Experiment -9

Spectophotometric Determination of Iron

Aim: Spectophotometric determination of Iron conc. using 'Beer - Lambert Law'.

Requirements:

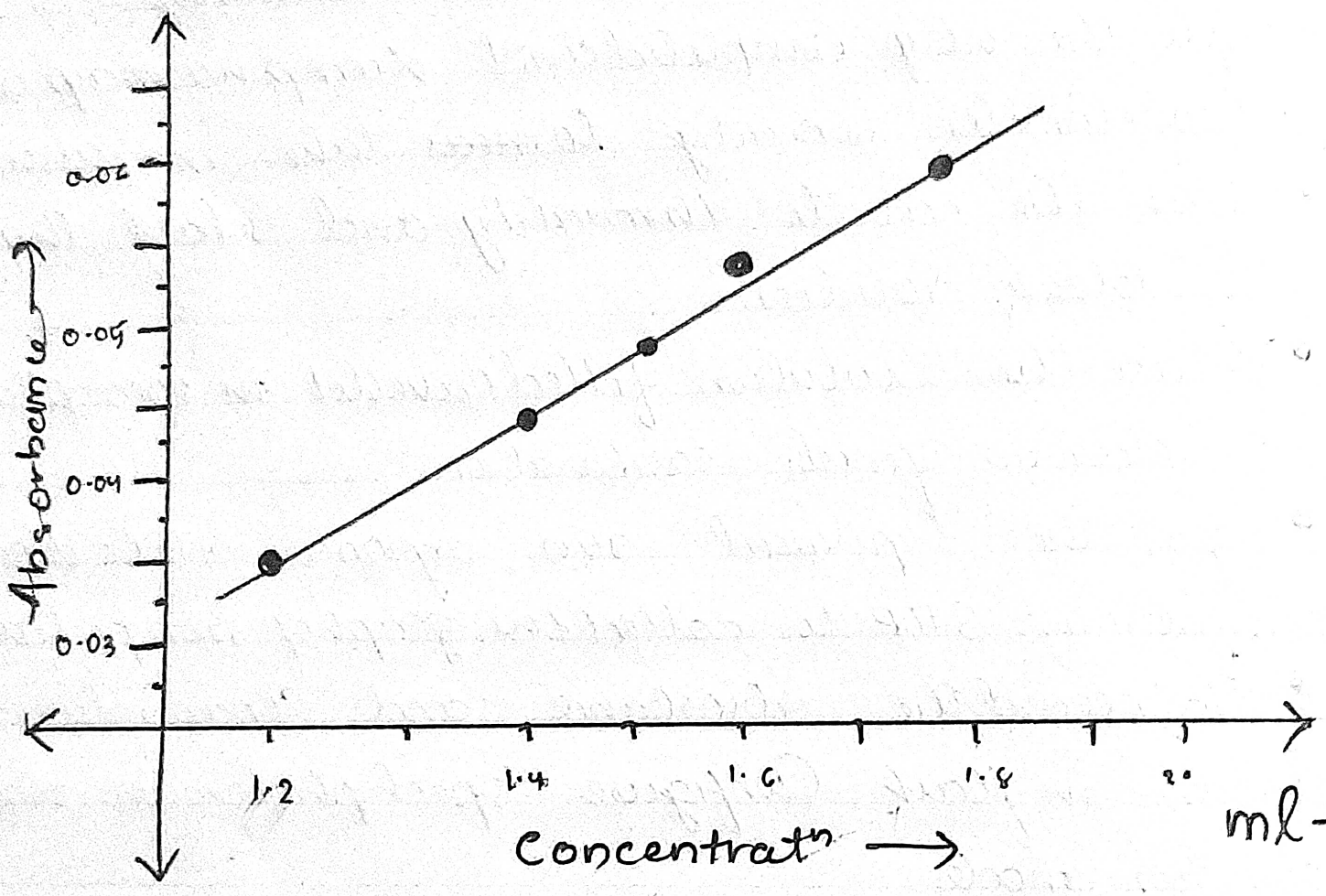
- Spectophotometer
- Cuvette
- Flask (500ml and 250ml)
- Iron sample
- Hydroxylamine hydrochloride
- Ortho-phenantrolin

