

Date Name Group

Lab report from the practical lesson on biochemistry

Topic: Reactions of saccharides. Thin layer chromatography. Polarimetry

Task 1: Analysis of unknown sample of saccharide by means of color reactions

Principle:

| Reaction | Chemical principle of the test | Main reagent components | Positively reacting substances |
|------------------------------|--------------------------------|-------------------------|--------------------------------|
| Molisch reaction | | | |
| Bial reaction | | | |
| Selivanov reaction | | | |
| Benedict reaction | | | |
| Barfoed reaction | | | |
| Reaction with Schiff reagent | | | |
| Reaction for starch | | | |

Molisch reaction:

| | Test tube 1 FRUCTOSE | Test tube 2 MALTOSE | Test tube 3 UNKNOWN | Test tube 4 BLANK |
|--------|-------------------------|------------------------|------------------------|----------------------|
| Result | | | | |

Bial reaction:

| | Test tube 1 XYLOSE | Test tube 2 GLUCOSE | Test tube 3 UNKNOWN | Test tube 4 BLANK |
|--------|-----------------------|------------------------|------------------------|----------------------|
| Result | | | | |

Selivanov reaction:

| | Test tube 1 GLUCOSE | Test tube 2 FRUCTOSE | Test tube 3 SUCROSE | Test tube 4 UNKNOWN | Test tube 5 BLANK |
|--------|------------------------|-------------------------|------------------------|------------------------|----------------------|
| Result | | | | | |

Benedict reaction:

| | Test tube 1 GLUCOSE | Test tube 2 MALTOSE | Test tube 3 SUCROSE | Test tube 4 ASCORBIC ACID | Test tube 5 UNKNOWN | Test tube 6 BLANK |
|--------|------------------------|------------------------|------------------------|---------------------------------|------------------------|----------------------|
| Result | | | | | | |

Barfoed reaction:

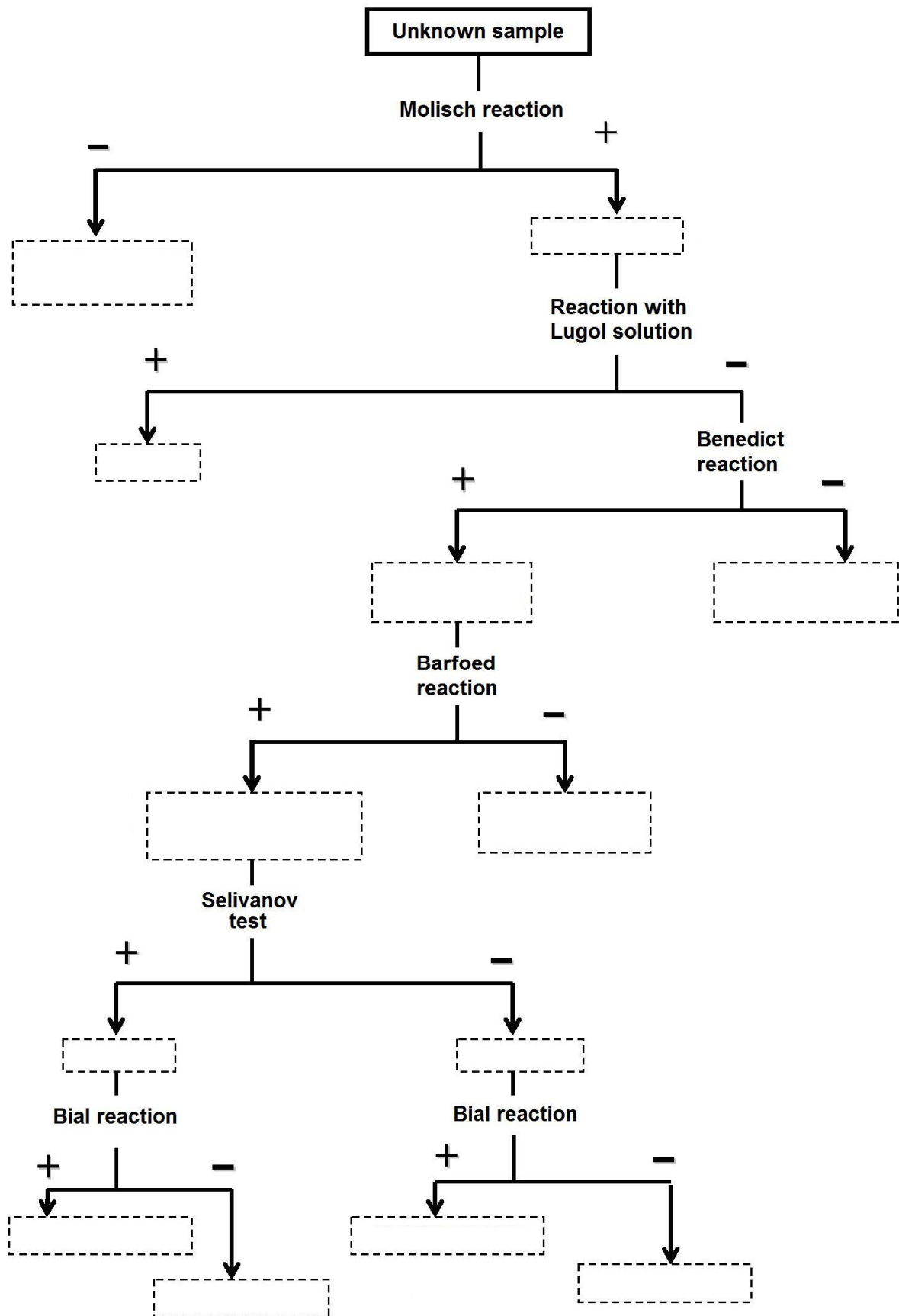
| | Test tube 1 GLUCOSE | Test tube 2 MALTOSE | Test tube 3 SUCROSE | Test tube 4 UNKNOWN | Test tube 5 BLANK |
|--------|------------------------|------------------------|------------------------|------------------------|----------------------|
| Result | | | | | |

