**ФГБОУ ВО НОВОСИБИРСКИЙ ГАУ**
**БИОЛОГО-ТЕХНИЧЕСКИЙ ФАКУЛЬТЕТ**

# Essential of biochemistry

**Методические разработки по выполнению лабораторных работ**

**Новосибирск 2017**

**УДК 557.1(075)**
**ББК 28.072, я73**

**Кафедра ветеринарной генетики и биотехнологии**

**Составитель: Короткевич О.С., д.б.н., профессор, Коновалова Т.В., ст. преподаватель**
**Рецензент: Бокова Т.И., д.б.н., профессор, зав. кафедры химии НГАУ**

**Essential of biochemistry: методические разработки по выполнению лабораторных работ /** сост. Короткевич О.С., Коновалова Т.В.: Новосиб. гос. аграр. ун-т. Биолого-технологический факультет.- Новосибирск, 2017. - 21 с

Методические разработки по выполнению лабораторных работ предназначены для бакалавров, магистров и аспирантов очной и заочной форм обучения Биолого-технологического факультета, обучающихся по направлению подготовки 19.03.04. Технология продукции и организация общественного питания, 19.03.02. Продукты питания растительного происхождения, 36.03.02. Зоотехния, 06.03.01 Биология.

Представлены лабораторные работы по основным разделам курса «Essential of biochemistry», вопросы для контроля и библиографический список.

Методические разработки по выполнению лабораторных работ утверждены и рекомендованы к изданию на заседании учебно-методического совета БТФ (протокол № 2 от 1.03.2017)

© Новосибирский государственный аграрный университет, 2017

**СОДЕРЖАНИЕ**

1.THE PROPERTIES OF BUFFER SOLUTIONS ..... 4
1.1.WORK № 1. BUFFER ACTION OF A SOLUTION..... 4
1.2. WORK № 2. THE INFLUENCE OF DISSOLVING ON THE pH OF BUFFER SOLUTION AND BUFFER CAPACITY..... 4
1.3.WORK № 3. BUFFER CAPACITY OF BIOLOGICAL LIQUIDS..... 5
2. THE METHODS OF THE RECEIPT COLLOIDAL SOLUTIONS …..... 6
2.1. WORK № 4. THE OBTAINED OF ROSIN AND PHENOPHTALEINE COLLOIDAL SOLUTIONS..... 6
2.2. WORK № 5. THE OBTAINED OF HYDROXIDE FERRUM SOL..... 6
2.3. WORK № 6. THE OBTAINED OF SULPHUR SOL.....7
2.4. WORK № 7. THE OBTAINED OF PRUSSIAN BLUE SOL.....7
2.5. WORK № 8. THE DETERMINE OF COLLOID CHARGE.....8
3. PROTEINS..... 9
3.1.WORK № 9. Precipitation of proteins with the help of ammonium sulfate..... 9
3.2. WORK №10. Biuret test..... 9
3.3.WORK № 11. Precipitation of proteins with the help of sodium chloride and magnesium sulfate..... 9
3.4.WORK № 12. Precipitation of proteins with the help of heavy metals ions.....10
4. QUALITATIVE REACTIONS ON THE PROTEINS.....11
4.1.WORK № 13. Ninhydrin reaction.....11
4.2.WORK № 14. Xanthoproteic reaction.....11
4.3.WORK № 15. Nitroprusside test.....11
4.4.WORK №16. Millon’s test.....12
4.5. WORK № 17. Hopkins-Cole test.....12
5. MEAT PROTEINS.....13
5.1.WORK №18. Isolation of albumin fraction..... 13
5.2.WORK №19. Isolation of globulin fraction.....13
5.3.WORK №20. Isolation of skleroproteins.....14
6.ENZYMES.....14
6.1.WORK № 21. Thermolabile of ferments.....14
6.2.WORK № 22. The influence of activators and inhibitors.....15
6.3.WORK № 23. Specific action of yeast enzyme.....15
6.4.WORK №24. The influence of pH on the enzymes activity.....16
7.VITAMINS (Vitamin C).....18
7.1. Work №25. Qualitative reaction for vitamin C.....18
7.2.Work №26. Quantitative determination of vitamin C.....19

1.THE PROPERTIES OF BUFFER SOLUTIONS

## 1.1. WORK № 1. BUFFER ACTION OF A SOLUTION.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid.

**PRACTICE COURSE.** In a conical flask or a cylinder 4 ml of 0.1 n solution of acetic acid and 16 ml of 0,1 n solution of sodium acetate are measured and then mixed. Four test-tubes are labeled by numbers. 5 ml of the prepared buffer mixture are poured into test-tubes 1 and 3. Each of test-tubes number 2 and 4 is filled by 5 ml of distilled water. Then 1-2 drops of phenolphthalein are added into test-tubes number 1 and 2 and their content is titrated from burette by alkali, counting the number of drops until the solution attains pink color. 1-2 drops of congo red are added into test-tubes number 3 and 4 and titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue.

Can you explain why it is necessary to add more alkali into test-tube 1 than into test-tube 2? Why do we add less acid into the 4th test-tube than into the 3d test-tube? Write this.