Procedure:

- Prepare two groups of solution one standard iron concentration solution in 500ml another unknown conc.
- Transfer the solution into series of 50ml volumetric flask and add different known amount of iron to the flask and also 2 unknown amount in two flask.
- Add 'Hydroxylamine hydrochloride which reduce iron from 3^+ oxidation state to 2^+ oxidation state.
- Add 'Ortho-phenanthroline' which react with the (2+) iron and form colour complex (orange)
- Leave it as for 10min.

- Then dilute flasks with distilled water and shake it.
- Connect spectrophotometer to Laptop, open the logger pro software then calibarate the instrument.
- After the setup completed and Lamp warmup is over spectrophotometer is ready to measure take measurement.
- Clean the cuvette throughly and start the process with 'Blank Solution'.
- Place the solution filled cuvellet in spectrophotometer and click on finish calibaration.
- After the experiment run a graph will be shown then presson STOP to collect the graph of Ironspectra
- To record the absorbance and concentration of solution in flask Configure spectrophotometer under collection mode
- Check absorbance Vs concentration and change unit to milligram of iron per 50 milliliter.
- Select individual wavelength (highest generation on spectra i.e 510nm) under the dropdown menu and click on Clear selection to remove anything that might be previously checked off.
- Click on Ok once the graph setup to collect absorbance and concentration value.
- Then take series of standard and to construct calibaration curve.

- Take the cuvette, rinse it with the solution to be measured, take solution and place it in spectrophotometer
- Click on Collect.
- We can see the absorbance actively being displayed and data point showing on graph.
- Click on Keep.
- Enter the concentration value of the solution
- Keep repeating this process for all the concentration
- After getting the required graph for all known concentration now, measure the ~~three~~ unknown sample in the spectrophotometer and repeat the process that is followed for known concentration.



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Experiment -10

Qualitative Analysis of Carbohydrate

Aim: Perform some common test to analyse the properties and quality of 4 known carbohydrate.

Requirements:

- Glucose
- Lactose
- Sucrose
- Starch
- distilled water
- Molisch reagent
- Conc. H_2SO_4
- fehling sol" A & B
- Benedict reagent
- Tollens reagent
- Iodine reagent
- Glass & plastic ware

Theory:

Carbohydrates are large biological molecules consisting of carbon, hydrogen and oxygen atoms having general formula $C_n(H_2O)_n$. They are one of the most important nutrient present in a food. During the process of photosynthesis carbohydrate are produced from carbon dioxide and water in the presence of chlorophyll and sunlight. Glucose, Lactose, Sucrose and starch are comonly known carbohydrate. Some tutors use to know and learn about the properties of carbohydrate, those are Solubility test, Molisch's test, Fehlings test, Benedict's test, Tollens test, Iodine test

Procedure and Observation :

Solubility test

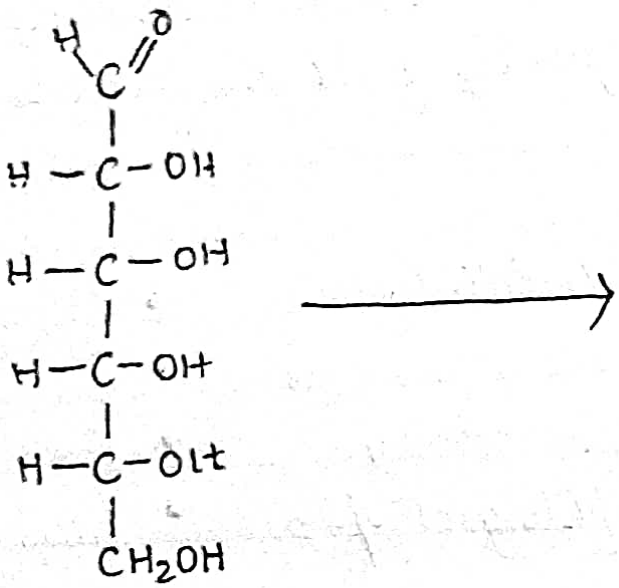
- Take a small amount of glucose, lactose, sucrose and starch in four test tube A, B, C and D.
- Now, add a small volume of distilled water to to the test tubes and shake it well.

