Lab 4: BENZYLIDENE ACETALS AS A PROTECTING GROUPS

CHM 2123- Section **B00-**Section **D**

Demonstrators:

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Department of chemistry
University of Ottawa

Introduction:

Carbohydrates are one of the major sources of energy. Carbohydrates can be divided into monosaccharides and polysaccharides. Oligosaccharides and glycoconjugates are some examples of complex carbohydrates which have a very important role in the DNA, RNA and cell structures. Because of The multifunctional and multi-chiral nature of carbohydrates, a very high technique is needed for purification process to prevent any side reactions. Usually protecting groups are used to prevent any of the side reaction to happen, such as keto-enol tautomerization that could occur when dissolving a monosaccharide in aqueous base. Therefore, it is a challenge in oligosaccharide chemistry to develop appropriate protecting groups for certain functional groups while allowing the other functional groups to react. Acetals are known as stable reagents to reducing conditions, therefore in this experiment Acetal is used as a protecting group, to protect ketone, aldehyde and alcohol groups while the unprotected functional groups can go under the reaction. At the end of the reaction all of the protected functional groups can be regenerated by adding an excess amount of water and aqueous acid (H3O+). This experiment should be performed under anhydrous conditions with tetrahydrofuran to obtain a good yield and that is because the presence of water would force the equilibrium back to reactants. In this experiment, benzylidene acetal has been created at the C4 and C6 alcohols of glucose, to block these two sites and let the reaction proceeds further.

In this experiment, benzaldehyde dimethyl acetal is protonated by Camphorsulfonic acid to generate the oxonium which is then trapped by one of the hydroxyl groups of the Methyl-α-D-glucopyranoside to form a mixed acetal. Methanol is then eliminated through an acid catalysis to produce an oxonium ion that is trapped again by another hydroxyl group to give the final product.

This experiment is divided into two parts, Part A and Part B. In Part A of the experiment many important techniques have been used such as reflux,TLC plates, extraction, filtration and recrystallization. Many chemical reactions need heat to proceed. A reflux is an apparatus that is used to maintain a continuous reaction of forming and breaking bonds without losing any of its reactants, and products. When the reaction gets into the boiling point, it's very important to have a reflux system that is settled with a water flush going from the bottom part into the upper part of the condenser to condense the vapors and reform it into its liquid phase that would drip back into the heated flask and by this, no vapour would be lost when the solution is boiling. Therefore a higher yield would be obtained at the end of the experiment. A thin-layer chromatography (TLC) is used to detect any impurities in our target compound. In general there are two types of TLC plates, normal and reverse-phase plates. A non-polar solvent is used in the normal type of TLC where the more polar functional groups have increased interaction with the plate, and the less polar and nonpolar compounds travel further up the plate with a higher R_f value compared to the polar compounds. While in this experiment a reverse-phase plate is used in this experiment where it is similar to the normal type except that the surface of the hydroxyl groups of silica have been replaced with non-polar alkyl chains. Therefore, the more polar compounds would travel further with higher R_f values than the nonpolar ones.

Gravity Filtration is another technique that has been used in part A. Filtration is a physical separation of solids and liquids. In order to increase the yield of the desired products, the beaker and the sample should be washed with the proper solvent. Extraction has been used in this experiment in order to separate polar and nonpolar compounds by

forming an aqueous and an organic phase. Extraction has been preceded by using an extraction funnel. The solution has been washed with water 3 times using 10 mL for each extraction. Water has been used in the extraction part because its polar and it is known that the starting material Methyl-α-D-glucopyranoside is soluble in water but is not soluble in organic phase. Recrystallization technique is also used in this experiment to form the final pure product which is 4,6-O-benzylidene-alpha-D-glucopyranoside. Using the proper solvent in the recrystallization process is very important. The solute must be insoluble in the solvent at room temperature but much more soluble in the solvent at higher temperatures. Same happened to impurities, where it must be soluble in a specific solvent at room temperature or insoluble in the solvent at a high temperature. In this experiment dichloromethane was used as the solvent to dissolve 4,6-O-benzylidene-alpha-D-glucopyranoside while hexane was used to dissolve any impurities and to form the crystals. DCM is much more molar polar than hexane, therefore DCM is the proper solvent to use to dissolve the target product that is needed to be collected because it is already mentioned that the final product is more soluble in polar phase. The mechanism that shows the formation of the pure product is explained by the following:

