C3807 Laboratory practice in Chemistry of Natural Polymers

Instruction for laboratory experiments

Radka Bačovská, Ladislav Pospíšil

Přírodovědecká fakulta, Masarykova univerzita

Tutorials for Exercises of natural polymers chemistry, C3807, were created thanks to financial support within the project FRMU 2018. All experiments were tested in laboratories of the Faculty of Science, Masaryk University. Exercise is also supplemented by an excursion to CEITEC VUT. Students will learn about common and modern trends in the field of natural polymers. The exercise is related to the subject C3804 Natural polymers. It is necessary strictly follow safety rules during working in lab. Some experiments will work with hazardous and irritating substances. The equipment must be checked by the teacher before starting the chemical process. Students are required to become familiar with the safety rules for handling chemicals (eg safety data sheets on Sigma Aldrich, Acros Organics, etc.).

Book content

CARBOHYDRATES

Carbohydrates are a large and very diverse group of natural substances belonging to the group of polyhydroxy derivatives of carbonyl compounds (aldehydes or ketones). According to the number of monosaccharide units, they are divided into: monosaccharides, oligosaccharides (2-10 molecules) and polysaccharides.

Low-molecular-weight carbohydrates are water-soluble and have more or less sweet taste. Macromolecular polysaccharides are mostly tasteless and are only slightly soluble in water (starch, agar), or completely insoluble (cellulose).

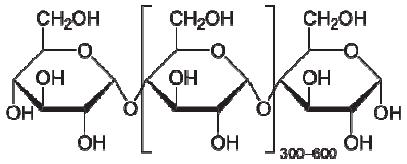
Task. n. 1: POLYSACHARIDES – STARCH

Starch is the most important storage polysaccharide of plants. It is mainly associated with potatoes in our geographical zone, but corn is the most important crop worldwide.

Starch occurs in plants as heterogeneous particles, not as solutes. It has different shapes and sizes depending on the type of crop. Some crops (eg wheat) have grains of two different sizes.

Fig. 1: Graing of strarch

Chemically, starch is poly (1,4) α-D-glucopyranose in the form of a linear macromolecule of amylose or branched amylopectin, their ratio varies according to plant species.



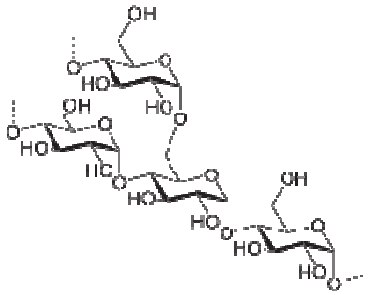


Fig. 2: Amylosa *Amylopectin*

Only amylose gives a typical color reaction with Lugol's reagent (iodine solution in KI).

Starch can be chemically and enzymatically cleaved in the backbone into oligosaccharides to monosaccharides. Chemical cleavage is usually acid catalyzed by common inorganic acids (HCl, H2SO4).

A. QUALITATIVE EXPERIMENTS OF SACCHARIDES

a. Fehling reaction

The oxidation-reduction reaction takes place only in the group of so-called reducing sugars. These are sugars which can have a free, oxidation-capable, carbonyl group. These may be monosaccharides, disaccharides, trisaccharides or higher carbohydrates. For a visible reaction (change of color of the copper compound from blue to yellow-red), there must be a sufficient concentration of terminal meres in the solution which may have a free, oxidative-capable, carbonyl group. If this is not the case, it is called non-reducing sugar. The oxidation-reduction reaction

usually proceeds only up to the degree of reduction of Cu + 2 to Cu + 1. In case of higher concentration of reducing saccharide, reduction reaction can finish up to Cu.

$$2\ \text{-COH} + O_2 + 2\ Cu^{+2} \rightarrow \text{-COOH} + 2\ Cu^{+1}$$

$$2\ \text{-COH} + O_2 + 2\ Cu^{+1} \rightarrow \text{-COOH} + 2\ Cu$$

Equipments:

test tube, spoon, 2 pipettes, beaker, stander, heating ring, heating network

Samples:

Carbohydrate samples

Chemicals:

Fehling reagent I - 4 g $CuSO_4.5H_2O$ in distilled water, fill up to 100 ml.

Fehling reagent II - 20 g $KNaC_4H_4O_6\ 4H_2O$, 15 g NaOH in distilled water, fill up to 100 ml.

Workflow:

1. Put solid carbohydrates (few grains on the spatula) into test tubes and dissolve it in distilled water.
2. Add 1 ml of Fehling reagent I and 1 ml of Fehling reagent II.
3. Place the tube in a water bath and heat until the color changes.
4. Prepare a blank - pour only distilled water instead of carbohydrate.

Conclusion:

As a result, indicate which sample contained a reducing and which non-reducing carbohydrate, describe the color change.

b. Tollens reaction

Tollens reagent is prepared when silver nitrate reacts with concentrated ammonium hydroxide solution to form an ammonium complex.

$$AgNO_3 + 2\ NaOH \rightarrow Ag_2O\ (s) + 2\ NaNO_3 + H_2O$$

$$3\ NH_4OH + AgNO_3 \rightarrow [Ag(NH_3)_2]OH + 2\ H_2O + NH_4NO_3$$

$$2\ Ag^+[NH_3]^0 + \text{R-HCO} \rightarrow 2\ Ag^0 + \text{R-COO}^-NH_4^+ + 2\ NH_3 + H_2O$$

Equipments:

test tube, spoon, 2 pipettes, beaker, stander, heating ring, heating network

Samples:

Carbohydrate samples

Chemicals:

$AgNO_3$, NaOH, NH_4OH, H_2O

Workflow:

1. Add a solution of NaOH and AgNO3 to the sample of carbohydrates (in the form of an aqueous solution) in the tube, resulting in a brown Ag2O precipitate.
2. Add concentrated NH4OH to the tube to dissolve the brown Ag2O precipitate.
3. Be careful, if the reaction takes longer, explosive Ag3N can be produced. In the reaction with aldehyde groups in carbohydrates, the ammonium salt of the carboxylic acid and reduced silver are formed. The reduced silved is observed as a silver mirror on the wall of the test tube.
4. Compare the experiment with presented carbohydrates with the blank, eg. add only water instead of the carbohydrate sample.

Conclusion:

As a result, indicate which sample contained a reducing and which non-reducing carbohydrate.

c. Selivan reaction

The essence of the reaction is dehydration of saccharides by concentrated acids to 5-hydromethylfural, which subsequently reacts with resorcinol with color change.

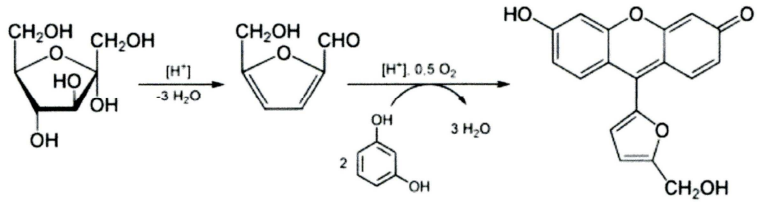


Fig. 3: Selivan reaction

The Ketoses presence gives a red color, aldoses react more slowly. Aldoses can react the same after a while, when aldose isomerizes to ketosis in solution.

Equipments:

test tube, spoon, 2 pipettes, beaker, stander, heating ring, heating network

Samples:

Carbohydrate samples

Chemicals:

0,5 % resorcinol v 10 % HCl, conc. HCl

Workflow:

1. Add a drop of HCl to the carbohydrate solution in the tube.
2. Add a drop of resorcinol solution and observe the color change to red.
3. Compare with the blank

Conclusion:

Give a description of the color change as a result.