[We can see that glucose lactose and sucrose are soluble in water whearas starch is insoluble]

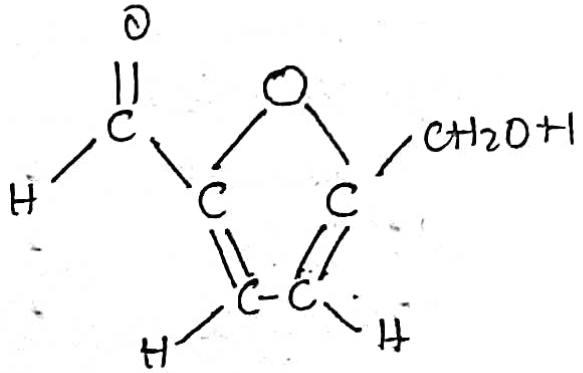
Molisch's test

- Take a small amount of aqueous solution of glucose, lactose, sucrose and suspension of starch in 4 test tube A, B, C and D respectively.
- Using a dropper add a few drop of Molisch's reagent to test tube A, B, C, and D.
- Take the test tube A and pour a small amount of concentrated sulphuric acid slowly along side of test tube
- Similarly pour concentrated sulphuric acid to other test tube B, C, and D

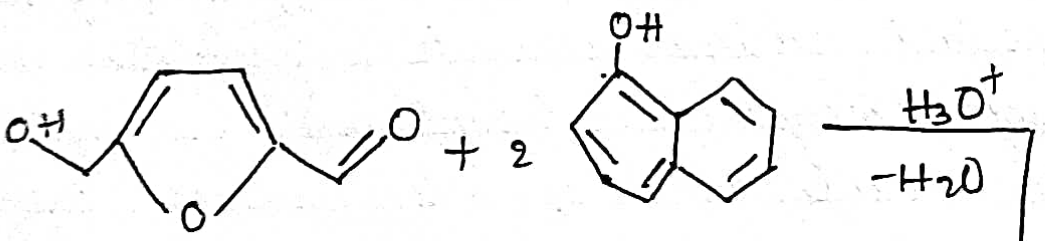
[Concentrated sulphuric acid dehydrates carbohydrate to form furfuraldehyde or its derivative which further react with α-napth-ol present in molisch's reagent to form coloured product that appear as purple ring at the interface between the acid layer and test layer.]



{carbohydrate}

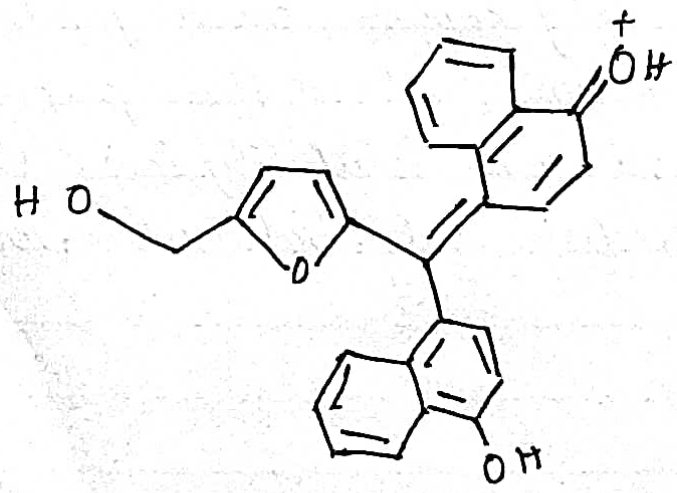


5-Hydroxymethyl furfural



furfural

α-napthol



Purple-Coloured Dye.