## 1.2. WORK № 2. THE INFLUENCE OF DISSOLVING ON THE pH OF BUFFER SOLUTION AND BUFFER CAPACITY.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solution of phenolphthalein ($C_{20}H_{14}O_2$); universal indicator; 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid.

**PRACTICE COURSE.** Take three test-tubes. Each of them is filled by 2 ml of 0.1 n solution of acetic acid and 2 ml of 0,1 n solution of sodium acetate. The content the first test-tube remains undiluted. The content of the second test-tube is watered down two times; equal volume of water (4 ml) is added to the buffer mixture. The content of the third test-tube is diluted 4 times; 12 ml of water are added. All the solutions from each of the test-tubes are mixed and used in the experiment.

1. Three test-tubes are labeled by numbers and 2 ml of buffer solutions are put into the 3 labeled test-tubes in the following way: the first test-tube has undiluted; the second test-tube solution is watered 2 times and in the third test-tube solution is diluted 4 times. Then three drops of universal indicator are added into each test-tube and the reaction is examined by the coloring degree of the solutions. Does pH change after watering of a buffer solution? If not, then why?

2. Three test-tubes are labeled by numbers and 2 ml of buffer solutions are put into the 3 labeled test-tubes in the following way: the first test-tube has undiluted; the second test-tube solution is watered 2 times and in the third test-tube solution is diluted 4 times. 2-3 drops of phenolphthalein are added into each test-tube and the content is titrated from burettes with alkali, counting the drops until the solution attains pink color.

**1.3.WORK № 3. BUFFER CAPACITY OF BIOLOGICAL LIQUIDS**.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); universal indicator 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid; water; milk; blood serum; saliva; urine.

**PRACTICE COURSE.** 1. Approximately pH of different examined liquids is determined in the glasses the help of universal indicator. It is done to make sure that they have practically neutral reaction, i.e. their pH is about 7.

5 ml of investigated liquids are poured into separate test-tubes, then 2-3 drops of phenolphthalein are added to each of them and their content is titrated from burettes with alkali, counting the drops until the solution attains pink color. Will you put down the results in notebooks.

After that again 5 ml of investigative liquids are poured into separate test-tubes, then 2-3 drops of congo red are put down into each of them and the obtained content is titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue. Results are written down in notebooks.

Which of the investigated liquids has the greatest buffer capacity? Regarding which substances (acid or alkali) is the buffer capacity more expressed in biological liquids? Which buffer systems are in the blood serum?

**General scheme of the experiment (1)**

| Reagents | Water | Milk | Blood serum | Saliva |
|---|---|---|---|---|
| pH | | | | |
| Quantity | 5 ml+ | 5 ml+ | 5 ml+ | 5 ml+ |
| Congo red | 2-3 drops+ | 2-3 drops+ | 2-3 drops+ | 2-3 drops+ |
| 0.1 n solution of sodium hydroxide | | | | |
| Results | | | | |
| Conclusions | | | | |

**General scheme of the experiment (2)**

| Reagents | Water | Milk | Blood serum | Saliva |
|---|---|---|---|---|
| Quantity | 5 ml+ | 5 ml+ | 5 ml+ | 5 ml+ |
| Phenolphthalein | 2-3 drops+ | 2-3 drops+ | 2-3 drops+ | 2-3 drops+ |
| 0,1 n solution of hydrochloric acid | | | | |
| Results | | | | |
| Conclusions | | | | |

**WILL YOU PLEASE WASH THE USED LABORATORY DISHES AND HANDS AFTER THE EXPERIMENTALS! CLEAN YOUR WORKING PLACES!**

## 2. THE METHODS OF THE RECEIPT COLLOIDAL SOLUTIONS

### 2.1. WORK № 4. THE OBTAINED OF ROSIN AND PHENOPHTALEINE COLLOIDAL SOLUTIONS.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid.

**PRACTICE COURSE.** In a conical flask or a cylinder 4 ml of 0.1 n solution of acetic acid and 16 ml of 0,1 n solution of sodium acetate are measured and then mixed. Four test-tubes are labeled by numbers. 5 ml of the prepared buffer mixture are poured into test-tubes 1 and 3. Each of test-tubes number 2 and 4 is filled by 5 ml of distilled water. Then 1-2 drops of phenolphthalein are added into test-tubes number 1 and 2 and their content is titrated from burette by alkali, counting the number of drops until the solution attains pink color. 1-2 drops of congo red are added into test-tubes number 3 and 4 and titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue.

Can you explain why it is necessary to add more alkali into test-tube 1 than into test-tube 2? Why do we add less acid into the 4th test-tube than into the 3d test-tube? Write this.

### 2.2. WORK № 5. THE OBTAINED OF HYDROXIDE FERRUM SOL.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solution of phenolphthalein ($C_{20}H_{14}O_2$); universal indicator; 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid.

**PRACTICE COURSE.** Take three test-tubes. Each of them is filled by 2 ml of 0.1 n

solution of acetic acid and 2 ml of 0,1 n solution of sodium acetate. The content the first test-tube remains undiluted. The content of the second test-tube is watered down two times; equal volume of water (4 ml) is added to the buffer mixture. The content of the third test-tube is diluted 4 times; 12 ml of water are added. All the solutions from each of the test-tubes are mixed and used in the experiment.

