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THE AMINO ACID COMPOSITION OF PROTEINS

Complete tasks 1-5 using a solution of egg white, gelatine and peptone (a product of partial protein digestion by pepsin containing 600-3000 Da polypeptides). Record all results in the table below, indicating a positive (+) or negative (-) result.

TEST	PROTEIN		
	EGG WHITE	GELATINE	PEPTONE
BIURET			
XANTHROPROTEIN			
MILLON			
ADAMKIEWICZ-HOPKINS			
CYSTEINE			

Task 1. Biuret test

The aim of the test is to demonstrate the presence of protein in solution based on the colour reaction resulting from the combination of copper ions with peptide bond.

Protocol: Add 4cm^3 of the cooper reagent to 1cm^3 of a 1% protein solution. The presence of the protein is evidenced by appearance of a violet or pink colour.

Task 2. Xanthroprotein test

The aim of the test is to demonstrate the presence of aromatic amino acids in test solution based on the colour reaction, whose products are nitro-derivatives of benzene rings which are present in aromatic amino acids.

Protocol: Add 1cm^3 of concentrated HNO_3 to 1cm^3 of 1% protein solution and heat a mixture for 30 seconds. The precipitate of sedimenting protein stains yellow.

Task 3. Millon test

The aim of the test is to demonstrate the presence of tyrosine in test solution based on the colour reaction, whose products are mercury-derivatives of nitrated tyrosine.

Protocol: Add a few drops of Millon's reagent to 1cm^3 of 1% protein solution and heat a mixture carefully above the burner. Proteins with nitric acid form an insoluble precipitate, which turns red during heating. If the red colour doesn't appear, add few more drops of Millon's reagent and heat again.

Warning! With excess of the Millon's reagent, the colour disappears during heating!

Task 4. Adamkiewicz-Hopkins test

The aim of the test is to demonstrate the presence of tryptophan in examined solution based on the colour products of tryptophan's indole ring with glyoxylic acid condensation.

Protocol: Add 1cm^3 of concentrated CH_3COOH to 1cm^3 of 1% protein solution. Mix the received solution and make a sublayer by slow pouring of 1cm^3 of concentrated H_2SO_4 over the wall of the tube. A violet ring is formed on the border between two layers.

Task 5. Cysteine test

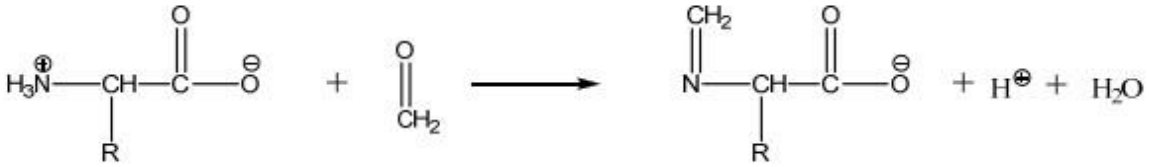
The aim of the test is to demonstrate the presence of cysteine in examined solution based on the formation of sulphur hydride (H_2S) and lead sulphide (PbS).

Protocol: To 1 cm^3 of 1% protein solution add 1cm^3 of 20% NaOH and a few drops of 2mol/dm^3 of lead (II) acetate and heat the mixture. The liquid darkens. Cool the mixture and add concentrated HCl carefully to it. Sulphur hydride is evolved.

Under the reaction conditions no sulphur hydride is formed from methionine and the test result is negative.

Task 6. Measurement of amino acids by formol titration using the Sörensen method

The aim of the test is to determine the content of nitrogen (amino acids) in the received sample. Formaldehyde (methyl aldehyde) reacts with amine groups or ammonium ion to give methylene derivatives. For each amine group or ammonium ion, a hydrogen ion is released and can be titrated with the base in the presence of phenolphthalein. The reaction of amino acids with formaldehyde proceeds according to the equation below:



Protocol: Take 5 cm^3 of the amino acid solution to the beaker, add 0,5ml of phenolphthalein and carefully add dropwise 0,1mol/dm^3 NaOH until it appears a pink colour, then add 5cm^3 of phenolphthalein neutralized formaldehyde (formalin) solution. Mix. The solution turns discoloured. Titrate with 0,1mol/dm^3 NaOH until the pink colour reappears.

Calculations: 1 cm^3 of 0,1mol/dm^3 NaOH used in titration responds to 1,4 mg of nitrogen. Calculate the nitrogen content in the received sample.

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