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LIPIDS

(physico-chemical properties, determination of the acid value)

Task 1

The aim of this activity is to determine the acid value.

Procedure

Add 5 g of examined fat to the Erlenmeyer flask. Heat the flask in a water bath at 50°C until the sample will be completely melted. **Carefully** add 25 cm^3 of the alcohol-ether mixture and mix it.

To the clear solution add 5 drops of phenolphthalein and titrate with 0.1 M NaOH to achieve a stable color of the indicator in the Erlenmeyer flask.

Calculate the acid value (acid number, AN) of tested fat, using the information that the acid number is the mass of potassium hydroxide (KOH) in milligrams, that is required to neutralize free fatty acids in 1 g of fat.

The acid number is calculated as such:

$$\mathrm{AN} = \frac{5{,}611a}{g}$$

where:

a – volume of 0,1 mol/dm^3 KOH used to titration of fat sample (read from burette)

g – sample weight (5g)

5,611 – mass KOH [mg] in 1cm^3 of standard solution (0,1 mol/dm^3 KOH)

Task 2

The aim of this activity is to demonstrate the presence of unsaturated aldehyde (acrolein) in the examined fat sample.

Under the drainage substances glycerol loses 2 molecules of water and is transformed into unsaturated aldehyde – acrolein, which has unpleasant, specific and very easily recognizable smell.

Procedure

Add a pinch of potassium bisulfide and 2 drops of oil to the test tube. Carefully heat the tube until the appearance of an

irritating odor characteristic for acrolein. Put a strip of filter paper moistened with a solution of diamminesilver(I) complex at the mouth of the test tube. Filter paper becomes black by reducing Ag^+ ions to metallic silver by vapor emitted acrolein.

Precipitating metallic silver on the filter paper is black, because it is in the colloidal form.

Task 3

The aim of this activity is to detect the presence of multiple bonds in the examined sample.

Unsaturated fatty acids are oxidized under the influence of $KMnO_4$. The reaction allows to detect a multiple bond in the hydrocarbon chain of fatty acid and determines its location, because the oxygen derived from $KMnO_4$ is attached at the location of the bond and leads to degradation of the chain into fragments, which can be easily identified by physico-chemical methods.

Procedure

Add 5 cm^3 of 0,5 mol/dm^3 Na_2CO_3 and one drop of oil to the test tube. Heat the sample in a water bath at approx. 50°C for 2 minutes. Add 0,1 mol/dm^3 of $KMnO_4$ by drops shaking the tube after the addition of each drop of permanganate. $KMnO_4$ decolorises, and the end of the reaction is recognized when the color of solution is slightly pink.

Task 4

The aim of the activity is to determine the presence of the lipid oxidation products (rancidification), such as aldehydes and free fatty acids.

Unsaturated fatty acids are sensitive to the oxidation. Under the influence of light and trace amounts of metals (which are catalysts for reactions, unsaturated fatty acids break down into their metabolites: aldehydes and short-chain fatty acids. Aldehydes give rancid acids piercing, disagreeable and acrid smell, while short-chain fatty acids decrease the pH.

Procedure

a) Demonstrating the presence of aldehydes:

Add 0,5 cm^3 of rancid oil and 2 cm^3 of saturated NaCl solution to the test tube **A.**

Add 0,5 cm^3 of fresh oil and 2 cm^3 of saturated NaCl solution to the test tube **B.**

Heat the test tubes (A and B) over the burner until boiling,

and then add 5 drops of Schiff reagent to each tube. Compare the developed colors of the samples.

Schiff reagent is a specific compound used to detect aldehydes - when aldehyde is present a characteristic purplish-red color develops. The reagent does not react with ketones.

b) pH test for fats:

Add drops to the 3 wells of porcelain plate according to the following points:

1) To the first well: 3 drops of distilled water.
2) To the second well: 2 drops of distilled water and 1 drop of rancid oil.
3) To the third well: 2 drops of distilled water and 1 drop of fresh oil

Afterwards, add 1 drop of bromophenol blue (the indicator) to all three wells. Observe the color of the indicator and explain the result of an experiment.

Bromophenol blue is an acid-base indicator and its useful range lies between pH 3.0 and 4.6. At pH ≤ 3.0 is yellow, at pH ≥ 4.6 is blue.

Task 5

The aim of this activity is to examine the solubility of lipids in water and organic solvents.

Procedure

Add the following components to 4 test tubes:

1) To the 1st test tube: 2 cm^3 of distilled water
2) To the 2nd test tube: 2 cm^3 of ethanol
3) To the 3rd test tube: 2 cm^3 of acetone
4) To the 4th test tube: 2 cm^3 of chloroform

Next, add 5 drops of oil to each test tube and shake them until obtaining emulsion. Observe the differences in the behavior of fat in the respective solvents. Briefly heat the test tubes, in which there were quick stratification of the liquid, in a water bath and shake again. Observe the results of the experiment.

Task 6

The aim of this activity is the examination of the solubility of dyes in lipids.

Add 5 drops of Sudan III to 2 cm^3 of distilled water. Next, add 1 cm^3 of oil and shake the content of this test tube. Observe

the behavior of the dye relative to the oil and aqueous phases.

Task 7

The aim of this activity is the observation of the effects of the substances, which decrease the surface tension at the interface of the organic phase (fat) and water, on the emulsification process.

Procedure

Add the reagents sequentially listed in the following table to 7 test tubes:

	1	2	3	4	5	6	7
water	3 cm^3	3cm^3	3 cm^3	3 cm^3	3 cm^3	3 cm^3	3 cm^3
oil	4 drops	4 drops	4 drops	4 drops	4 drops	4 drops	4 drops
NaOH mol/dm^3		1 drop		1 drop			
fatty acid			pinch	pinch			
detergent					1 drop		
bile						1 drop	
protein							1 drop

Vigorously shake each tube for 30 sec. to obtain the emulsion (the mixture of oil and water).

Evaluate stability of these emulsions after 15, 30, 60 sec. and after 2 minutes. Emulsions in which there are two visible immiscible phases (water and oil) are considered to be unstable. Note the results in the table below:

Number of test tube	Emulsion stability after given times			
	15 sec.	30 sec.	60 sec.	2 min.
1				
2				
3				
4				
5				
6				
7				