1. Three test-tubes are labeled by numbers and 2 ml of buffer solutions are put into the 3 labeled test-tubes in the following way: the first test-tube has undiluted; the second test-tube solution is watered 2 times and in the third test-tube solution is diluted 4 times. Then three drops of universal indicator are added into each test-tube and the reaction is examined by the coloring degree of the solutions. Does pH change after watering of a buffer solution? If not, then why?
2. Three test-tubes are labeled by numbers and 2 ml of buffer solutions are put into the 3 labeled test-tubes in the following way: the first test-tube has undiluted; the second test-tube solution is watered 2 times and in the third test-tube solution is diluted 4 times. 2-3 drops of phenolphthalein are added into each test-tube and the content is titrated from burettes with alkali, counting the drops until the solution attains pink color.

## 2.3. WORK № 6. THE OBTAINED OF SULPHUR SOL.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); universal indicator 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid; water; milk; blood serum; saliva; urine.

**PRACTICE COURSE.** 1. Approximately pH of different examined liquids is determined in the glasses the help of universal indicator. It is done to make sure that they have practically neutral reaction, i.e. their pH is about 7.

5 ml of investigated liquids are poured into separate test-tubes, then 2-3 drops of phenolphthalein are added to each of them and their content is titrated from burettes with alkali, counting the drops until the solution attains pink color. Will you put down the results in notebooks.

After that again 5 ml of investigative liquids are poured into separate test-tubes, then 2-3 drops of congo red are put down into each of them and the obtained content is titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue. Results are written down in notebooks.

Which of the investigated liquids has the greatest buffer capacity? Regarding which substances (acid or alkali) is the buffer capacity more expressed in biological liquids? Which buffer systems are in the blood serum?

**2.4. WORK № 7. THE OBTAINED OF PRUSSIAN BLUE SOL**.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); universal indicator 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid; water; milk; blood serum; saliva; urine.

**PRACTICE COURSE.** 1. Approximately pH of different examined liquids is determined in the glasses the help of universal indicator. It is done to make sure that they have practically neutral reaction, i.e. their pH is about 7.

5 ml of investigated liquids are poured into separate test-tubes, then 2-3 drops of phenolphthalein are added to each of them and their content is titrated from burettes with alkali, counting the drops until the solution attains pink color. Will you put down the results in notebooks.

After that again 5 ml of investigative liquids are poured into separate test-tubes, then 2-3 drops of congo red are put down into each of them and the obtained content is titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue. Results are written down in notebooks.

Which of the investigated liquids has the greatest buffer capacity? Regarding which substances (acid or alkali) is the buffer capacity more expressed in biological liquids? Which buffer systems are in the blood serum?

**2.5. WORK № 8. THE DETERMINE OF COLLOID CHARGE**.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, burettes, different graduated pipettes, eye pipettes.

**REACTIVES:** 0.1 n solution of acetic acid; 0.1 n solution of sodium acetate; solutions of congo red ($C_{32}H_{22}N_6O_6Na_2S_2$) and phenolphthalein ($C_{20}H_{14}O_2$); universal indicator 0.1 n solution of sodium hydroxide; distilled water; 0.1 n solution of hydrochloric acid; water; milk; blood serum; saliva; urine.

**PRACTICE COURSE.** 1. Approximately pH of different examined liquids is determined in the glasses the help of universal indicator. It is done to make sure that they have practically neutral reaction, i.e. their pH is about 7.

5 ml of investigated liquids are poured into separate test-tubes, then 2-3 drops of phenolphthalein are added to each of them and their content is titrated from burettes with alkali, counting the drops until the solution attains pink color. Will you put down the results in notebooks.

After that again 5 ml of investigative liquids are poured into separate test-tubes, then 2-3 drops of congo red are put down into each of them and the obtained content is titrated by 0.1 n solution of hydrochloric acid, counting the drops until the solution becomes blue. Results are written down in notebooks.

Which of the investigated liquids has the greatest buffer capacity? Regarding which substances (acid or alkali) is the buffer capacity more expressed in biological liquids? Which buffer systems are in the blood serum?

**WILL YOU PLEASE WASH THE USED LABORATORY DISHES AND HANDS AFTER THE EXPERIMENTALS! CLEAN YOUR WORKING PLACES!**

## 3. PROTEINS

**3.1.WORK № 9. Precipitation of proteins with the help of ammonium sulfate**.

**APPARATUSES:** support with test-tubes, funnel with filter, different graduated pipettes.

**REACTIVES:** diluted egg-white (dissolve one egg-white in 230 ml of distilled water and 100 ml of saturated solution of NaCl. Filter this solution through 3 beds of gauze), crystalline ammonium sulfate, saturated solution of ammonium sulfate, 10% solution of NaOH, 1% solution of $CuSO_4$.

**PRACTICE COURSE.** 2-3 ml of diluted egg-white put in the test-tube and add 2-3 ml of saturated solution of ammonium sulfate. Mix contents of test-tube and observe the reaction of globulins precipitation. After 5-7 min filter the contents of test-tube. Albumins remain in the filtrate and globulins are on the filter. Add crystalline ammonium sulfate in the filtrate while it will be undissolved for albumins precipitation. Filter the contents of test-tube and do the biuret test with 2-3 ml of filtrate. Negative reaction shows the absence of proteins in the filtrate and their full precipitation.

