



DEPARTMENT OF BIOCHEMISTRY

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Body fluids Milk and bile

Experiment 1. Chemical composition of milk

Protocol.

Obtaining of milk proteins

Take $10\,\mathrm{cm}^3$ of milk to beaker, add $20\,\mathrm{cm}^3$ H_2O and 7 drops of glacial CH_3COOH . Caseous sediment of casein and lipids will precipitate. Drain sediment of casein and lipids with 2 filter papers and remove. Add $1,5\,\mathrm{cm}^3$ 10% Na_2CO_3 to obtained filtrate, pH of sample should be 8 (check with strip indicator). Boil the solution. Precipitated sediment of lactoalbumin and lactoglobulin filtrate with filter paper and remove.

Use obtained filtrate for further determinations:

1. Detection of lactose

Take 1 cm 3 of filtrate to glass tube and add 1 cm 3 of Benedict or Fehling reagent. Boil for few minutes. As positive result of presence of lactose red sediment of Cu_2O will be formed.

2. Detection of Cl- ions

Take 1 cm 3 of filtrate to glass tube, add 4 drops of concentrated HNO $_3$, and 0,1 mol/dm 3 AgNO $_3$. As positive result white, caseous precipitate of AgCl will be formed.

3. Detection of Ca+2 ions

Take 1 cm³ of filtrate to glass tube and add 0.5 cm³ ammonium oxalate (szczawian amonu - $(NH_4)_2C_2O_4$). As positive result white cloudiness of calcium oxalate (CaC_2O_4) will be formed.

4. Detection of PO_4^{-3} ions

Take 1 cm 3 of filtrate to glass tube, add 1 cm 3 of concentrated HNO $_3$ and 0.5 cm 3 ammonium molybdate solution (molibdenian amonu). Heat it carefully over the burner to boil. As positive result yellow precipitate of ammonium phosphoromolybdate will be formed.

Experiment 2. Detection of fat in milk

Protocol.

1. Detection of fat in milk

Take 3 cm 3 of "Phenoloftalein" milk and add 2 cm 3 of pancreatic lipase. After mixing the solutions incubate in 40° C. Observe when milk will decolor, explain the course of experiment.

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Experiment 3. Detection of bile acids

Protocol.

Reaction of Hay with "sulfur flower" (kwiat siarkowy)

Take 2 glass tubes and add 2 $\rm cm^3$ of water to each. Add 1 drop of bile to one tube. Add "sulfur flower" to both tubes and compare the results. "Sulfur flower" will fall down in tube with bile - explain this reaction.

2. Emulsifying properties of bile

Take 2 glass tubes and add $5~{\rm cm^3}$ of water and few drops of oil to both. Add one drop of bile to one tube. Both tubes mix vigorously and observe the behaviour of emulsion in both tubes.

3. Reaction of Pettenkofer - detection of bile acids

Take 1 cm 3 of bile, add few cristals of sacharose and gently 1 cm 3 of concentrated $\rm H_2SO_4$. Red ring will be formed on the border between solutions.

4. Detection of bilirubin - reaction of Gmelin

Take 1 cm^3 of bile and add gently concentrated HNO_3 . Coloured rings of products of bilirubin oxidation will be formed on the border of solutions.