B. DETERMINATION OF STARCH GRAVIMETRICALLY

Equipments:

Erlenmeyer flask 250 ml (1x), funnel, frit (larger pores), measuring cylinder 100 ml, beaker 250 and 600 ml, pH paper, mixer, cooker

Samples:

chicken sausages „Striptease“, pate „Májka“

Chemicals:

8% KOH in ethanol, ethanol (96% and 50%)

Workflow:

1. Place a 100 ml Erlenmeyer flask in a water bath (600 ml beaker on a magnetic stirrer).
2. Weigh first the whole sausage (pulled) or the pate package.
3. Weigh 2,5 - 3 g of homogenised meat from pulled sausage or pate. Put it into an Erlenmeyer flask.
4. Add 80 ml of 8% Alcoholic KOH solution to the meat sample.
5. Bring the water bath to boil and heat the contents of the Erlenmeyer flask while stirring occasionally in a water bath for 45 min.
6. Add 25 ml of 50% hot ethanol to the fully dissolved sample. Starch grains will precipitate and leave to settle to the bottom.
7. Carefully discard the top layer of the solution. Decant the deposited starch 3 times with 30 ml of hot 50% ethanol. Monitor the efficiency of the decantation using a pH paper, when the pH should drop to neutral.
8. Quantitatively transfer the contents of the conical flask to a pre-dried and weighed frit and vacuum filter. Rinse the conical flask thoroughly with hot 50% ethanol.
9. Wash the precipitate on the frit with 20 ml of cold 96% ethanol and allow air to pass through the sample frit for 10 minutes. Place the sample frit in an oven at about 100 ° C and dry to constant weight.

Evaluation and conclusion:

Calculate the starch content of the sample:

$$w(\text{škrob}) = \frac{\text{hmotnost škrobu na fritě v gramech}}{\text{navážka masného vzorku v gramech}} \; 100$$

As a result, give the starch content of the products analyzed (sausage / pate) expressed as the arithmetic mean of three parallel determinations. Compare the two tested products with each other and try to compare them with the values declared by the manufacturer.

C. EVIDENCE OF STARCH AND SOLANIN IN POTATOES

The principle of the Lugol reaction is the formation of a starch-I^{3-} complex.

$$I_2(l) + I^-(aq) \leftrightarrow I^{3-}(aq)$$

$$\underline{I^{3-}(aq) \leftrightarrow \text{škrob } I^{3-} \text{ complex}}$$

$$I_2(l) + I^-(aq) \leftrightarrow \text{škrob } I^{3-} \text{ complex}$$

Equipments:

Petri dish, 4 capillaries, knife, tweezers, filter paper, slide, microscope

Samples:

potato sprouted and not sprouted

Chemicals:

Concentrated H_2SO_4, concentrated CH_3COOH, 1% aqueous formaldehyde solution, **0,5%** H_2O_2, Lugol's solution (5 g I2 and 10 g KI in 85 ml distilled water)

Workflow:

1. Cut a thin slice from the potato tuber (1x sprouted, 1x un sprouted) and place on a petri dish or glass slide.
2. Gradually drip on the slice into a row of 1-3 drops of conc. H_2SO_4, conc. CH_3COOH, 1% formaldehyde solution, 0.5% H_2O_2, and Lugol's solution.
3. The presence of solanine is indicated by a reddish coloration, the presence of starch is shown by a dark blue coloration.

Conclusion

As a result, provide a comparison of germinated and non-germinated tubers. Try to monitor with photos (from your mobile phone).

D. DETERMINATION AMYLOSE AND AMYLOPECTINE IN STARCH

Equipments:

plastic bowl, beaker 800 ml, measuring cylinders 500 and 250 ml, funnel, gauze, blender, scales, thermometer, glass rod, indicator paper, dropper

Samples:

potato (200 g)

Chemicals:

0.9% NaCl, 35% HCl, 0.01M and 0.2M NaOH, Lugol's solution

Workflow:

1. Homogeneize 200 g of washed, sliced potato in a mixer and transfer to 800 ml beaker filled with 500 ml saline.
2. Mix the suspension 3 times within 5 minutes, filter through gauze several times into a graduated cylinder and allow to settle.
3. Carefully discard the liquid and decant the settled starch 3 times with 150 ml of saline, then with 150 ml of 0,01 M NaOH and finally 3 times with 150 ml of distilled water.
4. Allow the starch to dry on air until the next lesson, weigh and make evidence using Lugol's solution, see section C

5. Transfer the isolated starch to an Erlenmeyer flask, add 1.5 ml of 35% HCl, and 35% HCl dropwise to adjust the pH 6-7. Check the pH with indicator paper.

6. Allow the sample to stand until the next lesson, when a white gel (amylopectin) appears at the bottom of the beaker. Decant the amylopectin and slowly filter it on a Büchner funnel. Dry amylopectin sample to constant weight at room temperature.

Conclusion:

As a result, give the percentage yield of starch obtained from 200g of potato, give the percentage of amylose and amylopectin in the sample and their relative proportion.

E. STARCH ENZYMATIC HYDROLYSIS

Equipments:

250 ml beaker, cooker, test tubes, dropper, measuring cylinder, dropping plate

Samples:

human saliva

Chemicals:

potato starch, Lugol solution

Workflow:

1. Pour 5 ml of starch sebum and 4 ml of human saliva diluted with distilled water 1: 1 into 2 tubes using a graduated cylinder.

2. Leave the first tube as a reference, when blue Lugol solution is added.

3. Place the second tube in a water bath (250 ml beaker) and heat gently.

4. At regular intervals, collect a sample from both the reference and heated test tubes and observe the color of the samples after adding a drop of Lugol's solution. The color of the reference sample will be blue to indicate the presence of starch, and the color of the heated sample should change color depending on the rate of enzymatic degradation of starch by human saliva.

Conclusion:

Report the color change of the reaction as a result.

TASK 2: WOOD AND ITS POLYSACHARIDE

The chemical structure of wood consists of 40-50% cellulose, 20-30% hemicellulose, 20-30% lignin, 1-15% other organic substances (terpenes, fats, waxes, tannins, pectins, sterols, resins), 0.5% inorganic substances and water. Cellulose and hemicellulose are the polysaccharides. Lignin is a high molecular weight substance.

Cellulose is the most widespread BIOPOLYMER on the Earth. It forms the basic building component of plants. Cellulose consists of ß-D-glucose molecules linked by a ß-1,4 glycoside bond.

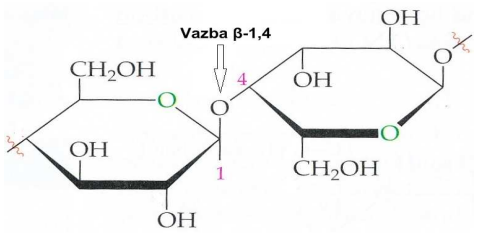


Figure 6: ß-D-Glucose, ß-1,4 glycoside bond

Thanks to its fibrous structure, cellulose has great strength. Cellulose is hygroscopic. Its solubility is very limited and it often breaks down the backbone. The cellulose backbone can be cleaved chemically (acid catalysis) and enzymatically (cellulose enzyme). Solubility depends on the molecular weight of the cellulose.

Cellulose is isolated from wood for commercial purposes by removing other components (lignin, hemicellulose, oils). Cellulose fiber is used in the paper and textile industry.

Shorter cellulose molecules, often substituted and branched, are called hemicelluloses. Hemicellulose forms straight, linear chains containing both pentoses and hexoses. Lignin is a high molecular weight polyphenolic amorphous substance. Lignin ensures wood cell walling.

Tab.: Components of wood[1]

Component	Cotton [%]	Coniferous wood [%]	Deciduous wood [%]
Cellulose	90-94	50-58	52-54
Pentosa	1,5-2,0	11,0	25,0
Lignin	2,0-3,0	26,0-28,0	17,0
Pectin substances	2,0	1,0	1,5-2,0
Proteins	1,5-2,0	0,5-0,8	0,5-0,8
Fats and waxes	0,5-1,0	1,0-2,0	1,0-2,0
Ash	1,0	0,25-0,5	0,25

A. DETERMINATION OF THE SOLUBILITY OF CELLULOSW IN SODIUM HYDROXIDE

Equipments:

Erlenmeyer flask 250 ml (2x), filter paper, Büchner funnel, aspirator, Petri dish (2x), measuring cylinder 100 ml, funnel (3x), spoon, oven, beaker, cooker, thermometer up to 100 °C, pH papers, scissors

Chemicals:

1% NaOH, 10% CH_3COOH, cellulose (cotton sheath, sisal, jute or hemp rope…)

Workflow:

1. Weigh 2.0 g of cellulose into an Erlenmeyer flask, add 100 ml 1% NaOH (60 °C), mix it.
2. Place the funnel on the flask and heat in a boiling water bath for about 1 hour. Mix the contents of the flask regularly at ten minute intervals.