The sediment of albumins together with filter put in the test-tube and dissolve in 4-5 ml of distilled water. Filter the solution of albumins and do biuret test with filtrate.

**3.2. WORK №10. Biuret test**

**APPARATUSES:** support with test-tubes, different graduated pipettes.

**REACTIVES:** diluted egg-white, 30% or 10% solution of NaOH, 1% solution of $CuSO_4$

**PRACTICE COURSE.** Take 2-3 ml of diluted egg-white in the test-tube, add an equal volume of 30% or 10% solution of NaOH, mix thoroughly and add 2-3 drops of 1% solution of $CuSO_4$. Mix it well until a purple-violet or pinkish-violet colour develops.

**3.3.WORK № 11. Precipitation of proteins with the help of sodium chloride and magnesium sulfate**.

**APPARATUSES:** support with test-tubes, funnel with filter, different graduated pipettes.

**REACTIVES:** diluted egg-white (dissolve one egg-white in 230 ml of distilled water and 100 ml of saturated solution of NaCl. Filter this solution through 3 beds of gauze), crystalline sodium chloride, crystalline magnesium sulfate, 1% solution of acetic acid, 10% solution of NaOH, 1% solution of $CuSO_4$.

**PRACTICE COURSE.** Two test-tubes are labeled by numbers and 2-3 ml

of diluted egg-white put into the 2 labeled test-tubes. Than add in the first test-tube crystalline sodium chloride and in the second test-tube - crystalline magnesium sulfate while these solutions will be full saturated. After 2-3 min filter the contents of both test-tube. Albumins remain in the filtrate. Add to filtrates of each test-tube 4-6 drops of 1% solution of acetic acid and note the albumins precipitation. Filter the contents of two test-tubes after 5 min and check up filtrates on proteins absence with the help of biuret test.

**3.4.WORK № 12. Precipitation of proteins with the help of heavy metals ions**.

**APPARATUSES:** support with test-tubes, glass sticks, different graduated pipettes.

**REACTIVES:** diluted egg-white (dissolve one egg-white in 230 ml of distilled water and 100 ml of saturated solution of NaCl. Filter this solution through 3 beds of gauze), 5% solution of acetous lead, 5% solution of $CuSO_4$.

**PRACTICE COURSE.** Two test-tubes are labeled by numbers and 2 ml of diluted egg-white put into the 2 labeled test-tubes. Than add drop-by-drop in the first test-tube 5% solution of acetous lead and in the second test-tube - 5% solution of $CuSO_4$. Observe the precipitation of proteins in the both test-tubes. Make summary.

**WILL YOU PLEASE WASH THE USED LABORATORY DISHES AND HANDS AFTER THE EXPERIMENTALS! CLEAN YOUR WORKING PLACES!**

## 4. QUALITATIVE REACTIONS ON THE PROTEINS

### 4.1.WORK № 13. Ninhydrin reaction

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, thermostat (37ºC).

**REACTIVES:** 0,2% solution of ninhydrin in alcohol or acetone, diluted egg-white (dissolve one egg-white in 500 ml of distilled water and filter this solution through 3 beds of gauze), distilled water.

**PRACTICE COURSE.** Take 2-3 ml of diluted egg-white in the test-tube, add 10-12 drops of 0.2% solution of ninhydrin and mix it. Then put this test-tube into water-bath on 5 min. Observe the occurrence of blue-violet color in the solution.

This reaction is wide-spreaded in biochemical laboratory for quantitative determining of amino acids in biological liquids (blood, urine, liquor and so on).

### 4.2.WORK № 14. Xanthoproteic reaction

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, thermostat or water-bath (37ºC).

**REACTIVES:** 0.1% solution of phenol, diluted egg-white (dissolve one egg-white in 500 ml of distilled water and filter this solution through 3 beds of gauze), distilled water, concentrated azotic acid ($HNO_3$), 20% solution of NaOH or ammonia.

**PRACTICE COURSE.** Two test-tubes are labeled by numbers. Then put in the first test-tube 2 ml of 0.1% solution of phenol and in the second test-tube – 2 ml of diluted egg-white. Each of test-tubes is added 10 drops of concentrated azotic acid. Mix thoroughly each of these test-tubes and incubate them during 10 min into water-bath (37ºC). Observe the occurrence of yellow color in these solutions. After cooling test-tubes add cautious excess of 20% solution of NaOH or ammonia in each of them. The solutions' color transfers into orange.

This reaction helps to determine the presence of cyclic amino acids (phenylalanine, tyrosine, tryptophane).

### 4.3.WORK № 15. Nitroprusside test

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, water-bath (100ºC).

**REACTIVES:** diluted egg-white (dissolve one egg-white in 500 ml of distilled water and filter this solution through 3 beds of gauze), distilled water, 30% solution of NaOH, 10% solution of nitroprusside Na

**PRACTICE COURSE.** Take 1 ml of diluted egg-white in a test-tube and add 1 ml of 30% solution of NaOH. Heat test tube to the boiling and then cooling it. Add 5 drops of 10% solution of nitroprusside Na in this test-tube and mix all solutions. Record your result.

The nitroprusside test is specific for cycteine, the only amino acid containing a sulfhydryl group (-SH). The group reacts with nitroprusside in alkaline solution to yield a red complex.