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fehling's test

- Take a small quantity of aqueous solution of glucose, lactose, sucrose and suspension of starch in four test tube A, B, C and D respectively
- Using a dropper add a small quantity of fehlings solution A into the test tube A, B, C and D
- Now, using another dropper, add a small quantity of fehling's solution B into the test tubes A, B, C & D
- Heat the test tube in a boiling water bath for sometime.

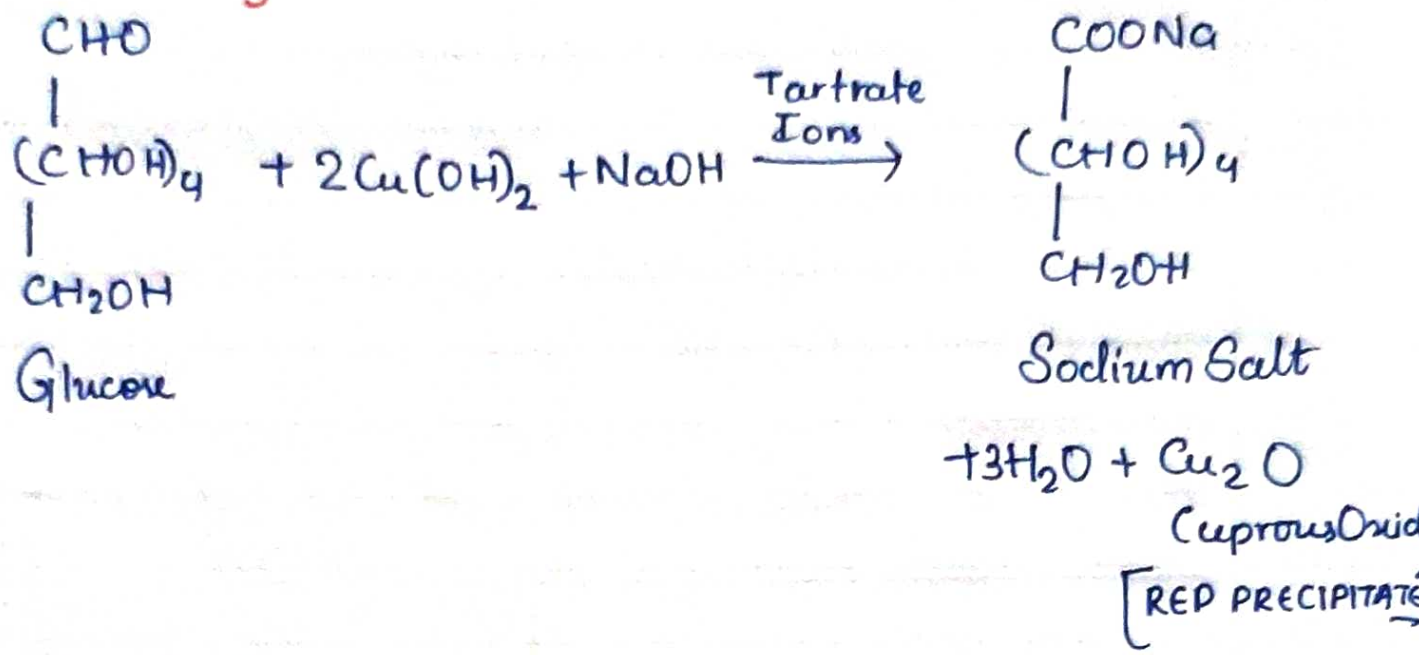
[The reducing sugar glucose and lactose reduce the Copper(II) ion in the test reagent to form red precipitate of cuprous oxide whereas no such precipitate is formed by the non-reducing sugar sucrose and starch]