3. Filter the contents of the flask through pre-weighed filter paper on a Büchner funnel.
4. Wash the flask with 60 ml of hot water and pour onto a filter.
5. Wash the filter cake with 50 ml of 10% CH_3COOH and hot water until the filtrate is neutral. Use a pH paper to check the pH of the filtrate.
6. Remove the filter cake from a Büchner funnel, place in a pre-weighed Petri dish with lid and dry for at least 90 minutes in a oven heated to 103 ° C ± 2 ° C to constant weight.

Evaluation and conclusion:

Determine the insoluble cellulose content of the sample according to the formula:

$$unsoluble\ cellulose(\%) = \frac{mass\ of\ soluble\ cellulose(g)}{total\ sample\ mass(g)}\ 100$$

Calculate the content of insoluble cellulose calculated as absolutely dry cellulose from the formula:

$$f = \frac{100}{100 - vlhkostcelulosy(\%)}$$

The soluble cellulose content drawn on to absolutely dry cellulose can then be determined by recalculating:

$$C_{soluble}(s) = 100 - C_{unsoluble}(s)$$

As a result, give the soluble and insoluble cellulose content. Compare several samples (filter paper, cotton wool, swabs, cartridge, sheath, etc.)

B. DETERMINATION OF WOOD MOISTURE

Equipments:

oven, analytical scales, exicator, beakers, petri dishes (2x), teaspoon

Test sample:

wood sawdust

Workflow:

1. Weigh a dry beaker on an analytical scale. Weigh approximately 1 g of sawdust.
2. Cover the beaker with a petri dish and allow to dry at 105 ° C for 2 hours.
3. Leave the beaker and the lid to cool and weigh the beaker containing the contents again on an analyticalscale.
4. Repeat this procedure at 1 hour intervals until the sample weight is within 0.2 mg.

Evaluation and conclusion:

Determine the moisture content of the wood sample using the formula:

$$v = \frac{B - C}{B - A} 100 \quad (\%)$$

where v = wood moisture in %,
A = weight of the empty beaker in g
B = mass, in g, of the sample beaker before drying
C = mass, in g, of the sample beaker after drying
Express the moisture content of the wood by the arithmetic mean of two parallel determinations.

C. DETERMINATION OF α CELLULOSE (by the Tappi method)

Equipment:

beaker 250 ml (2x), measuring cylinder 10 ml, 50 ml (2x), 100 ml, spoon, watch glass, aspirator, filter cup S1 (2x), beaker 400 ml, funnel medium (2x), oven

Chemicals:

10% CH_3COOH, 17.5% NaOH, filter paper (as a cellulose source), cellulose

Workflow:

1. Weigh approximately 3 g of cellulose into a 250 ml beaker on an analytical scale.
2. Add 35 ml of 17.5% NaOH at 20 ± 0.2 °C to cellulose and allow to stand for 3 minutes.
3. Then use a glass rod to mix and pulp for 3 minutes.
4. After 3 minutes, add an additional 10 ml of 17.5% NaOH and pulp for 2.5 minutes.
5. Add 10 ml of 17.5% NaOH again and pile for 3 minutes.
6. Next, add 10 ml of 17.5% NaOH and wait for only 1 minute.
7. Cover the beaker with a watch glass and allow to stand for 25 minutes.
8. Add 25 ml of water at 20 ° C and mix well with a spoon (approx. 1 minute)
9. Filter the pulped cellulose through a pre-weighed and dried frit.
10. Wash the filter residue with 300 ml of water at 20 ± 0,2 °C with constant suction.
11. Stop suction and pour 40 ml of 10% CH_3COOH at 20 ± 0,2 °C onto the filter residue and allow to react for 5 minutes.
12. Aspirate the acid and wash the residue on the frit again with 300 ml of 20 ° C water.
13. Dry the crucible and residue in an oven at 103 ± 2 ° C until constant weight.
14. After cooling in an exicator, weigh.

Evaluation and conclusion:

Determine the content of α cellulose (αCTAPPI) in [%] according to the formula:

$$aC_{TAPPI} = \frac{m_1}{n_1} 100$$

where m_1 = mass α of cellulose after drying in g,

n_1 = weight of cellulose for determination in g.

D. DETERMINATION OF α CELLULOSE (using Klauditze method)

Principle:

$$C_6H_{10}O_5 + 4\ K_2Cr_2O_7 + 16\ H_2SO_4 \rightarrow 6\ CO_2 + 6\ K_2SO_4 + 4\ Cr_2(SO_4)_3 + 21\ H_2O$$

Equipments:

Erlenmeyer flask 100 ml (4x), measuring cylinder 5 ml, 10 ml (2x), 50 ml, 100 ml, spoon, measuring flask 100 ml (4x), pipette 5 ml, 20 ml, aspirator, frit S1 (2x), 600 ml beaker, cooker, 250 ml titration flask (2x), medium funnel (2x).

Chemicals:

96% H_2SO_4 p.a., 17,5 % NaOH**,** 0,2 M $Na_2S_2O_3$, 1 M $K_2Cr_2O_7$ – toxic!!!

Workflow:

1. Weigh 3,0 g cellulose into a 75 ml conical flask on an analytical scale.
2. Add 40 ml of 17.5% NaOH at 20 ± 0.2 °C
3. Shake it in your hand until the contents of the flask are shredded and homogenized (approx. 10 minutes).
4. Allow to stand for 15 minutes at 20 °C, then shake it vigorously by hand (approx. 1 minute) and allow to stand for another 15 minutes at 20 °C. Mix it thoroughly with a spoon (1 minute) and filter through a filter crucible with suction.
5. Wash the filter cake with 50 ml of 20 ° C water.
6. Pour the wash water filter solution into a 100 ml volumetric flask and make up with distilled water.

7. Pipette 1 ml of filtrate into a 50 ml Erlenmeyer flask, add 2 ml of 1 M $K_2Cr_2O_7$ and 2 ml of concentrated H_2SO_4.
8. Boil it for 1 minute (hot stone!).
9. Cool the Erlenmeyer flask under running water.
10. Pour the contents of the flask into a 25 ml graduated flask, make up to the mark with distilled water, mix.
11. Pipette 25 ml of solution into the titration flask, add 100 ml of water and 10 ml of 5% KI. Leave it in the dark for 5 min.
12. Titrate 0.1 M $Na_2S_2O_3$ to the yellow color of the solution, then add 5 ml of starch wax and titrate to blue color.

Results:

Total mass of α–cellulose ($AC_{Klauditz}$) in %

$$AC_{Klauditz} = 100 - RS$$

$$RS = \frac{V_1 \ 0{,}000675 \ N}{n_2} \ \text{from} \ 100$$

where V_1 = consumption of 0,1 M $Na_2S_2O_3$ by titration in ml,
n_2 = weight of cellulose for determination in g,
z = dilution (100)
M = molarity of $Na_2S_2O_3$ solution,
0.000675… cellulose conversion factor

Conclusion:

The total mass of α–cellulose as the average from 3 titration results.

E. DETERMINATION OF HOLOCELLULOSE IN WOOD (Wise method)

Equipments:

Erlenmeyer flask 250 ml (2x), funnel, spiral mixer (2x), weighing bottle (2x), water bath, measuring cylinder 25, 50, 250 ml, oven, analytical scale, desiccator, filter paper, cooker, Büchner funnel (1x) , Petri dish (2x), 400 ml beaker, spoon, aspirator

Chemicals and material:

wood sawdust, ice CH_3COOH, $NaClO_4$

Workflow:

1. Weigh approximately 1.5 g of sawdust into an Erlenmeyer flask.
2. Add a magnetic stirrer.
3. Add 160 ml boiling water, 2.5 ml g glacial acetic acid and 2.5 g $NaClO_4$ to the sawdust.
3. Place the flask in a boiling water bath and heat for 40 minutes while stirring.
4. After 40 min. add 2.5 ml glacial acetic acid and 2.5 g $NaClO_4$ again and heat for a further 40 min.
5. After another 40 min. add 2.5 ml glacial acetic acid and 2.5 g $NaClO_4$ again and heat for a further 40 min.
6. After delignification, cool the contents of the flask with a stream of cold water.
7. Filter the mixture through pre-weighed filter paper (analytical scale) on a Büchner funnel. Wash the filter cake with 2 x 50 ml cold water and then with 20 ml acetone.
8. Dry the isolated Holocellulose filter cake on a petri dish in an oven heated to 105 ° C until constant weight.

