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PHYSICOCHEMICAL PROPERTIES OF PROTEINS

Task 1. Determination of the isoelectric point of protein

The aim of the test is to determine the isoelectric point of Casein based on its behaviour in solutions with different pH.

Protocol: To 6 tubes, measure the volumes of standardized solutions of CH_3COOH and distilled water given in the table, thus creating different reaction environments. Then add 1cm^3 of 0,5% solution of Casein in 0,1mol/dm^3 CH_3COONa to each tube. After adding each drop, shake the tube. Set aside the tubes for 30 minutes and after that time observe changes in the turbidity of the solutions. Make note of the results in the table: no turbidity (-) or different degrees of turbidity (+,++,+++). Calculate the pH in each tube using the Henderson-Hasselbalch equation.

In which pH is the highest turbidity? What is the value of isoelectric point for Casein?

COMPONENT [cm^3]	TUBE NUMBER					
	1	2	3	4	5	6
0,01mol/dm^3 CH_3COOH	0,6	1,3	-	-	-	-
0,1mol/dm^3 CH_3COOH	-	-	0,3	1	8	-
1mol/dm^3 CH_3COOH	-	-	-	-	-	1,6
Distilled H_2O	8,4	7,7	8,7	8	1	7,4
0,5% Casein	1	1	1	1	1	1
Turbidity						
pH						

Task 2. Salting out of Albumins and Globulins

The aim of the test is to separate the mixture of serum proteins through the salting out process, which uses the chemical properties of individual proteins.

Protocol: Add an equal volume of saturated solution of $(NH_4)_2SO_4$ to 5cm^3 of serum. Filter the solution after precipitation of the flocculent globulin sediment. Add $(NH_4)_2SO_4$ *in substantia* in portions to the filtered supernatant and shake the tube until at the bottom of tube crystals of insoluble $(NH_4)_2SO_4$ will remain. The sediment of albumins is precipitated. After filtration, examine the solubility of the sediment in H_2O. Check the filtrate for protein presence using precipitation method with trichloroacetic (TCA) or sulfosalicylic acid.

Task 3. Precipitation of proteins using inorganic and organic acids

The aim of the test is to observe the action of concentrated inorganic and some organic acids on proteins. These reactions may result in protein denaturation.

Protocol: For each of 3 test tubes add 1cm^3 of 1% egg white solution. Then, to 1,2 and 3 tube enter the 0,5cm3 of concentrated HCl, HNO_3 and H_2SO_4 respectively. Shake the contents of each tube. In test tubes containing HCl and H_2SO_4, the sediment of denatured protein dissolves (salts of proteins with these acids strongly dissociate). However, in test tube containing HNO_3, the sediment doesn't disappear after shaking.

Add 2cm^3 of 1% egg white solution to two other tubes. Then add dropwise 5% TCA solution to the 1 tube and 20% sulfosalicylic acid solution to 2 tube. A white sediment is precipitated. TCA and sulfosalicylic acid form insoluble salts with proteins and therefore they are used to remove proteins from biological fluids. These compounds in higher concentrations cause protein denaturation.

COMPONENT [cm^3]	**TUBE NUMBER**				
	1	**2**	**3**	**4**	**5**
1% protein solution	1	1	1	1	1
HCl	0,5	-	-	-	-
HNO_3	-	0,5	-	-	-
H_2SO_4	-	-	0,5	-	-
5% TCA	-	-	-	dropwise	-
20% sulfosalicylic acid	-	-	-	-	dropwise
Sediment					
Sediment after shaking					

Task 4. Precipitation of proteins using heavy metal cations

The aim of the test is to observe the action of heavy metal salts (Cu, Hg, Pb) on proteins depending on pH.

Protocol: To 3 test tubes add 2cm^3 of 1% protein solution at pH=3 and to other 3 tubes add 2cm^3 of the same proteins at pH=8. Then to each tube add dropwise (avoid excess of the reagent!) reagents listed in the table below. Record in the table the formation (+) or absence (-) of sediment. Explain the reaction results in each tube, taking into account the environment and ionizing of the relevant groups.

REAGENT	**EGG WHITE**	
	pH=3	**pH=8**
10% $CuSO_4$		
5% $HgCl_2$		
5% $(CH_3COO)_2Pb$		