$OH^-$

$$Na_2(CN)_5Fe(NO)\cdot 2H_2O + HS\text{-}CH_2CH\text{-}COO^- \rightarrow\rightarrow\rightarrow \text{red complex}$$
$$NH_3^+$$

**4.4.WORK №16. Millon's test**

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, water-bath (100°C).

**REACTIVES:** Millon's reagent (contains mercury and $HNO_3$), diluted egg-white (dissolve one egg-white in 500 ml of distilled water and filter this solution through 3 beds of gauze), distilled water.

**PRACTICE COURSE.** Take 2 ml of diluted egg-white in a test-tube and add 3 drops of Millon's reagent. Using a test tube holder, place the test tube into the boiling water bath. Heat the solution just to the boiling point, then use a test tube holder to remove the test tube from the bath and place it in the support. Record your result.

Millon's test is a test specific for tyrosine, the only amino acid containing a phenol group, a hydroxyl group attached to a benzene ring. In Millon's test the phenol group of tyrosine is first nitrated by nitric acid in the test solution. Then the nitrated tyrosine complexes mercury (I) and mercury (II) ions in the solution to form either a red precipitate or a red solution, both positive results.

**4.4.WORK № 17. Hopkins-Cole test**

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes.

**REACTIVES:** concentrated sulfuric acid, Hopkins-Cole reagent, diluted egg-white (dissolve one egg-white in 500 ml of distilled water and filter this solution through 3 beds of gauze), distilled water.

**PRACTICE COURSE.** Take 2 ml of diluted egg-white in a test-tube and add 2 ml of Hopkins-Cole reagent. If a purple ring does not form at the interface of the two layers in the test tube, gently tap the side of the test tube once to slightly mix the two layers. If a purple ring still doesn't form, consider it a negative result.

Holding the test tube with 2 ml of diluted egg-white at a 45° angle cautiously and slowly add 30 drops of concentrated sulfuric acid down the inside wall of the test tube. Do not mix the two layers that form. Record your observations.

The Hopkins-Cole test is specific for tryptophan, the only amino acid containing an indole group. The indole ring reacts with glyoxylic acid in the presence of a strong acid to form a violet cyclic product. The Hopkins-Cole reagent only reacts with proteins containing tryptophan. The protein solution is hydrolyzed by the concentrated sulfuric acid at the solution interface. Once the tryptophan is free, it reacts with the glyoxylic acid to form the violet product.

# 5. MEAT PROTEINS

## 5.1.WORK №18. Isolation of albumin fraction

**Instruments:** Shuthelah-machine, glasses, chemical, scissors, funnel, test tubes, glass, chemical, pipette 2 mL, gauze.

**Reagents:** 10% solution of sodium hydroxide, 1% solution of copper sulphate, crystalline ammonium sulfate, 6% solution of trichloroacetic acid.

**Proceedings.** In a beaker placed 8-10 g of ground muscle, pour a triple volume of distilled water and shaking at Shuthelah pan for 20 minutes and extracted.

Extract was separated by filtration through three layers of cheesecloth into another beaker, and the remaining muscle pulp stored for later extraction of salt. In the aqueous extract of proteins transferred sarcoplasm - Myogit, mioalbumin and myoglobin, which carry out these reactions.

Poured into four test tubes with 2 ml of the resulting extract. In the first test tube for determination of protein in the extract, carried out biuret reaction (ie, 2 ml of the liquid is added 4 ml 10% solution of sodium hydroxide, mixed and then add 2-3 drops of 1% solution of copper sulfate) . The appearance of blue-purple staining indicates the presence of the protein.

In the second tube is added to saturation of ammonium sulfate powder. In the sediment with the fall of albumin. In the third test tube was reacted to precipitation of proteins with salts tezhelyh metals (2 ml test liquid was added dropwise 1% solution of copper sulfate and observe the precipitation of proteins).

For a complete removal of proteins from biological fluids using trichloroacetic acid, so in the fourth tube to 2 ml of extract was added 2-3 drops of 6% solution of trichloroacetic acid, shaken and observed precipitation of the protein.

**Remember! Trichloroacetic acid can precipitate only proteins and their degradation products (urea, uric acid, amino acid amides, amino acids, low molecular weight peptides, etc.) remain in solution. It is necessary for a separate determination of protein and nonprotein (residual) nitrogen in the tissues.**

## 5.2.WORK №19. Isolation of globulin fraction

**Instruments:** Shuthelah-machine, glasses, chemical, funnel, tripod with test tubes, test tubes at 2 ml gauze.

**Reagents:** a saturated solution of ammonium sulfate, 10% solution of ammonium chloride, 10% solution of sodium hydroxide, 1% solution of copper sulphate.

**Proceedings.** After extraction, water balance muscle slurry is placed in a glass and pour 20 ml of 10% solution of ammonium chloride, then extracted by shaking for 30 minutes.

Through three layers of gauze filtered saline extract. Keep in mind that it pass globulin fraction proteins - myosin and actomyosin. For further investigation, preserve muscle tissue residue on the filter.

The resulting saline extract of muscle poured into three vials of 2 ml. In order to establish the presence of protein in the first test tube conducting biuret reaction (ie, 2 ml of the liquid is added 4 ml 10% solution of sodium hydroxide, mixed and then add 2-3 drops of 1% solution of copper sulfate).