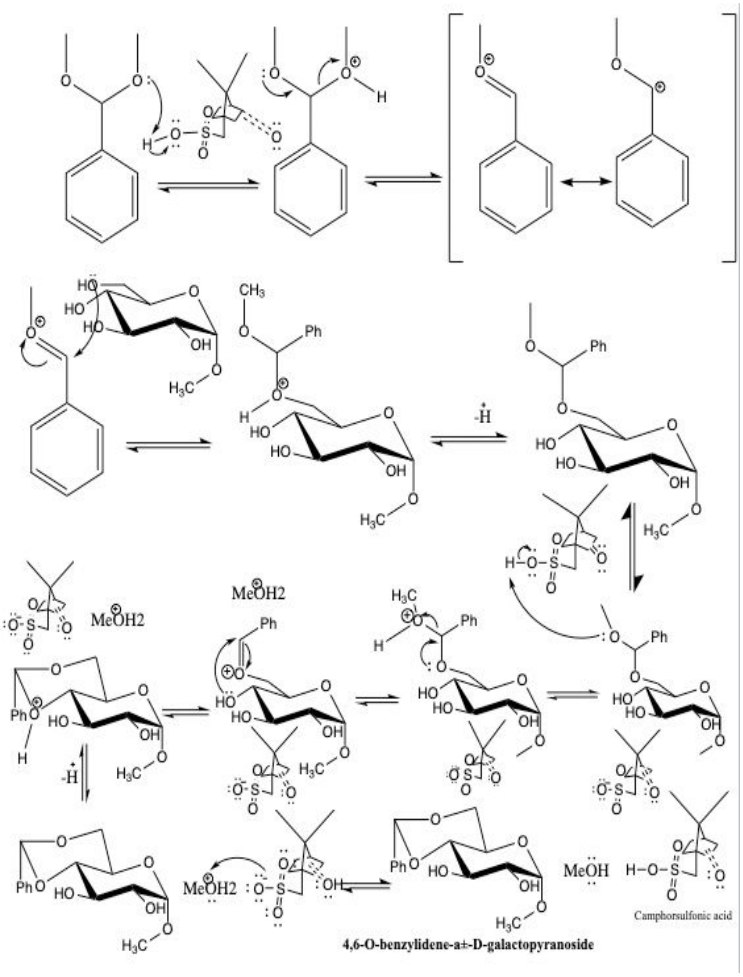
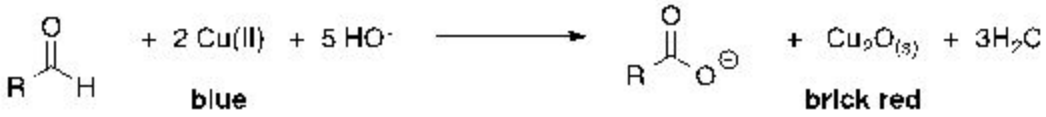


Figure 1.Acid-catalyzed benzylidene acetal formation

In part B of the experiment, Benedict's test for reducing sugars was performed. A reducing sugar is known to be any sugar that can act as a reducing reagent and has a hemiacetal group . Specifically, a reducing sugar is a type of carbohydrate that contains a free aldehyde or ketone group which can be detected through a benedict's test through a notable change in the color. Benedict's solution is known as a chemical solution that changes its color in the presence of reducing sugars.



