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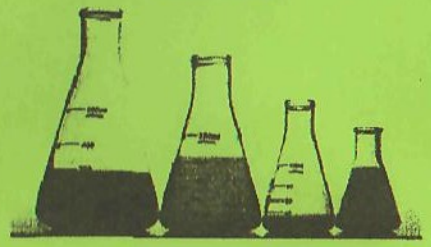
"KHANGALASSKY ULUS (DISTRICT)" MUNICIPAL DISTRICT AUTHORITY

XXIV МЕЖДУНАРОДНАЯ ОЛИМПИАДА ШКОЛЬНИКОВ «ТУЙМААДА»

XXIV INTERNATIONAL SCHOOL OLYMPIAD «TUYMAADA»

ХИМИЯ

CHEMISTRY



II (экспериментальный) этап

Second (experimental) round

Старшаялига

Senior league

Yakutsk, 2017

Safety information

Please, observe the following rules when working in a chemical laboratory:

- You must plan your work. Any experiments, which is not given in a problem, are prohibited.
- Keep work area neat and free of any unnecessary objects.
- Wear a full-length, long-sleeved laboratory coat or chemical-resistant apron, secure long hair.
- Do not eat and drink in a laboratory!
- Never touch, taste, or smell any reagents.
- Wash hands before and after work.
- All experiments with smelling and toxic substances must be carried out in a fume cupboard.
- Always use a spatula or scoopula to remove a solid reagent from a container. Donotdirectlytouchanychemicalwithyourhands.
- Weigh out or remove only the amount of chemical you will need. Do not return the excess to its original container, but properly dispose of it in the appropriate waste container.
- Use a holder at heating of solutions or solid substances.
- Never point the open end of a test tube containing a substance at yourself or others.
- Never lean over dish where liquid is boiling or is heating.
- When it is necessary to smell chemicals in the lab, the proper technique is to cup your hand above the container and waft the air toward your face.
- If it is necessary, determine smell only in air flow from open dish.
- Add concentrated acid to water slowly. Never add water to a concentrated acid.
- Place chemical waste in appropriately labeled waste containers.
- With all the emerging issues immediately contact with the members of the jury.
- Immediately report any spills, accidents, or injuries to a teacher.

Reagents and equipment

Reagents

Reagent	Volume, ml
2% solution of $FeCl_3$ in 0.5 M HCl	25
2% solution of ninhydrin in acetone	25
Isopropanol : water : 25% ammonia (20:2:1)	10
n-butanol : water : 25% ammonia (5:1:1)	10
Acetone : water (3:2)	10
Diluted milk	10
Folin-Ciocalteu reagent	10
Feling reagent	50

Laboratory glassware and equipment

Glassware/Equipment	Amount
Beaker or flask	6
1-2 ml graduated pipette	1
5 mlgraduatedpipette	2
Cuvette for photometry	1
Beaker with height not less than 12 cm	2
Petri dish	1
Filter paper strip	1
Paper clips (for applying drops)	3
Test tubes	3
Graduated test tubes	6
Drying cabinet	1 for room
Photocolorimeter	1 for 4 persons

XXIV International School Olympiad "Tuymaada-2017"
Experimental round

Introduction

Milk is a widely distributed food product that can be consumed both directly and as part of other products.

Most important component which determines high worth of milk is protein that is generally represented by casein possessed all eight irreplaceable amino acids.It is casein that causes the fullness of taste of milk and dairy products. Due to high content of protein milk is valued for its good enterosorbing properties. Milk especially effectively binds phenols and cations of heavy metals. It is why milk is recommended for people have contact with harmful chemicals.

In this work, you are invited to:

- identify amino acids and phenols in mixture by ascending paper chromatography (Problem 1);
- quantitatively evaluate of protein content in a milk sample.

Choose order of execution by yourself.

Problem 1

DETERMINATION OF AMINO ACIDS AND PHENOLS MIXTURES BY CHROMATOGRAPHY

Mixture of four different organic compounds from the next list is given: resorcinol, glycine, alanine, α-naphthol, 4-nitrophenol and cysteine.

Tasks:

1. Write structural formulas of resorcinol, glycine, alanine, α-naphthol, 4-nitrophenol and cysteine.
2. Conduct separation of given mixture by ascending paper chromatography and identify individual components of the mixture.

Individual detecting and separation are conducted for each organic substance class in accordance with items 1.1 – 1.3. In order to save time, we recommend conducting experiments in **parallel**.