Benedict's Test

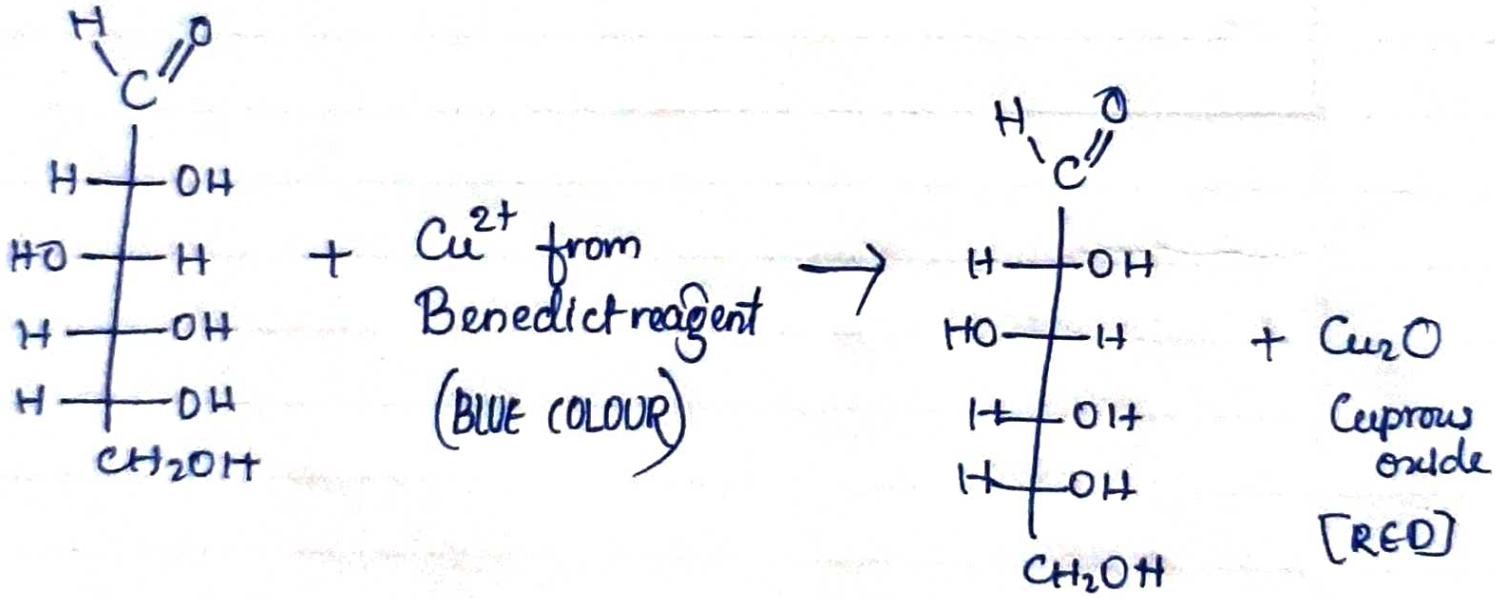
- Take a small quantity of aqueous solution of glucose, lactose, sucrose and suspension of starch in 4 test tube A, B, C, D respectively
- Using a droper add a small quantity of Benedicts reagent into test tube A, B, C, D
- Heat test tube in boiling water bath

[Reducing sugar glucose and lactose reduce the Cu(II) ion in test reagent to from red precipitate whereas no precipitate is formed by non-reducing sugar sucrose and starch.]

Fehling's :-



Benedict's :-



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Tollen's Test

- Take a small quantity of aqueous solution of glucose, sucrose, lactose & suspension of starch in testtube
- Add a small quantity of Tollen's reagent in testtube
- Heat the testtube in boiling water bath for sometime

[The reducing sugar glucose & lactose reduce silver ion in test reagent to elemental silver appearing as a silver mirror on the inner surface of vessel whereas no such silver mirror is produce by non reducing sugar sucrose and starch]

Iodine test

- Take a small quantity of aqueous solution of glucose sucrose lactose and suspension of starch in testtube,
- Add a small quantity of iodine in testtube A, B, C, D

[Iodine react with stach to form blue colour starch iodine Complex whereas no such complex is formed other three samples.]

Conclusion

By the expt we derive many properties of many carbohydrate and also learnt their quality

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