Benedict's solution is a mixture of sodium or potassium citrate, sodium carbonate, and copper sulfate. Usually most monosaccharides such as glucose and lactose are known to be reducing sugar with some of disaccharides, oligosaccharides, and polysaccharides. Disaccharides are formed from two monosaccharides such as sucrose which is formed of fructose and glucose. Sucrose is considered to be nonreducing disaccharide. When Benedict's solution is added to a monosaccharide such as glucose, and then is heated, the solution changes to orange red brick color or to more brownish color and that is because that the copper (II) ions are reduced to copper (I) and that would lead to the color changing and that would be observed by positive result. Also it is good to mention that copper (I) ions are not soluble in water therefore it forms precipitate in the solution. While in the case of sucrose, Benedict's test would give negative results and that would imply the absence of the reducing agent where there were no change in the color of solution. But it is good to mention that hydrochloric acid can be added to the solution of sucrose to break this sugar into glucose and fructose. By this, since glucose is a reducing sugar, a yellow-brownish color should be observed indicating the presence of reducing sugar. As a result, the blue color would indicate the absence of reducing agent, low trace of reducing sugar is detected by green and yellow color with some precipitate, while a high amount of reducing sugar is detected by the red-brownish color with the formation of precipitate.

Table1. Table of reagents.

Reagent name:	**Quantity**	**Molar Mass (g/mol)**	**Number of moles: (mol)**	**Density:**	**Boiling point: °C**	**Equivalents:**
Methyl-α-D-glucopyranoside	0.5 g	194.18 g/mol	0.00257	N/A	389	1
Tetrahydrofuran	10 mL	72.11 g/mol	0.123	0.889 g/cm^3	66 °C	47.85
Benzaldehyde dimethyl acetal	0.70 mL	152.19 g/mol	0.00466	1.014 g/L	89 °C	1.81

Camphorsulfonic acid	0.10 g	232.29 g/mol	0.0043	N/A	N/A	1.67
hexanes	10 mL	86.18 g/mol	0.076	0.655 g/cm^3	68 °C	29.57
ethyl acetate	30 mL	88.11 g/mol	0.307	0.902g/cm^3	77 °C	119.45
Distilled water	32 mL	18.02 g/mol	1.78	1 g/cm^3	100 °C	N/A
dichloromethane.	3-5 mL	84.93 g/mol	N/A	1.33 g/cm^3	39.6 °C	N/A
Triethylamine	Few drops	101.19 g/mol	N/A	0.726 g/cm^3	89 °C	N/A
Sodium sulfate Na_2SO_4	N/A	142.04 g/mol	N/A	N/A	N/A	N/A
Sucrose	0.1 g	342.297 g/mol	0.0003	1.59 g/cm^3	N/A	N/A
Glucose	0.1 g	180.156 g/mol	0.00055	1.56 g/cm^3	N/A	N/A
Hydrochloric acid	Few drops	36.46 g/mol	N/A	1.18g/L	108.6 °C	N/A
Sulfuric acid	N/A	98.079 g/mol	N/A	1.84 g/cm^3	337 °C	N/A
Benedict's solution	12 mL	475.74 g/mol	N/A	N/A	N/A	N/A
4,6-O-benzylidene-α-D-galactopyranoside	0.91 g	282.29 g/mol	0.00322	N/A	N/A	1.25

Procedure:

-Refer to CHM2132 protocol as given on TopHat.

- Modifications: scratching the becker in order to get crystals in a fast way.

-*Benzylidene Acetals As A Protecting Groups*. (n.d.). doi: https://app.tophat.com/e/896767/assigned

OBSERVATIONS AND RESULTS:

Table2. Table of observations of Part A and Part B:

Part A: Material used:	**Observations:**	**Part B:**	**Observations:**

Methyl-α-D-glucopyranoside	White powder	Milk	White liquid
Camphorsulfonic acid	Pale brown powder	Diet coke	Brown translucent solution
Hexanes	Transparent solution	Sucrose	White powder
ethyl acetate	Transparent solution	Glucose	White powder
4,6-O-benzylidene-α-D-galactopyranoside	White powder	Benedict's solution	Translucent solution

Key steps: part A

Table3. Key steps of part A.

During reflux	While powder were dissolved in the solution and the solution was bubbling
VisualiziNG TLC plates	It is observed that the reaction is complete and all of the starting material has been reacted.
Adding hexane.	White precipitate formed.
Filtration	Residue remained in the flask
Extraction	Organic layer formed in the top, and the aqueous layer (transparent) was formed in the bottom and that is because water density is higher than the density of ethyl acetate.
Adding Na2SO4	Getting rid of any water trace.
Evaporating ethyl acetate	A thick syruped is formed
Adding hexane slowly	Crystals were formed slowly

Key steps part B:

Table4. Key steps of part B.