Reaction with the Schiff reagent:

| | Test tube 1 GLUCOSE | Test tube 2 FORMALDEHYDE | Test tube 3 UNKNOWN | Test tube 4 BLANK |
|--------|------------------------|-----------------------------|------------------------|----------------------|
| Result | | | | |

Reaction for demonstration of starch:

| | Test tube 1 GLUCOSE | Test tube 2 STARCH | Test tube 3 UNKNOWN | Test tube 4 BLANK |
|--------|------------------------|-----------------------|------------------------|----------------------|
| Result | | | | |



Discussion to particular color reactions and analysis of unknown sample:

Task 2: Thin layer chromatography of saccharides

Principle:

Evaluation:

1. Draw a scheme of the developed chromatogram. Notice all the spots present and their colors after detection. Measure the distance of the solvent front from the start and also the distance of the center of each spot from the start. Use these values to calculate the R_f for each saccharide and summarize the results in the table.
2. Try to identify the saccharide in the unknown sample on the basis of comparison of its R_f value with the R_f of the standards.

Scheme of chromatogram:

| | Spot color | Distance a (start – center of spot) in cm | Distance b (start – solvent front) in cm | R_f |
|----------------|-------------------|---|--|----------------------|
| Galactose | | | | |
| Maltose | | | | |
| Lactose | | | | |
| Fructose | | | | |
| Unknown sample | | | | |

Conclusion:

Thin layer chromatography indicates that the unknown sample No. is

Summary of results and discussion of the analysis of the unknown sample both by means of the color reactions and the thin layer chromatography:

Task 3: Inversion of sucrose

Principle:

Results:

Optical activity of the *original sucrose* solution: $\alpha = \dots\dots\dots$

Optical activity of the *hydrolysate of sucrose*: $\alpha = \dots\dots\dots$

Evaluation:

1. Calculation of the original concentration of sucrose:

$$w \text{ (g/l)} = \frac{\alpha \times 100}{[\alpha]_D^{20^\circ\text{C}} \times l} \quad l = 0.2 \text{ m; specific rotation for sucrose: } [\alpha]_D^{20^\circ\text{C}} = +66.5^\circ$$

$$w \text{ (g/l)} = \dots\dots\dots$$

2. Calculation of the optical activity of invert sugar after hydrolysis of sucrose (verification of hydrolysis completion):

Sucrose $c \text{ (mol/l)} = \dots\dots\dots$ (MW of sucrose: 342)

Predicted mass concentration of the hydrolysis products glucose and fructose:

Glucose $w \text{ (g/l)} =$ fructose $w \text{ (g/l)} = \dots\dots\dots$ (MW of glucose = MW of fructose: 180)

Predicted optical activity of the solution after hydrolysis:

$$\alpha = [\alpha]_{D \text{ D-glucose}}^{20^\circ\text{C}} \times \frac{1 \times w}{100} + [\alpha]_{D \text{ D-fructose}}^{20^\circ\text{C}} \times \frac{1 \times w}{100}$$

Specific activity for D-glucose: $[\alpha]_D^{20^\circ\text{C}} = +52.5^\circ$; for D-fructose: $[\alpha]_D^{20^\circ\text{C}} = -92.4^\circ$

$\alpha =$

Conclusion:

(Was the hydrolysis of sucrose achieved? Was it complete?)