The appearance of blue-purple staining indicates the presence of protein. To 2 ml of saline extract of the second test tube add 2 ml of saturated ammonium sulfate solution. Celebrate the precipitation of myosin and actomyosin. The third tube is gradually poured distilled water until the water-insoluble precipitation of myosin and actomyosin.

### 5.3.WORK №20. Isolation of skleroproteins

**Products:** tiles, glasses, chemical, stand with test tubes, funnels, muslin.

**Reagents:** 10% solution of sodium hydroxide, 1% solution of copper sulfate, 6% solution of trichloroacetic acid.

**Proceedings.** After extraction with water and brine in the remainder of the muscle tissue contains skleroproteins (collagen and elastin).In a beaker place the remainder of muscle tissue, which is poured triple the amount of water and boil for 30 minutes, keeping the volume of liquid in a glass of periodic addition of hot water.

Collagen by boiling exposed shallow hydrolysis and converted into water-soluble gelatin. Filtered hot solution into a test tube and using biuret reaction in the filtrate establish the presence of gelatin.

To 2 ml of the liquid is added 4 ml 10% solution of sodium hydroxide, mixed and then add 2-3 drops of 1% solution of copper sulphate. The appearance of blue-purple staining indicates the presence of protein. Gelatin does not give a positive reaction to the tryptophan and tyrosine. Thin films of elastin fibers and remain on the filter.

## 6.ENZYMES

### 6.1.WORK № 21. Thermolabile of ferments.

**APPARATUSES:** support with test-tubes, 50 ml cylinders or conical flasks, different graduated pipettes, eye pipettes, alcohol lamp, thermostat (37°C), glass with ice.

**REACTIVES:** diluted saliva (take 20-25 ml of distilled water in mouth and collect it through paper filter in cylinder), 0.3% solution of NaCl, 1% solution of starch on the 0.3% solution of 0.3% solution of NaCl, reactive of Lugol (20g of KJ and 10 g of iodine dissolve in 100 ml of water, then this solution dissolves by distilled water as 1:5).

**PRACTICE COURSE.** Three test-tubes are labeled by numbers and 2-3 ml of diluted saliva is put into the 3 labeled test-tubes. The saliva from the first test-tube is boiling during 1-2 min. Than add 4-5 ml of 1% solution of starch in each of 3 test-tubes. Put test-tubes number 1 and 2 into thermostat (37°C) or water-bath on 10 min. The test-tube number 3 put in glass with ice on 10 min. After that reactive of Lugol is added in all test-tubes by 1 drop. Fill the table of experiment and make summary.

| Number of test-tube | Enzyme | Conditions of experiment | Substrate | Incubation | Colour with iodine |
|---|---|---|---|---|---|
| **1** | Amylase | Denaturated enzyme | Starch | 10 min, 37ºC | |
| **2** | Amylase | Native enzyme | Starch | 10 min, 37ºC | |
| **3** | Amylase | Native enzyme | Starch | 10 min, 0ºC | |

Summary:

**6.2.WORK № 22. The influence of activators and inhibitors**

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, thermostat (37ºC) or water-bath.

**REACTIVES:** diluted saliva, 1% solution of NaCl, 1% solution of starch on the 0.3% solution of NaCl, 1% solution of $CuSO_4$, distilled water, reactive of Lugol.

**PRACTICE COURSE.** Three test-tubes are labeled by numbers and 4-5 ml of 1% solution of starch is put into the 3 labeled test-tubes. Than in the test-tube number 1 add 1-2 ml of 1% solution of NaCl, in the test-tube number 2 – 1-2 ml of 1% solution of $CuSO_4$ and in the test-tube number 3 – 1-2 ml of distilled water. Each of test-tubes is added by 1-2 ml of diluted saliva. Mix thoroughly each of these test-tubes and incubate them during 10 min into water-bath (37ºC). After that add by 1 drop of reactive of Lugol in each test-tube and fill the table of experiment.

| Number of test-tube | Enzyme | Effector | Substrate | Incubation | Colour with iodine |
|---|---|---|---|---|---|
| **1** | Amylase | NaCl | Starch | 10 min,37ºC | |
| **2** | Amylase | $CuSO_4$ | Starch | 10 min,37ºC | |
| **3** | Amylase | $H_2O$ | Starch | 10 min, 37ºC | |

Summary:

**6.3.WORK № 23. Specific action of yeast enzyme**

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, alcohol lamp, thermostat (37ºC), porcelain mortar with pestle, funnels, paper filters.

**REACTIVES**: 0.5 g of dry yeast, 1% solution of starch on the 0.3% solution of NaCl, 1% solution of $CuSO_4$, 10% solution of NaOH, 2% solution of saccharose, distilled water.

**PRACTICE COURSE.** First of all take 0.5 g of dry yeast and triturate them in the porcelain mortar with pestle. Than add 5 ml of water and continue triturate yeast together with water. Filtrate the obtain mixture through fluted filter. Filter liquor contain yeast enzyme (saccharase).