Adding 1 mL of water to the sucrose	Sucrose dissolved easily and a transparent solution was formed
Adding 1 mL of water to the glucose	Glucose dissolved easily and a transparent solution was formed
Placing the test tubes in a steam hot bath for about 5 minutes	The diet coke in the F test tube became green in colour.

Placing the test tubes in a steam hot bath for about 5 minutes	The milk inside the M test tube turned orange with the forming of precipitates.
Placing the test tubes in a steam hot bath for about 5 minutes	The glucose inside the G test tube turned into brown colour with the forming of precipitates
Placing the test tubes in a steam hot bath for about 5 minutes	The sucrose inside the S test tube stayed the same (blue transparent solution)
Adding HCL to the S test tube	The solution bubbled slightly and turned into lighter blue color

TLC plates:

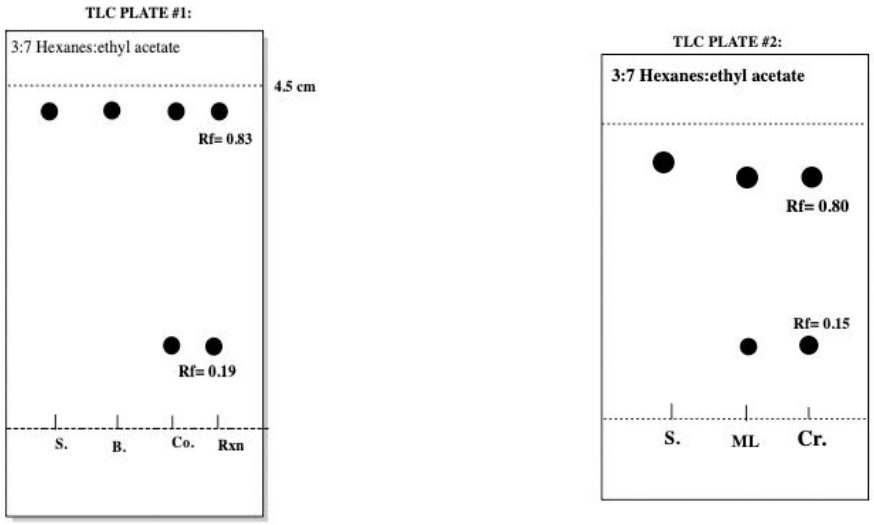


Figure1: TLC plates results with R_f values that shows the purity of the final product (4,6-O-benzylidene-alpha-D-glucopyranoside) compared to the starting material Methyl-α-D-glucopyranoside with a solvent system of 3:7 Hexanes:ethyl acetate.

Solvent is 3:7 Hexanes:ethyl acetate
S: starting material
B: Benzaldehyde dimethyl acetal
Co: Co-spot
Rx: reaction material

Solvent is 3:7 Hexanes:ethyl acetate
S: starting material
ML: mother liquor
Cr: final purified product

Compounds	**TLC PLATE #1**		**TLC PLATE #2**	
	Height (cm)	R_f **values:**	**Height (cm)**	R_f **values:**
Methyl-α-D-glucopyranoside	3.75	0.83	3.8	0.80

Benzaldehyde dimethyl acetal	3.75	0.83	----------	----------
Co-spot (spot 1)	0.85	0.19	----------	----------
Co-spot (spot 2)	3.75	0.83	----------	----------
Mother liquor (spot 1)	---------	----------	0.68	0.15
Mother liquor (spot 2)	---------	---------	3.8	0.80
Final purified product (4,6-O-benzylidene-α-D-galactopyranoside) (spot 1)	0.85	0.19	0.68	0.15
Final purified product (4,6-O-benzylidene-α-D-galactopyranoside) (spot 2)	3.75	0.83	3.8	0.80

Compound	**Molar mass (g/mol)**	**Quantity(g or mL)l**	**Number of moles**	**Yield (%)**	**Melting point °C**
Starting material Methyl-α-D-glucopyranoside	194.18 g/mol	0.5 g	0.00257	------	--------
Purified compound 4,6-O-benzylidene-α-D-galactopyranoside	282.29 g/mol	0.91 g	0.00322 mol	125 %	164-165 °C