Evaluation and conclusion:

Determine the content of holocellulose in wood according to the relation:

$$H = \frac{V}{n} 100 \quad (\%)$$

$$H_S = H \quad \text{f} \quad (\%)$$

$$f = \frac{100}{100 - v}$$

where H = Holocellulose content in wood in%
HS = wood holocellulose content in% calculated on wood dry matter
V = mass of fibrous material (holocellulose) on the crucible in g
n = weight of the wood sample in g
f = conversion factor for dry matter
v = wood moisture in%
As a result, prove the content of holocellulose in the wood and wood dry matter.

F. DETERMINATION OF LIGNIN IN WOOD (Komarov method)

Equipments:

weighing bottle, funnel, spoon, measuring cylinder 25 ml and 250 ml, beaker 100 ml, oven, condenser (2x) , cooker, bar, hour glass

Chemicals:

72% H_2SO_4, sawdust dried at 105 ° C (from previous task)

Workflow:

1. Weigh 1 g of dry sawdust into a beaker.
2. Add 15 ml of 72% H_2SO_4, cover with a watch glass and place on a water bath at 25 ° C for 2 hours. Mix the sample regularly.
3. Then pour the mixture gently into a 500 ml boiling flask with 150 ml of water and reflux for 1 hour.
4. Allow lignin as a dark brown powder to settle on the bottom of the flask and filter through a pre-weighed frit. Wash the filter cake with 50 ml of hot water.
5. Dry the isolated lignin frit in a oven at 105 ° C to constant weight

Calculation:

The lignin content of wood can be determined according to the formula:omůcky:

$$L = \frac{V_L}{n} 100 \quad (\%)$$

where L = wood lignin content in%,
VL = mass of lignin on the crucible in g,
n = weight of the sawdust sample in g.

Conclusion:

Report the calculated lignin content of the sample. Based on the table in the theoretical part, estimate whether sawdust came from a deciduous or coniferou

G. SIMULATION OF PERGAMEN PAPER PRODUCTION

Equipments:

600 ml beakers (2x), tweezers, filter paper

Chemicals:

96% H_2SO_4, NH_3

Workflow:

1. Mix 30 ml 96% H2SO4 with 15 ml ice-cold distilled water in a glass beaker (large beaker). Cool the prepared solution to about 5 ° C in an ice bath.
2. Cut two equally sized pieces from the filter paper.
3. Immerse the first part in cold H_2SO_4 solution using tweezers and place it immediately in the beaker with distilled water and 10 drops of NH_3 immediately after removal.

Leave the paper to wash for at least 5 minutes and then dry on a smooth surface.

Conclusion:

Compare the properties of paper immersed and not immersed in H_2SO_4 (mechanical strength, elasticity, wettability).

H. PREPARATION OF CELLULOSE NITRATE

Equipments:

250 ml Erlenmayer flask, glass rod

Chemicals:

HNO_3, H_2SO_4, ethanol, camphor, acetone

Workflow:

1. Carefully prepare the nitration mixture in an Erlenmeyer flask in a fume cupboard by mixing 12 ml of HNO_3 with 20 ml of H_2SO_4.
2. Cool the nitration mixture to 20 ° C in an ice water bath.
3. Put about 1 g of cotton (cellulose) into the nitration mixture, close the flask and mix for about 5 minutes.
4. Carefully remove the cotton wool from the nitration mixture into a beaker of water and wash it (change the water at least twice).
5. Dry the washed nitrated cotton wool between the filter papers and dry in a slightly switched-on oven (or let it dry until the next exercise).
6. Divide the nitrate cellulose into 4 parts.
7. Compare the solubility of nitrocellulose and cellulose in acetone.
8. Compare the solubility of nitrocellulose and cellulose in ethanol.
9. Weigh the third piece to the weight of 0,5 g nitrocellulose. Dissolve 0,1 g camphor in 8 ml acetone in a test tube. Add 0.5 g of ethanol-moistened nitrocellulose to the prepared solution. Close the tube and mix thoroughly.
10. Pour the viscous solution onto a petri dish, store in a fume cupboard and allow to dry for the next lesson. A translucent celluloid film is formed on a Petri dish.
11. Carefully place the fourth (remaining) piece of cotton wool on the annealing net (bold on the open hand) and light it.

Conclusion:

Compare the solubility of cellulose and nitrocellulose, record the cellulose nitration process by chemical equation (including preparation of nitration mixture).

PROTEINS AND AMINO ACIDS

Proteins are the basis of all known organisms where they perform the building functions (collagen, keratin, elastin), transport (hemoglobin), catalytic (enzymes, hormones), protective and defensive (immunoglobin, fibrin, fibrinogen), locomotor (actin, myosin). The basic structural particles of proteins are amino acids. Some amino acids are capable of being produced by the body, others must be consumed in the diet (the so-called essential amino acids). In proteins, amino acids are linked to each other by amino groups -NH_2 and carboxyl groups -COOH by an amide bond -NH-CO-, which is called a peptide bond.

We distinguish between primary, secondary, tertiary and, in some more complex proteins, quaternary structure of protein chains.

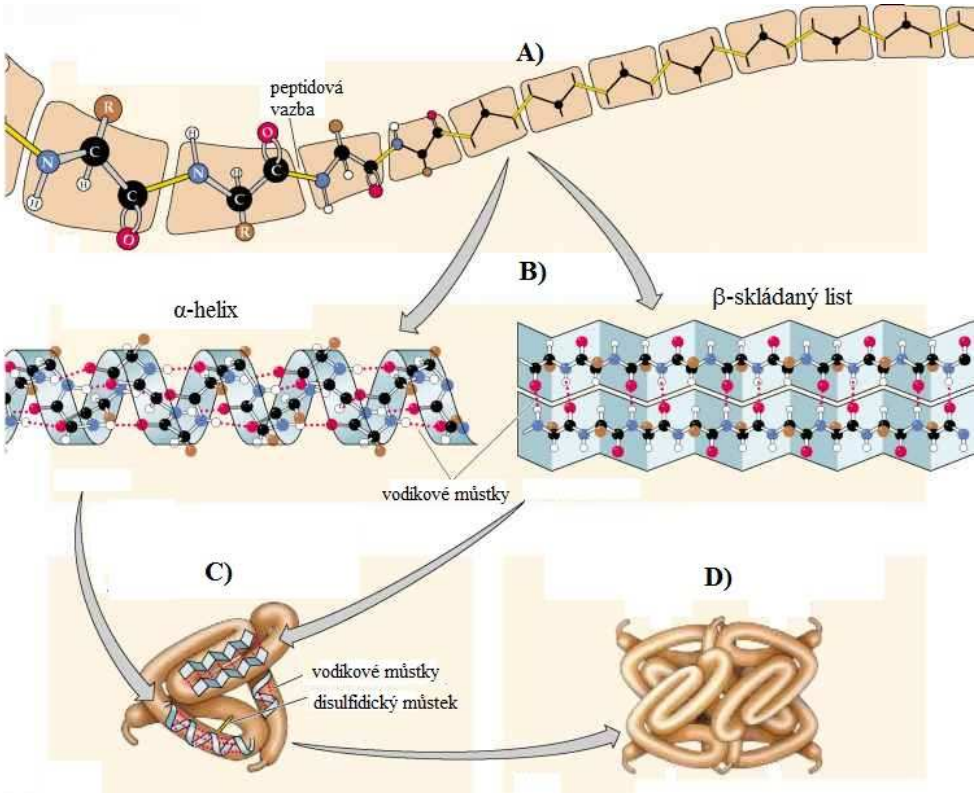


Fig. 7: protein structure - A) primary; B) secondary; C) tertiary; D) quaternary

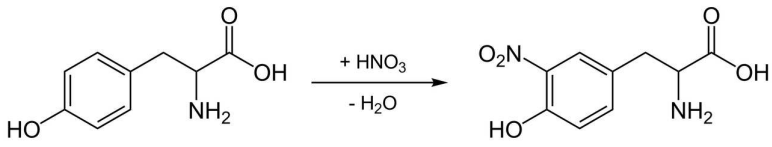
Task 3: PROOF OF PROTEINS / AMINO ACIDS

Biuret reaction

The biuret reaction is a reaction in which the protein is detected using a mixture of sodium hydroxide solutions and copper sulphate. The color turns to blue-violet when proteins are detected. By biuret reaction we prove peptide bonds by which amino acids bind to each other. These form a colored complex - biuret - in an alkaline environment with copper salts.