Two test-tubes are labeled by numbers and 1 ml of saccharase is put into each of them. Than add 1 ml of 2% solution of saccharose in test-tube number 1 and 1 ml

of 1% solution of starch into test-tube number 2. Put all test-tubes into water-bath (37ºC) on 15 minutes. After that do Trommer test. Take two clean test-tube and add in the first of them 5 drops of solution from the test-tube number 1. In the second test-tube fill 5 drops of solution from the test-tube number 2. Than add 10 drops of 10% solution NaOH and 1-3 drops of 1% solution of $CuSO_4$ (II). Mix all solutions and heat them to the boiling. Red colour of solution shows us that glucose is present.

| **Number of test-tube** | **Enzyme (saccharase), ml** | **Solution of saccharose, ml** | **Solution of starch, ml** | **Reaction of Trommer (+ or -)** |
|---|---|---|---|---|
| **1** | 1 | 1 | - | |
| **2** | 1 | - | 1 | |

Summary:

**6.4.WORK №24. The influence of pH on the enzymes activity**

**APPARATUSES:** support with test-tubes, different graduated pipettes, eye pipettes, thermostat or water-bath (37ºC).

**REACTIVES:** 0,2M solution of $Na_2HPO_4$ (solution A), 0,1M solution of citric acid ($C_6H_8O_7 \cdot H_2O$) (solution B). Buffer solutions (pH 5,0 : 515ml of solution A mix with 485 ml of solution B; pH 6,8 : 772,5 ml of solution A mix with 227,5 ml of solution B; pH 8,0 : 972,5 ml of solution A mix with 27,5 ml of solution B), diluted saliva, 1% solution of starch, reactive of Lugol.

**PRACTICE COURSE.** Take three test-tubes and labeled them. Fill test-tubes by 2-3 ml of buffer solutions with different values of pH (5,0; 6,8; 8,0). Add 2-3 ml of diluted saliva and 4-5 ml of 1% solution of starch in each of test-tube. Mix solutions in each test-tube and incubate them during 10 min into water-bath (37ºC). After that add by 1 drop of reactive of Lugol in each test-tube and fill the table of your experiment.

| **Number of test-tube** | **Enzyme** | **pH medium** | **Substrate** | **Incubation** | **Colour with iodine** |
|---|---|---|---|---|---|
| **1** | Amylase | 5,0 | Starch | 10 min, 37ºC | |
| **2** | Amylase | 6,8 | Starch | 10 min, 37ºC | |
| **3** | Amylase | 8,0 | Starch | 10 min, 37ºC | |

Summary:

**WILL YOU PLEASE WASH THE USED LABORATORY DISHES AND HANDS AFTER THE EXPERIMENTALS! CLEAN YOUR WORKING PLACES!**

**Summary of Digestive Enzymes**

**The digestive enzymes in the table below are summarized according to type of food that they digest.**

| FOOD TYPE | ENZYME | SOURCE | PRODUCTS |
|---|---|---|---|
| CARBOHYDRATES | Salivary amylase<br>Pancreatic amylase<br>Maltase | Salivary glands<br>Pancreas<br>Small intestine | Maltose<br>Maltose<br>Glucose |
| PROTEINS | Pepsin<br>Trypsin<br>Peptidases | Stomach mucosa<br>Pancreas<br>Intestinal mucosa | Peptides<br>Peptides<br>Amino acids |
| FATS | Lipase | Pancreas | Fatty acids and glycerol |

**The table below shows digestive enzymes grouped by source of the enzyme.**

| SOURCE | ENZYME | FOOD | PRODUCT |
|---|---|---|---|
| MOUTH (salivary glands) | Salivary amylase | Polysaccharides | Maltose |
| STOMACH | Pepsin | Proteins | Peptides |
| PANCREAS | Pancreatic amylase<br>Trypsin<br>Lipase | Polysaccharides<br>Proteins<br>Fats | Maltose<br>Peptides<br>Fatty acids and glycerol |
| SMALL INTESTINE | Maltase<br>Peptidases | Maltose<br>Peptides | Glucose<br>Amino acids |

## 7.VITAMINS (Vitamin C)

The most commonly discussed vitamin, ascorbic acid (vitamin C) is thought to function in hydrogen transfer and regulation of intracellular oxidation reduction potentials. Ascorbic acid is required for normal amino acid metabolism of certain drugs By virtue of its enediol group; ascorbate is a strong reducing compound. Although plants and most animals can synthesize this vitamin, humans cannot, and dietary ingestion is essential. Major dietary sources include fruits (especially citrus) and vegetables (tomatoes, green peppers, cabbage, leafy greens, potatoes).

Ascorbic acid is important in the formation and stabilization of collagen by hydroxylation of praline and lysine for cross-linking and the conversion of tyrosine to catecholamines (by dopamine, β-hydrolase). It increases the absorption of certain minerals, such as iron. Ascorbic acid deficiency is known scurvy. This condition is characterized by hemorrhagic disorders, including swollen bleeding gums, as well as impaired wound healing and anemia.

The reference range for ascorbic acid is 0.4 to 0.6 mg/dL in blood.

The usefulness of ascorbate in preventing cold has lead to the common practice of megadose intakes of vitamin C.

Ascorbic acid is a physiologic reducing agent and many methods are based on the oxidation of ascorbic acid to dehydroascorbic acid or on its reducing properties.

