. Measure the pH of all solutions and calculate the theoretical values using (if possible) the 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 3: Relationship of buffer capacity on the molar concentration of buffer

1. In the vessel marked as 'stock buffer' prepare 10 ml of phosphate buffer 1:1, c = 0.1 mol/l. Make it the same way as in Task 1, container No. 1.
2. In the container marked with No. 5 prepare 10 ml of phosphate buffer 1:1, c = 0.04 mol/l in the following way: measure 4 ml of the 'stock buffer' from the previous step and add 6 ml of distilled water.
3. Measure the pH of both mixtures and record the values.
4. To both mixtures, add 1 ml of HCl, c = 0.1 mol/l (mark as 5A), or 1 ml of NaOH, c = 0.1 mol/l (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 of 5B with 1B, respectively. Explain the differences.

Task 4: Electrochemical cell

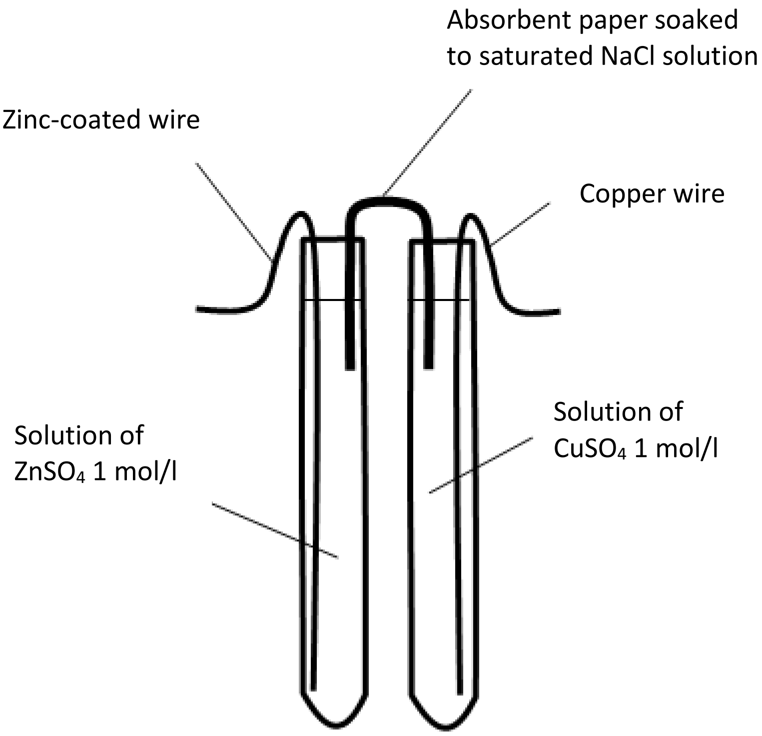
Equipment requirements

1. Analog voltmeter HD-075 1 V
2. Copper wire and zinc-coated steel wire (each about 15 cm long)
3. Solution $c(CuSO_4)$ = 1 mol/l 
4. Solution $c(ZnSO_4)$ = 1 mol/l 
5. Saturated solution of NaCl

6. Cables with connectors and crocodile clips, absorbent paper
7. Emery paper

Procedure

1. Assemble Daniell's electrochemical cell:



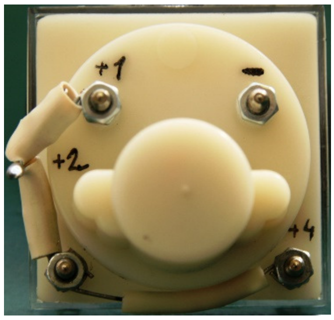
   a. Place two long glass test tubes in adjacent positions of a tube rack, one almost filled with a solution of copper sulfate (1 mol/l), and the other with a solution of zinc sulfate (1 mol/l).

   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 end reaches the copper sulfate solution and the other end 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 the salt bridge. The electrodes must be perfectly clean; they can be cleaned with emery paper if necessary (but care should be taken 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 the position "–".

A 

B 

Front (A) and rear (B) view on the HD-075 1 V voltmeter . The rear side connectors are designed for the 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 the voltage would be if the cell worked with 100% efficiency.
4. Remove both electrodes from the solutions, wash them with distilled water and dry them carefully. Immerse them in 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

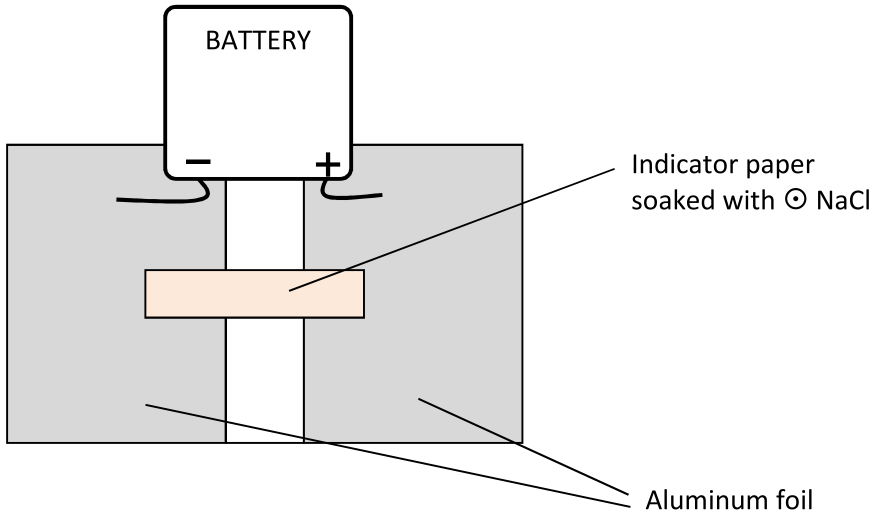
Equipment requirements

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

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 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 on the indicator paper.



Task 6: Electrochemical series of metals

Equipment requirements

1. Solution $c(AgNO_3) = 2$ mol/l
2. Solution $c(CuSO_4) = 1$ mol/l
3. Solution $c(ZnSO_4) = 1$ mol/l
4. Copper wire (about 5 cm)
5. Petri dishes
6. Silver wire (about 5 cm)
7. Zinc-coated steel wire (about 5 cm)

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 a 5 cm long piece of wire. Always clean before use: this is best done by immersion in HCl solution from your basic set of chemicals. Then, immerse one end of the wire in the selected solution in a Petri dish for about 30 s. Stir the solution with the wire for 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.