Xanthoprotein reaction

A protein providing a positive response to xanthoprotein reaction must have an aromatic nucleus such as tyrosine or tryptophan. The xanthoprotein reaction is nitration, here is the nitration of tyrosine.



Equipments:

filter paper, glass rod, beaker, test tubes, cotton wool, funnel, burner, tongs

Chemicals:

0.1M NaOH, 0.05M $CuSO_4$. $5H_2O$, conc. HNO_3

Samples:

the egg white

Workflow:

1. Dilute the egg white with water 1: 2, mix it and filter through a cotton ball in the funnel.
2. Divide the filtrate into two tubes. The contents of the tubes are as follows:

a. First tube: To 1 ml of the protein filtrate, add 1 ml of 0.1 M NaOH and 1 ml of 0.05 M $CuSO_4$. $5H_2O$. Typical blue-violet coloration for biuret reaction is formed.

b. Second tube: add 1 ml of HNO_3 to 2 ml of protein filtrate and gently heat on a water-bath until a precipitate is formed. Carefully add a drop of NaOH to the precipitate, first neutralizing the reaction between HNO_3 and NaOH, observing the formation of orange xantoprotein with an excess of NaOH.

Conclusion:

Record observations in the report.

Task 4: ACID HYDROLYSIS OF PROTEINS (denaturation of collagen)

Collagen is a complex protein, forming approx. 25 - 30% of bone weight and approx. 36% by weight of leather. Thus, bones and skins are the main raw materials for the manufacture of glues and gelatins, both denatured collagen. Gelatin has more molecular weight and higher purity, whereas glue has more degraded molecular weight (i.e., lower Mw) and lower purity.

In both cases, the first step of production is the leaching of slightly denatured collagen from bones or skins in a slightly alkaline environment (pH approx. 10) to a so-called glutinous solution.

Since this technological step is time consuming, we start from industrially produced collagen.

Equipments:

250 ml flask, reflux condenser, stirrer, pipette, 100 ml graduated cylinder, oil bath with bath temperature control, petri dishes, universal indicator paper

Samples:

Collagen (indursty produced)

Chemicals:

Conc. H_2SO_4, 2 M NaOH, 2 M H_2SO_4

Workflow:

1. Prepare a suspension of 5 g of industrial collagen in 150 ml of water in a flask.
2. Add 5 drops of concentrated H_2SO_4 to the suspension.
3. Heat for 30 min while stirring.
4. Allow the contents of the flask to cool to room temperature and neutralize with 2 M NaOH, checking with pH indicator paper.
5. Remove 10 ml of the cooled sample into a Petri dish.
7. If jelly does not form, place the solution in a rotary evaporator and concentrate it.
8. After reducing the volume. try creating a so-called glue galert.
9. Repeat the procedure, adding 10 ml of 2 M NaOH instead of H_2SO_4 and neutralize with 2 M H_2SO_4 after reaction.

Conclusion: Describe viscosity.

Task n. 5: DETERMINATION OF AMINO ACIDS / PROTEINS

A. DETERMINATION OF FREE AMINO ACIDS

Equipments:

spectrophotometer, 1 ml and 5 ml graduated pipettes, cooker, 500 ml beaker, stoppered tube (10x), small funnel (10x), graduated flask

Chemicals:

96% ethanol, phosphate buffer (pH = 7.2), ninhydrin solution, complexon III solution, alanine solution

Ninhydrin solution (freshly prepared): Weigh 0,2 g ninhydrin into a 50 ml graduated flask, dissolve in 30 ml ethanol, add 0,4 ml CH_3COOH and make up to the mark with ethanol.

Alanine solution: Weigh 1.5 g of alanine (record accurate weight). Transfer quantitatively to a 250 ml graduated flask, dissolve in distilled water, make up to the mark and mix.

Sample:

Toma Relax Orange juice (declared protein content about 0.6%)

Workflow:

1. **Calibration curve for alanine:**

   a. Pipette 2 - 3 - 4 - 5 - 6 - 7 ml of alanine solution into 6 tubes with stopper and make up to 8 ml with distilled water.

   b. Add 3 ml of phosphate buffer and 1 ml of ninhydrin reagent to each tube.

   c. Mix tube contents, place small funnels in tube throat and immerse all tubes in boiling water bath for 10 min.

   d. Carefully remove the tubes and cool by immersion in an ice beaker.

   e. Add 0.1 ml complexon III solution, 5 ml ethanol and mix well to each sample.

   f. Pipette 1.0 ml of the dark blue sample into 100 ml graduated flasks from each tube and make up to the mark with distilled water.

   g. Measure the absorbance on the spectrophotometer at 570 nm against the blank.

2. **Sample:**

   a. Pipette 3 ml juice and 5 ml distilled water into the tube, add 3 ml phosphate buffer and 1 ml ninhydrin solution, mix.

   b. Place a small funnel into the neck of the tube and immerse the entire tube in a boiling water bath for 10 min. Cool the contents of the tube in an ice water bath.

   c. Add 0.1 ml of the complexone III solution and 5 ml of ethanol to the sample. Mix well.

   d. Collect 1 ml of sample into a 100 ml graduated flask and make up to the mark with distilled water.

   e. Measure the absorbance on the spectrophotometer at 570 nm against the blank.

Blind experiment:

Carry out the blank preparation as well as the real sample preparation, only in point 1, instead of 3 ml juice and 5 ml distilled water, pipet only 8 ml distilled water into the tube.

Conclusion:

The amount of aminoacids ($mg.l^{-1}$) :

$$w_N = \frac{m_a \, r}{V} \, 1000$$

where m_a = amount of alanin from calibration curve in mg,
r = digree of dilution,
V = sample volume in ml.

As a result, give a graph of absorbance versus alanine concentration at 570 nm (calibration curve) with the point corresponding to the measured value of the real sample. Indicate how many mg of amino acids are present in 1 liter of juice, compare with the value on the product packaging.

B. DETERMINATION OF ACTIVE ACIDITY OF DAIRY PRODUCTS

Equipments:

pH-meter, beaker 50 ml (3x)

Sample:

OLMA milk, whipping cream Kunín, acidophilic milk, soft cottage cheese, Lučina cheese etc.

Workflow:

Perform the determination three times on two selected samples.

1. Pour the liquid sample into a 50 ml beaker or weigh 10 g of the solid sample, add 30 ml of distilled water and homogenise by shaking.
2. Immerse the pH meter electrode in the sample to be analyzed.
3. After stabilizing, read the pH value on the instrument scale.

Evaluation and conclusion:

As a result, give the arithmetic mean of the active acid values of both samples. Determine the characteristics of the dairy product by comparing the measured values with the values in the table.

Tab. pH value of some milk products.

pH value	Milk product characterization
6,5 – 6,7	Milk sweet
6,3 – 6,4	Less Sour milk
5,4 – 6,2	Sour milk
6,8 – 7,1	Milk probably diluted with water or with the addition of alkalis, or milk from sick dairy cows or old milk with proteolytic decomposition
4,6	Precipitation of casein from milk (isoelectric point)
5,1	Cream for making sour butter
6,3	Cream for the production of partially acidified butter
5,2	Yoghurt acidity limit value

B. DETERMINATION OF CASEIN ACIDITY

The acidity of casein can be expressed as the number of milliliters of a standard solution of 0.1 M NaOH needed to neutralize the acidic components per g of casein.