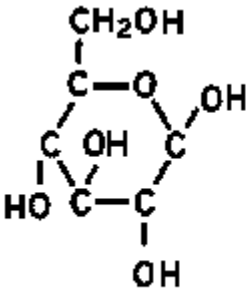
### 7.1. Work №25. Qualitative reaction for vitamin C

**Apparatuses:** support with test-tubes, different graduated pipettes

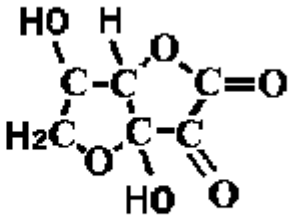
**Reactives:** 10% solution of NaOH, 10% solution of HCl, 5% solution of tetrapotassium hexacyanoferrate (potassium ferrocyanide) / $K_4Fe(CN)_6$ /, 1% solution of $FeCl_3$, 1% solution of vitamin C.

**Practice course.**

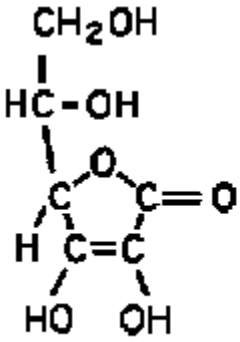
Two test-tubes are labeled. Put 5 drops of 1% solution of vitamin C in the first test-tube and 5 drops of distilled water in the second test-tube. Then add in each of these test-tubes 1 drop of 10% NaOH and 1 drop of 5% solution $K_4Fe(CN)_6$. Mix thoroughly and add 3 drops of 10% solution of HCl and 1 drop of 1 % solution of $FeCl_3$. Fill the table of experiment and make summary.



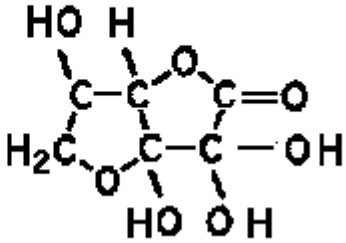
D-Glucose



Monodehydro ascorbate



Ascorbic acid



Dehydro ascorbate

**General scheme of the experiment**

| Reagents | First test-tube | Second test-tube |
|---|---|---|
| 1% solution of vitamin C | 5 drops | - |
| Distilled water | - | 5 drops |
| 10% NaOH | 1 drop | 1 drop |
| **5%** $K_4Fe(CN)_6$ | 1 drop | 1 drop |
| | | |
| 10% HCl | 3 drops | 3 drops |
| 1% $FeCl_3$ | 1 drop | 1 drop |
| **Result** | | |

**7.2.Work №26. Quantitative determination of vitamin C**

**Apparatuses:** support with test-tubes, different graduated pipettes, porcelain mortar with pestle, 25ml cylinders, 25 and 50 ml conical flasks, scales, scissors, scalpel

**Reactives:** 10% solution of NaCl, 0.001n solution of sodium salt 2,6-dichlorphenolindophenol, needles, potatoes.

**Practice course.**

Weigh 1 g of needles and thoroughly cut very small them. Than add 20 ml of distilled water drop by drop and filter all through paper filter. Take two conical flasks and fill them by 10 ml of filtrate. Add 2-3 drops of 10% solution of NaCl. Titrate the contents of flasks using 0.001n solution of 2,6-dichlorphenolindophenol to pink colour of solution, which could not disappears during 30 sec .

Weigh 5 g of potatoes, cut it and than triturate in porcelain mortar. Add to biomass 3 drops of NaCl and 15 ml of distilled water. Put all content in conical flask and titrate with the help of 0.001n solution of 2,6-dichlorphenolindophenol.

In a flask, 5 ml of milk are diluted in 15 ml of water. A milliliter of the 2% muriatic acid is poured to 5 ml of the solution obtained reaching a 15 ml volume by water. Cautiously agitating, the content of the flask is titrated from the microburette by the 0.001n solution of the 2.6 dichlorophenolindophenol until poor pink color appears and maintains for 0.5-1 min.

The level of the ascorbic acid (C) is calculated for the formula:

$$C=A\cdot K\cdot 5.28,$$

Where C is the level of ascorbic acid, mg%;

A is the amount of the 2.6 dichlorophenolindophenol process solution taken for the titrating, ml;

K is correction for the indicator titer;

5.28 is a permanent coefficient.

In the raw milk a vitamin C concentration averages 0.3-2 mg%. However, with the product stored and further processed the vitamin C level falls dramatically. To raise the vitamin C level, the milk is enriched, but if stored unpacked; the vitamin oxidizes fast by air oxygen.

The content of vitamin C in different substances

| Substance | Quantity of substance | Dilution | Quantity of 2,6-dichlorphenolindophenol | Recalculation | Result, mg% |
|---|---|---|---|---|---|
| Needles | 1 g | | | | |
| Potatoes | 5 g | | | | |
| Milk | 5 ml | | | | |

Quantity of 2,6-dichlorphenolindophenol is equal quantity of vitamin C in solution.

Составитель
Короткевич Ольга Сергеевна
Коновалова Татьяна Валерьевна

# Essential of biochemistry

**методические разработки по выполнению лабораторных работ**

Формат 60x84 1/16 1.5 усл.печ.л.

В авторской редакции
Компьютерная вёрстка О.С. Короткевич