1.1. Separation and determination of α-naphthol and 4-nitrophenol:

a) Take a filter paper strip with dimensions 2x12 cm. Make sure that there is the start and finish lines marked with pencil at a distance of 1 cm from one edge and at a distance of 0.5 cm from the other edge.

b) Transfer filter paper on a Petri dish. Gently apply a drop of the mixture to be analyzed on the start line with the tip of the paper clip. The drop must be spread out in the form of a circle with a diameter of not more than 4-5 mm. Cut the left and right corners of the bottom edge for uniform lifting of liquid on the filter paper.

c) Carefully, without soaking the walls of the dish, pour out the system of isopropyl spirit, water and 25% solution of ammonia in a volume ratio of 20: 2: 1 into a graduated beaker so that the thickness of the solvent layer does not exceed 1 cm.

d) Lower filter paper strip with the drop of analyzed mixture vertically into a beaker and close it by a Petri dish. *Ensure that the eluent is lifted evenly and strictly vertically through the filter paper!The spot of analyzed mixture must not be touch the solvent!*

e) Duration of chromatography is 30 – 40 minutes. Process should be stopped after solvent reaches finish line. Then gently take out paper from beaker and dry it on air. Spots of α-naphthol and 4-nitrophenol become yellow on air because they are partially oxidized. Circle the resulting spots with a pencil and measure their height - the distance between the center of the spot and the start line. Determine retardance factor R_f of spots according to the formula:

Problem 2

QUANTITATIVE DETERMINATION OF PROTEIN IN MILK BY PHOTOMETRIC METHOD OF LOWRY

2.1. Preparation of calibration solutions

Transfer 0.08% casein solution and distilled water in 4 numbered graduated test tubes with graduated pipette in accordance with the table:

Number of test tube	1	2	3	4
Volume of casein solution	1.25	2.50	5.00	10.00‘
Volume of distilled water	8.75	7.50	5.00	0

Plug test tubes and thoroughly mix solutions. Transfer 1.00 ml from each tubes to beakers or flasks using graduated pipette previously numbered them from 1 to 4.

2.2. Preparation of comparison solution

Mark one of the beakers or flasks as "0" and transfer there 1.00 ml of distilled water to prepare comparison solution.

2.3. Preparation of analyzed solution

Mark beaker or flask as "M" and transfer there 1.00 ml of diluted milk using graduated pipette.

2.4. Photometry

Execute the next operations:

a) Add into each beaker or flask "0" – "4" and "M" 8 ml of Feling reagent using graduated pipette, mix and leave for 10 min.

b) Add to each beaker or flask "0" – "4" and "M" 0.8 ml of Folin-Ciocalteu reagent, mix and leave them for 30 min.

c) Transfer solution "0" to a cuvette for photometry. The cuvette should be held at the side edges to avoid contamination from the fingers and damage to the transparent walls.

d) Open the cover of the cuvette compartment of the photometer and set the cuvette so that the transparent walls are positioned vertically with respect to the inscriptions on the photometer body. Close cover of the cuvette compartment.

e) After making sure that the photometer is tuned to a wavelength of 635 nm, press the "CAL" button. The value 0.000 should be displayed on the screen after pressing. Then open the cover

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$$R_f = \frac{l}{L}$$

where l - distance between the center of the spot and the start line; L – distance between start and finish lines.

f) Compare experimental values of R_f and reference values, make conclusion about presence of α-naphthol and 4-nitrophenol in the given mixture.

g) Evaluate R_f values for each spot and compare them with retardance factors given in the table:

R_f	Substance
0.87	4-nitrophenol
0.98	α-naphthol

1.2. Determination of resorcinol

Repeat items a) – e) from the previous experiment using system made of n-butanol, water, ammonia in a volume ratio of 5:1:1. After carrying out chromatography, put filter paper strip in a Petri dish and then in a drying cabinet for 4 – 5 minutes to remove all solvent. Apply evenly 2% solution of $FeCl_3$ in 0.5% solution of HCl using piece of cotton and place in a drying cabinet again until completely dry. If resorcinol is in the analyzed mixture, violet spot will appear on the chromatogram with $R_f = 0.82$.

1.3. Separation and determination of amino acids

Repeat items a) – e) of 1.1 in the system made of acetone and water in the ratio 3:2. After carrying out chromatography, put filter paper strip in a Petri dish and then in a drying cabinet for 4 – 5 minutes to remove all solvent. Apply evenly 2% solution of ninhydrin in acetone using piece of cotton on dried strip and place it again in a drying cabinet on a Petri dish until completely dry. Evaluate R_f for each spot and compare with reference data given in the table below:

R_f	Substance
0.72	Glycine
0.84	α-alanine
0.98	Cysteine

of the cuvette compartment and completely pour into the waste container the reference solution from the cuvette.

f) Fill the cuvette with "1" solution a third, carefully rinse the inner walls of the cuvette and pour the solution into the waste container. Fill the cuvette with the remaining "1" solution again, place the cuvette in the cuvette compartment and close the cover of the cuvette compartment.

g) Record the value of optical density that the device indicates. After removing the cuvette from the cuvette compartment, pour the test solution into a waste container.

Repeat operation f) – g) on solutions "1" – "4" and "M".

Wash the cuvette thoroughly with water after finishing work.

Tasks:

1. From the experimental data, draw a calibration line in the coordinates "concentration - optical density".

2. Using the calibration line, determine the concentration of protein in the analyzed milk in% if it is known that "diluted milk" was obtained by diluting the milk 100 times.

3. Feling reagent is prepared by dissolving of $CuSO_4$ in diluted solution containing sodium tartrate and NaOH. Please, explain processes which lead to change of color. If possible, support explanations with reaction equations.

4. What other components, besides protein, can influence on experiment result?