Equipments:

Erlenmeyer flask 250 ml, measuring cylinder 25 and 100 ml, medium funnel (2x), magnetic stirrer with heating, thermometer up to 100 ° C.

Chemicals:

0.15M NaOH, 1% phenolphthalein solution, 0.1M HCl (prepared titration solution)

Workflow:

1. Weigh 1 g casein and pour into a 250 ml conical flask.
2. Add 25 mL of 0.15M NaOH solution. Heat slightly (40 ° C) until it completely dissolved.
3. After reconstitution, add a few drops of 1% phenolphthalein and titrate with 0.15M HCl until discolored.

Evaluation and conclusion:

Perform the determination trhee times and give the result as the arithmetic mean of the values.

Note: Casein made from milk has an acidity in the range of 8.8 - 13 ml of 0.1 mol / l NaOH; casein in cheese has an acidity of 1.6 - 2.8.

C. DETERMINATION OF FAT CONTENT IN CASEIN

Equipments:

measuring cylinder 10 and 100 ml, medium funnel (2x), 50 ml Erlenmeyer flask, 100 ml beaker (2x), 150 ml divider (2x), 250 ml boiling flask, thermometer up to 100 ° C, cooker

Chemicals:

37% HCl, 96% ethanol, petroleum ether

Workflow:

1. Weigh 3 g casein into an Erlenmeyer flask and add 15 ml HCl and cover with a stopper.
2. Heat the mixture to 40 ° C in the water bath until the casein is dissolved.
3. After complete dissolution, cool the solution and add 10 ml of hot water.
4. Pour into a separating flask.
5. Put 40 ml of ethanol and pour the contents into a separatory funnel and repeate it with light petroleum (50 ml), which is also poured into the separatory funnel.
6. Shake the contents of the separating flask thoroughly (be careful to pressurize the apparatus). Drain the bottom layer into a 250 ml beaker. Pour the upper layer into a pre-weighed 250 ml cooking flask with cooking stones.
7. Return the bottom layer to the separating flask and add 20 ml light petroleum and 10 ml ethanol. Shake and add the light petroleum layer to the boiling flask.
8. Evaporate the light petroleum in a water bath. Dry the remainder of the light petroleum in a chemical oven at 80 ° C.
9. After cooling the flask in the desiccator, weigh it.

Evaluation and conclusion:

Determine the mass of fat in casein in %:

$$\text{Fat in casein} = \frac{mass\ of\ isolated\ fat}{mass\ of\ weighted\ fat}\ 100$$

D. DETERMINATION OF ASHESE IN CASEIN

Equipments:

temperature muffle furnace, analytical balance, desiccator, annealing crucible (2x), teaspoon

Chemicals:

casein

Workflow:

1. Weigh the crucible and the cooled crucible.
2. Weigh approximately 1,5 g casein on an analytical balance.
3. Put the sample in crucible over the gas burner and calcined in a muffle furnace at 500 ° C for 30 minutes.
4. Allow to cool for at least 5 minutes on a metal mesh.
5. Transfer the crucible to the desiccator and allow it to cool to room temperature. Then weigh the crucible.
6. Repeat this process to constant ash weight (range 0.2 mg).

Evaluation and conclusion:

Determine the ash content in casein in% by weight according to the formula:

$$\text{ash} = \frac{mass\ of\ ash\ in\ g}{mass\ of\ the\ total\ sample\ in\ g}\ 100$$

Write down the ash content and compare it with the casein ash value reported in the literature.

E. DETERMINATION OF CASEIN VISCOSITY

Equipments:

measuring cylinder 5 and 50 ml, funnel, beakers, thermometer up to 100 ° C, glass rod, stopwatch, burettes

Chemicals:

casein, 27% NH_3

Workflow:

Work in a fume hood !!

1. Weigh 2, 4, 6, 8 and 10 g ± 0.1 g casein into 150 ml beakers, add 20 ml water and 2 ml NH_3.
2. Heat the mixtures in a water bath at 60 ° C until the casein is completely dissolved while stirring with a glass rod.
3. Prepare 5 burettes in which to pour still warm casein solution. Work quickly (all solutions should be approximately equally warm).
4. Use a stopwatch to determine the times before 10 ml of casein solution flows out from the burettes.
5. Pour the residual casein solution out of the burettes, cool to 30 ° C and sensoryly evaluate with a glass rod (observe especially viscosity).

Evaluation and conclusion:

As a result, give a graph of the concentration of casein solution in the burette flow rate. Describe the appearance and behavior of each cooled casein solution.

F. DETERMINATION OF PROTEINS IN MILK (Pyne method)

Equipments:

titration flask (3x), 1 ml graduated pipette, measuring cylinders 5, 10 and 50 ml, pH-papers, filter paper, burette

Chemicals:

1% ethanolic phenolphthalein solution, saturated $C_2K_2O_4$ solution. H_2O, 0.143M NaOH, neutralized formaldehyde, 0.0025% fuchsin solution, milk

Workflow:

1. Pour 50 ml of milk into the titration flask using a graduated cylinder, add 0.5 ml of phenolphthalein solution and 2 ml of $C_2K_2O_4$. H_2O. Mix the contents of the flask and let it to stand the titration flask for 2 minutes.
2. Neutralize the solution in the flask with 0.143M NaOH (check pH with paper) and add 10 ml of neutralized formaldehyde. Mix the contents of the flask and let to stand it for 2 minutes.
3. Titrate the bleached solution with 0.143M NaOH to the color of the reference solution.
4. Prepare the reference solution in a second titration flask. To 50 ml of milk add 3 ml of fuchsin solution and mix.
5. Perform the titration three times.

Evaluation and conclusion:

The percentage of protein in milk may be determined by the conversion rate (0,348), based on the observed titration of NaOH:

% proteins = (volume 0,143M NaOH for neutralization during titration in ml) . 0,348

As a result, give the percentage of protein in the sample calculated as the arithmetic mean of the three titrations.

G. DETERMINATION OF PROTEINS IN MILK (Schulz method)

Equipments:

titration flask (3x), 1 ml graduated pipette, measuring cylinders 5, 10 and 50 ml, magnetic stirrer, pH-papers, filter paper

Chemicals:

1% ethanolic phenolphthalein solution, saturated $C_2K_2O_4$ solution. H_2O, 0.143M NaOH, neutralized formaldehyde, 5% $CoSO_4$ solution, milk

Workflow:

1. Pour 25 ml of milk into the titration flask using a graduated cylinder, add 0.5 ml of phenolphthalein solution and 1 ml of $C_2K_2O_4$. H_2O. Mix the contents of the flask and leave to stand for 2 minutes.
2. Neutralize the solution in the flask with 0.143M NaOH and add 5 ml of neutralized formaldehyde. Mix the contents of the flask and leave to stand for 2 minutes.
3. Titrate the bleached solution with 0.143M NaOH to the color of the reference solution.
4. Prepare the reference solution in a second titration flask. To 25 ml of milk add 1 ml of potassium oxalate solution and 0,5 ml of $CoSO_4$ solution, mix.
5. Perform the test three times.

Evaluation and conclusion:

The consumption of 0.143M NaOH in the determination of proteins by the Schulze method indicates the so-called protein titre, ie directly the percentage of proteins in the milk sample. As a result, give percentages by volume of protein in milk. Compare the result to that obtained from the Pyne protein determination.

Task n. 6: PRODUCTION OF CASEIN GALALITE

Equipments:

beakers of 100, 150 and 2 x 600 ml, Petri dish, strainer, cloth handkerchief, spoon, gloves

Chemicals:

milk, commercial casein, vinegar, formaldehyde

Workflow:

1. Measure 100 ml of vinegar and 150 ml of milk in a beaker.
2. Filter the resulting precipitate over a cloth handkerchief, squeezing out all possible liquid. Use gloves.
3. Divide the mass into three parts.
4. Add one third of the mass to 1% formaldehyde solution and boil for 5 minutes. Use gloves to filter through a cloth handkerchief. Shape a knob from the mass on the canvas and let it dry for the next lesson.
5. Shape a straight knob from the second and third thirds.
6. Place the second third in formaldehyde solution and allow to cure in a closed Petri dish for the next lesson.
7. Allow the third knob to dry only at room temperature.