Calculations:

- R_f values calculations:

$$R_f = \frac{\textit{distance travelled by the compound}}{\textit{Distance travelled by the solvent}}$$

TLC PLATE #1:

R_f for (starting material) = 3.75 cm / 4.5 cm = 0.83

R_f for (Benzaldehyde dimethyl acetal) = 3.75 cm / 4.5 cm = 0.83

R_f for (Co-spot, spot 1) = 0.85 cm / 4.5 cm = 0.19

R_f for (Co-spot, spot 2) = 3.75 cm/ 4.5 cm = 0.83

R_f for (reaction material spot 1)

R_f for (reaction material spot 2) = 0.85 cm / 4.5 cm = 0.19

TLC PLATE #2:

R_f for (starting material) = 3.8 cm / 4.5 cm = 0.80

R_f for (Mother liquor spot 1) = 0.68 cm/ 4.5 cm = 0.15

R_f for (Mother liquor spot 2) = 3.8 cm / 4.5 cm = 0.80

R_f for (Final purified product spot 1) = 0.68 cm / 4.5 cm = 0.15

R_f for (Final purified product spot 2) = 3.8 cm/ 4.5 cm = 0.80

Yield calculations:

-Mass of the watch glass = 45.66 g

-Mass of the watch glass with the purified product = 46.57

-Mass of the purified product (4,6-O-benzylidene-α-D-galactopyranoside) = 46.57 - 45.66 = 0.91 g

-Number of moles of the purified product = Mass of the purified product / Molar mass of the purified product = 0.91 g/ 282.29 g/mol = 0.00322 mol

- Number of moles of the starting material is = 0.00257 mol

Yield (%) = $\frac{\text{Actual yield}}{\text{Theoretical yield}} \times 100\%$

= ($n_{experiment}$ / $n_{theoretical}$) x 100% = 0.00322 mol/ 0.00257 mol x 100% = 125 %

Flow chart:

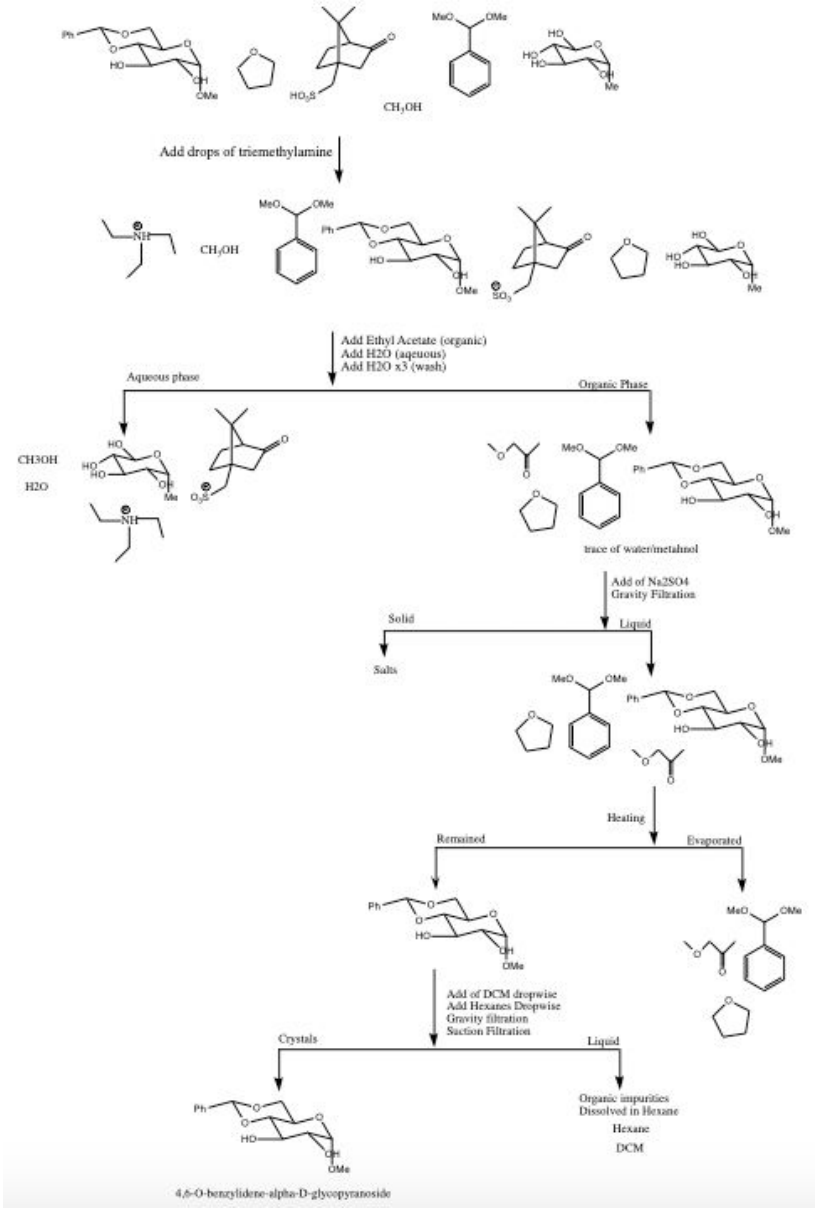


Figure2. Flow chart that shows the extraction process that has been done in experiment 4.

Discussion:

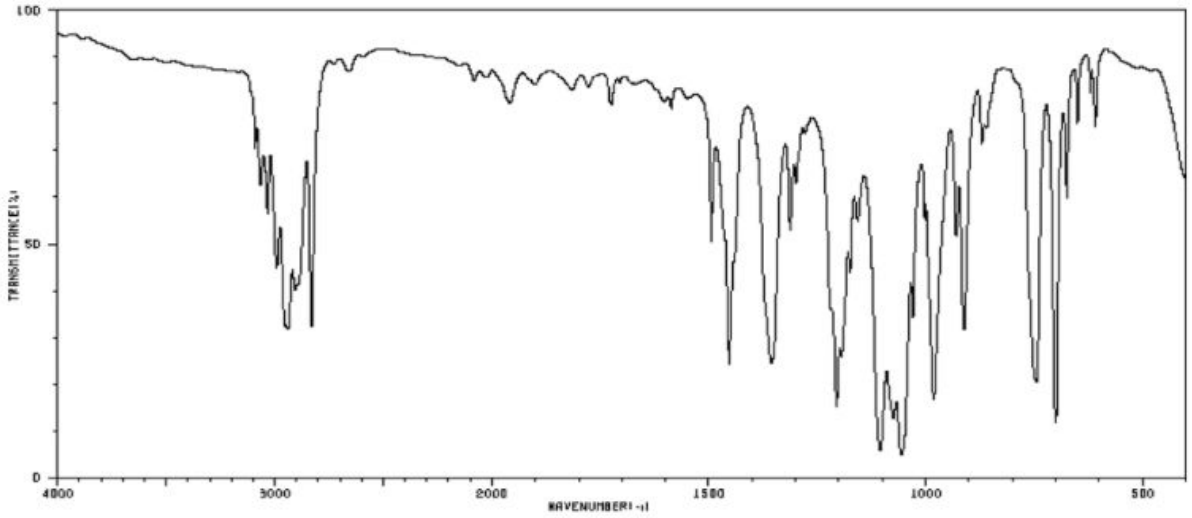
In the first part of the experiment, the purpose was creating benzylidene acetal at the C4 and C6 alcohols of glucose. The protection of this alcohol allowed the production of 4,6-O-benzylidene-alpha-D-glucopyranoside. This experiment used various methodologies which are critical for successful synthesis of the final product. At first, all of the reagents were added to the round bottom flask with a stirrer bar. Benzaldehyde dimethyl acetal was dissolved in tetrahydrofuran and then methyl-α-D-glucopyranoside (the starting material) was added with10-camphorsulfonic acid. This reaction had to be performed in anhydrous conditions, that is why tetrahydrofuran was added as a

solvent. In case of presence of any trace of water the equilibrium would be shifted back to reactants. Therefore,any trace of water whether through contamination or atmospheric water could lead to a lower yield. Since the equilibrium for acetal formation is weakly favoured, water could cause the equilibrium to go to back to reactants. The 10-camphorsulfonic acid is a strong acid which protonated dimethyl acetal to generate oxonium. The hydroxyl group of sugar then reacts with oxonium ion to produce mixed acetals. Then, acid catalysis takes place and the methanol is removed. The resulting oxonium ion is trapped by hydroxyl group of sugar via intramolecular reaction.

Reflux was set up once all the reagents were added to the round bottom flask. The heating was turned on and the refluxing commanced. This step is important to make sure that concentrations of all of the reagens remained the same throughout the reaction. As the solution was boiling, it was observed that the vapors were condensed and drip back into the flask. Therefore, the yield of the reaction would be protected from being reduced. After thirty minutes of the reaction, first TLC analysis was done using 3:7 hexanes:ethyl acetate which is considered a polar solvent. Observing the results, the products of reaction was observed to confirm that the reaction was successful and some product was produced. The Rf values _of methyl-α-D-glucopyranoside and benzaldehyde dimethyl acetal was too close to each other and was about 0.83. More polar compounds have travelled longer distances while non polar compounds (the product) which travelled only by a small distance and have Rf value of 0.19. This is because the solvent system contained more of the polar solvent (ethyl acetate) than of the non polar (hexanes). TLC plate was visualised in two steps. The first one was performed using UV light and the second one was done by dipping the TLC plate into methanolic solution of sulfuric acid and then dried using heating fan. Sugars and sulfuric acid undergo dehydration reaction in which carbon is formed. The elemental carbon turns black once all the water is taken away by the acid and evaporated by the application of heat.
After confirming the presence of reaction products, the mixture was cooled down to room temperature. Few drops of triethylamine was added to the reaction mixture to neutralize the acid before extraction steps. Extraction was performed using water as aqueous phase and ethyl acetate as organic phase. The solution was washed using 10 mL of water three times to increase the efficiency of extraction. Water phase was observed to be in the bottom because it has a higher density than ethyl acetate. The target product was collected in the organic phase. Na_2SO_4 was then added to the organic phase as a drying agent to remove any trace of water. The salts formed upon addition of the Na_2SO_4 were removed by gravity filtration. The solution was then heated to evaporate ethyl acetate that has a boiling point of 77°C, tetrahydrofuran (89 °C) and benzaldehyde dimethyl acetate (89 °C). Once only 1 mL of the thick syrup was left, the mixture was taken out from the heat source. Dichloromethane was added to the syrup that has formed. The solid particles have dissolved in dichloromethane and then hexane was added to force

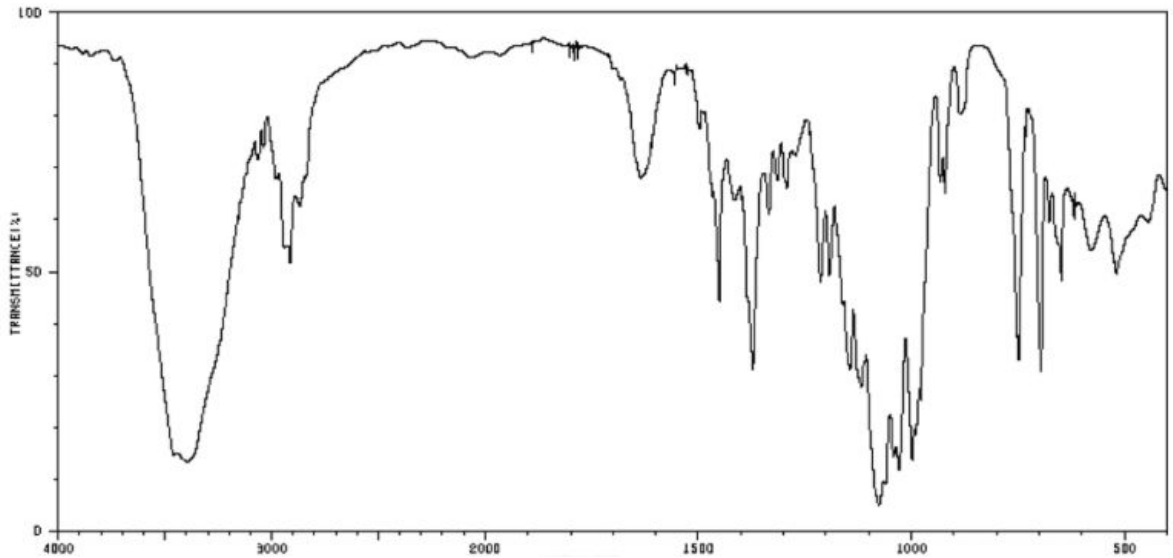
Infrared analysis:

Spectrum A:



In spectrum A, it indicate that the compound is aromatic ring. Also, the multiple strong peaks indicate alkyl C-H bonds. While the peak over 3000 cm-1 indicate aromatic C-H stretches. There is nothing showing O-H bonds. Therefore the compound can not be Methyl-α-D-glucopyranoside or 4,6-O-benzylidene-α-D-galactopyranoside. Therefore it could represent benzaldehyde dimethyl acetal.

Spectrum B:



Spectrum B:

The peak 1600-1650 indicates the presence of a carbon double bond on benzene ring. The peaks at 3400 indicate that the molecule contains O-H groups. This would exclude dimethyl acetal, since this compound does not have O-H bonds. There is another peak at around 3000, which confirms the presence of alkyl C-H bond. By observing spectrum B it can be concluded that this spectrum is IR analysis of 4,6-O-benzylidene-α-D-galactopyranoside

References:

-(1) Magnan, F. Exp. 1. In CHM2123 Laboratory of Organic Chemistry Manual, University of Ottawa: Ottawa, 2019; tab: "Experiment 4"

-(3) "Coefficient of Determination (R Squared): Definition, Calculation" Statistics How to.Available on https://www.statisticshowto.datasciencecentral.com/probability-and-statistics/coefficient-of-determination-r-square

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-*Benzylidene Acetals As A Protecting Groups*. (n.d.). doi: https://app.tophat.com/e/896767/assigned

Discussion:

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They yield obtained is %125 of 4,6-O-benzylidene-α-D-galactopyranoside which was calculated in the calculation part.

PART B:

The purpose of part B was to identify reducing sugars using Benedict's test. Benedict's solution is known as a chemical solution that changes its color in the presence of reducing sugars. The tube with glucose turned red brick, while sucrose tube remained clear blue. Milk turned orange with the observation of precipitate, while diet coke changed the color to slightly green.The positive result for this test is red brick color, and the negative result is blue color. Therefore, according to the results, glucose was identified as a reducing sugar, while sucrose was a non reducing sugar. That would be the result of presenting of free aldehyde and ketone groups in glucose but not in sucrose. Also, the results showed that milk contains reducing sugars as it changed in color and formed precipitate too. Diet coke color changed to green which indicates the presence of a slight amount of reducing sugar. The expected result for diet coke is negative test, that is why it is possible that the tube was contaminated with reducing sugars therefore it shows a positive result instead of negative results.In the case of sucrose, Benedict's test gave negative results and that would imply the absence of the reducing agent where there were no change in the color of solution. But it is good to mention that hydrochloric acid can be added to the solution of sucrose to break the sugar bonding and form two monosaccharides which glucose and fructose. Theoretically, glucose is a reducing sugar and it should give a yellow-brownish color which would indicate the presence of the reducing sugar, but in this experiment the results obtained for this part was different and the tube that HCl was added into it stayed blue and it did not change, and that could be because of some source of errors like any contamination in the tube, the initial amount used of sucrose was not enough to give the similar results as the theoretical part.

Error analysis:

In the first part of the experiment, the most probable sources of error are water contaminations and reflux set up. Water contamination would lead the equilibrium of the reaction to shift towards reactants, thus lowering the yield of the final product. Improper reflux set up can have water entering from the top and thus not ensuring cool surface at the bottom of the condenser. This way some vapour can leave the condenser and the concentration of some reactants could be reduced. Not adding enough of drying agent could leave some traces of water which will make the final product impure. Adding DCM or hexane fast might result in impure crystal formation.

Collecting aqueous phase instead of organic is also a source of error which could lead to the loss of the final product. In part B, the major source of error could be contamination of tubes with reducing sugars, incomplete reaction, not using the proper amount mentioned in the lab procedures, and not heating the samples enough.