Evaluation and conclusion:

Compare the appearance and hardness of the material.

FATS, OILS, WAXES

The chapter "Fats, oils and waxes" is included in this lesson because these materials play an important role in the field of restoration and conservation chemistry. Oxygen, temperature and UV radiation, which catalyses the polymerization process, so-called drying oils in paintings.

Fats (lipids) are esters of higher fatty carboxylic acids and a trihydric alcohol (glycerol). According to the physical state, we distinguish solid fats, which are predominantly saturated fatty acids, and oils whose physical state is liquid and which contain larger amounts of unsaturated fatty acids. Another basic division is the division into vegetable fats and animal fats.

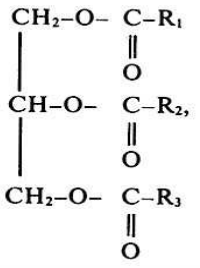


Fig. 4: Fat formula

Task n. 7: FAT CHARACTERISTICS

A. PEROXIDE NUMBER

Equipments:

250 ml Erlenmeyer flask + stopper (3x), graduated cylinders 5 and 50 ml, pipette 1 ml, spoon, funnel, 100 ml graduated flask, beaker 10 ml, cooker, thermometer.

Chemicals and solutions:

chloroform, CH_3COOH, KI solution - 5 g of KI is dissolved in 5 ml of water (freshly prepared), starch sebum (stock solution), titration solution of sodium thiosulphate, c ($Na_2S_2O_3$) = 0,002 mol.l-1

Sample:

butter, margarine, lard …

Workflow:

The determination is performed twice on the same sample.

1. Prepare 75 ml of a mixture of chloroform and CH_3COOH in 100 ml graduated flask 1: 1.5
2. Weigh approximately 8 ± 0,0001 g into an Erlenmayer flask.
3. Add 25 ml of chloroform-CH_3COOH and carefully (set low heating power on the cooker) to a maximum temperature around 40 ° C and heat at this temperature until the sample is dissolved.
4. Add (by pipette) 1.0 ml of KI solution, close the flask, mix gently and store in a dark place for 20 minutes.
5. Add 50 ml of distilled water and mix.
6. Add 5 ml of starch sebum and mix.
7. Titrate the contents of the flask with stirring by a standard solution of 0,002M $Na_2S_2O_3$ until the upper (aqueous) layer discoloures. If the bottom layer is purple, the flask is closed and the iodine present in the fat layer is shaken into the aqueous layer. This layer is then titrated to permanent decolorationy.
8. **Blank test:** Carry out in the same manner without sample adding. The mixture does not

Evaluation:

The peroxide number (PN), expressed in µg of oxygen per 1 g of fat, is calculated according to formula:

$$PN = \frac{(a-b)}{n} \; c \; f \; 8 \; 1000$$

where a = consumption of ref. $Na_2S_2O_3$ solution (c = 0,002 mol.l-1) for determination in ml,
b = consumption of rem. solution of $Na_2S_2O_3$ (c = 0,002 mol / l) per ml in blank,
c = concentration of standard solution of $Na_2S_2O_3$ in mol.l-1,
f = $Na_2S_2O_3$ volumetric solution factor,
n = test sample weight in g.

Conclusion:

The peroxide number is expressed by the arithmetic mean of two parallel determinations. The report shall also include a description of the sample used for the determination.

A. IODINE NUMBER

Equipments:

cooker, measuring cylinder 25 and 100 ml, beaker 150 ml, conical flask 300 ml with stopper (3x), spoon, funnel, pipette 1 ml

Chemicals and solutions:

chloroform, stock solutions: 0.1 mol / l iodmonobromide solution IBr (Hanus reagent), 20% KI solution, starch wax indicator, sodium thiosulphate solution, c($Na_2S_2O_3$) = 0,2M

Sample:

butter, margarine, shortening, lard,…

Workflow:

Perform the determination 3 times on the same sample.

1. Weigh 1 ± 0,0001 g of the sample into the conical flask.
2. Add 25 ml of chloroform and allow to dissolve; dissolution can be accelerated by gentle heating).
3. Add 25 ml of IBr solution, close the flask, mix with contents and store the flask in the dark for 1 hour
4. Then add 20 ml of KI solution and 100 ml of water.
5. Titrate the contents of the flask with stirring with a 0,2 M standard solution of sodium thiosulphate until an orange (orange-yellow) color is obtained.
6. Add 1 ml of starch sebum and titrate until discolored.

Blank: Perform in the same manner as the sample, omitting the test sample.

Evaluation:

The iodine number (IN) is given by:

$$IN = \frac{(a-b)\ f\ c\ 12{,}692}{n}$$

where: a = volumetric consumption. $Na_2S_2O_3$ solution in ml

b = consumption in ml of the standard $Na_2S_2O_3$ standard solution

f = $Na_2S_2O_3$ standard solution factor

c = concentration of the standard $Na_2S_2O_3$ solution

n = test sample weight in g.

Conclusion:

The iodine value is expressed by the arithmetic mean of two parallel determinations.

B. ACID NUMBER

Equipments:

cooker, metal spatula, measuring cylinder 50 ml, beaker 150 and 250 ml Erlenmeyer flask (2x), spoon, funnel

Chemicals:

96% ethanol, 3% ethanolic KOH solution, 0.1M ethanolic KOH solution, chloroform, 1% ethanolic phenolphthalein solution

Sample:

lard, vegetable oil, butter,…

Workflow:

Perform the determination 3 times with the same sample.

1. Weigh 20 g of the sample and transfer quantitatively to a 250-ml conical flask.
2. Pour 50 ml of ethanol and 50 ml of chloroform in a beaker. Neutralize the mixed solution to phenolphthalein until a slightly pink color with 3% ethanolic KOH.
3. Pour the neutralized solution onto the sample and heat the contents of the flask gently to the boil.
4. Add 5 drops of phenolphthalein solution and titrate while i tis hot with 0.1M ethanolic KOH until a pinkish violet color (30s).
5. Perform the tests three times.

Evaluation and conclusion:

The acid number (NA) can be determined as the mass of KOH (in mg) needed to neutralize 1 g of the sample.

$$NA = \frac{a\ c\ M}{n}$$

where: a = consumption of standard solution of 0,1 M KOH in ml,
c = concentration of the KOH standard solution (c = 0,1 mol.l-1),
M = the molar mass of KOH in g.mol-1,
n = sample weight in g.

Fatty acidity can be expressed in degree of acidity (SK), which indicates the consumption of KOH solution in ml to neutralize free acids in 100 g of fat. The following relationship applies between SK and NA:

$$SK = \frac{100\ NA}{56,11} = 1,7825\ NA$$

As a result, give the acid number and the degree of acidity as the arithmetic mean of each test.

C. SAPONIFICATION NUMBER

Equipments:

cooker, metal spatula, 250 ml Erlenmeyer flask (3x), measuring cylinder 50 ml, reflux condenser (2x), pipette 25 ml, spoon, funnel, cooking balls

Chemicals and solutions:

Stock solutions: 0.5 mol.l-1 ethanolic KOH solution, 0.5 mol.l-1 HCl, 1% phenolphthalein solution in ethanol

Sample:

lard, oil, shortening.

Workflow:

The determination is performed twice with the same sample.

1. Weigh 3 ± 0,001 g of the sample into an Erlenmeyer flask.
2. Pipette 25 ml of KOH solution and put several cooking stones.
3. Put a reflux condenser to the flask and cook it for 1 hour.
4. Then add about 5 drops of phenolphthalein indicator to the hot solution.
5. Titrate with 0.5 mol.l-1 HCl while it is hot until the pink (red) color disappears.

Blank: Perform in the same manner as the sample, omitting the test sample. The mixture does not boil, it only warms to boiling and then titrates.

Evaluation:

The saponification number (mg) in mg KOH per g of sample is calculated according to the formula:

$$NS = \frac{(V_0 - V_1)\ c\ f\ 56,1}{m}$$

where: V_0 = consumption of ml of standard solution of HCl (c = 0,5 mol.l-1),
V_1 = consumption of standard volumetric HCl solution (c = 0,5 mol / l), in ml,
c = concentration of HCl standard solution in mol.l-1,
f = HCl standard solution factor,
m = mass of the test sample in g.

Conclusion:

The saponification number is expressed by the arithmetic mean of the two determinations.
A description of the sample used for the determination shall also be included in the report.

D. ESTER NUMBER

The ester number indicates the number of mg KOH needed to saponify the esters in 1 g of fat. The ester number is calculated from the difference between the saponification number and the acid number:

EN = NS – SK

MODERN TRENDS IN POLYMER CHEMISTRY

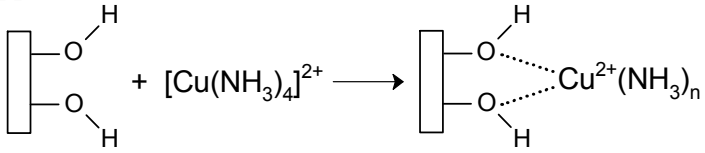
Current trends in the field of polymer chemistry (natural and synthetic polymers) focus not only on the recycling of polymeric materials, but also on the creation of nanostructures or replacement of the original materials.

Task n. 8: COPPER SILK

The principle of copper silk production was devised in 1857 by Eduard Schweizer, when pure cellulose was dissolved in a solution of copper (III) tetraamminium hydroxide $[Cu(NH_3)_4](OH)_2$, which can be prepared according to the equation:

$$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2 + Na_2SO_4$$
$$Cu(OH)_2 + 4NH_4OH \rightarrow [Cu(NH_3)_4(H_2O)_2](OH)_2 + 2H_2O$$

The binding of copper to cellulose follows this scheme:



The equilibrium of the reaction depends on the pH of the environment. In an acidic environment, the equilibrium shifts in favor of insoluble cellulose.

Equipments:

beakers, tweezers, Erlenmeyer flask (200 - 250 ml, 500 ml), Büchner funnel, filter flask, syringe and needle (capillary tube, plastic tube), pH paper, dropper.

Chemicals:

$CuSO_4.5H_2O$, NaOH, NH_3, H_2SO_4 or CH_3COOH

Saple:

Cellulose wadding - contains a part of the so-called viscose, which is regenerated cellulose
Cotton swabs - 100% cellulose
Handkerchief - 100% cellulose
Working jackets - 100% cellulose

Workflow:

Preparation of Schweizer reagent:

1. Prepare 250 ml of a 5% aqueous $CuSO_4$ solution.
2. Prepare 50 ml of an 8% aqueous NaOH solution.
3. Add the NaOH solution to the $CuSO_4$ solution. Mixing results in a blue $Cu(OH)_2$ precipitate.
4. Decant the precipitate with distilled water at least 3 times to remove soluble salts. Filter the precipitate on a Büchner funnel and transfer it to an Erlenmayer flask. Do not use frit!
5. Create a saturated solution of Schweizer reagent $[Cu(NH_3)_4](OH)_2$ by dissolving the $Cu(OH)_2$ precipitate in 70-100 mL of concentrated NH_3. Dissolve the precipitate in an Erlenmayer flask. If all of the $Cu(OH)_2$ does not dissolve, filter the remaining precipitate on a frit dedicated for this task.

Preparation of copper silk:

1. Add 1 g of cellulose (cotton wool, mantle, facial pads, tissue, etc.) to a flask containing 100 ml of filtered Schweizer reagent solution.
2. Shake the solution occasionally for 60 min and watch the sample dissolve.

Filter the undissolved cellulose on a dedicated frit, wash with water and dry in an oven at 150 ° C for 2 hours. Then consider and calculate the amount of dissolved cellulose. If the cellulose dissolves slowly, let it dissolve until the next exercise.

3. Prepare 100 ml of precipitation solution. Add H_2SO_4 dropwise to distilled water until an acidic solution of pH 2-3 is obtained (check pH with indicator paper)

4. Draw the Schweizer reagent / cellulose mixture into the syringe with the needle and the tube. Slowly add the solution into the precipitation solution.

5. Copper silk fibers are formed in an acidic solution.

6. Filter the precipitated fibers on a frit (Büchner funnel), dry for 2 hours at 150 ° C in an oven and weigh.

Conclusion:

Give your own observation of the course of the reaction, describe the resulting product. Note the weight of the precipitated fibers and the percentage yield with respect to the initial weight and the dissolved cellulose. Provide all necessary calculations.

Task n. 9: ELECTROSPINNING OF NATURAL POLYMERS

The electrospinning method allows to create nanofiber structures from a polymer solution. The arrangement is based on two opposing electrodes. The first electrode (spinning electrode, so-called emitter) is used for dosing of polymer solution and formation of nanofibres. The electrode can be in the form of a simple needle electrode (for smaller sample amounts) to large cylindrical or string electrodes rotating in a polymer solution. The second electrode, the collector, is used to store the fibers. Due to high electrical voltage (up to tens of kV), a cone shape, called Taylor cone, forms on a drop of polymer solution. By further increasing the electric field, the surface forces are destroyed and the fibres are created from the top of Taylor cone. The fibres transfer to collector. They are elongated and the rest of solvent is evaporated on this way.

Due to the chaotic movement of the fibers towards the collector, the resulting layer has a structure of randomly arranged fibers. The final structure of nonwoven textile is depend on polymer type and solution parameters (include viscosity, concentration, molecular weight of the polymer, solvent properties, surface tension or conductivity). The spinability of the solution is determined by the concentration of the fibrous polymer.

Electrospinning method is mostly used in biomedical applications. The ideal material is biocompatible, inflamable, nontoxic. It is usually more difficult to convert natural biopolymers into nanofibers by electrospinning than synthetic polymers. Natural polymer solutions often have too high viscosity and surface tension, which limit their spinnability. Electrospinning is used in medicine, agriculture, as a protective material.

Fig-: 5, 10: Nanospider TM (cit. http://academic.sun.ac.za/polymer/electrospinning.html, https://tech.ihned.cz/c1-23053440-nanotechnologie-z-liberce-maji-budoucnost-i-za-oceanem)

Equipments:

beakers, tweezers, Erlenmeyer flask (250 ml, 500 ml), Nanospider electrodes, Nanospider, napkins, stick, scales, graduated cylinder, pipette, scissors

Chemicals:

Organic solvent (THF, TFA)

Samples:

Polylactic acid (PLA)

Workflow:

1. Weigh PLA granules into an Erlenmayer flask with an electromagnetic stirrer and pour over the solvent until the resulting solution is 10%, 25% and 35%.
2. Place the flask on the electromagnetic mixer, let the PLA granulate dissolve (for the next exercise).
3. The spinning of the solution itself is carried out at UFE PřF MU using Nanospider TM.
4. Spin from the needle electrode first, where a sample of polymer solution is applied to the top of the electrode using a glass rod.
5. Select the most appropriate solution concentration.
6. Then spin the solution using a cylindrical electrode.
7. Cut the fiberized nonwoven sample together with the carrier fabric, allowing it to air dry.
8. Use SEM, describe the nanofiber structure, determine the fiber width (its average value depending on the concentration of the spinning solution).
9. Determine the wetting contact angle with the water drop.

Conclusion:

Report SEM images, fiber structure description, contact angle value.

Task n. 10: 3D PRINTING OF NATURAL POLYMERS

3D printing is the process of creating three-dimensional objects by laying continuous layers of polymeric materials. Several steps are required to print the whole product. The first step is to create a 3D model (eg using CAD software or a 3D scanner or photogrammetric software). This model must be converted in the second step to the printer format required. The next step is the suitable polymer selection.

The appropriate polymer is chosen on the base of its parameters (print head temperature, pad type and temperature). Subsequently, the printing material is coated in layers. Each time the layer is printed, the print head (or pad) moves one layer and the next layer begins printing again.

3D printing with polylactic acid (PLA) material will be realized as part of the excursion in CEITEC VUT.