Biochemistry

The building blocks of life
Carbohydrates

Test for reducing sugar – Fehling's test

Aims of the experiment

■ Introduction to the chemistry of sugars

■ To differentiate reducing and non-reducing sugars using Fehling's test.

■ Consideration of the indicating reaction as a redox reaction

■ Explanation of the colour change in the Fehling reagent due to a complex reaction

■ Consideration of reaction equilibria

Principles

The Fehling test was developed in 1848 by Herrmann Fehling. It is a indicating reaction for reducing groups such as aldehyde functions. It makes it possible to differentiate between reducing and non-reducing sugars. Originally, the Fehling test was also used to determine the sugar content in the blood of diabetics. Today, the Fehling test is particularly important as a didactic example for understanding the chemistry of sugars.

The principle of the Fehling test is based on the fact that the aldehyde group of sugar is oxidised by complexed copper ions to form acid. The red copper (I) oxide then precipitates, which is an indicator for the redox reaction.

Sugars can exist in aqueous solution as a ring shape or as an open chain molecule. Both forms exists in chemical equilibrium with each other. However, for the pentoses ($C_5H_{10}O_5$) and hexoses ($C_6H_{12}O_6$), equilibrium is shifted toward the ring shape by more than 99.9 %. An intramolecular semiacetal (Fig. 2) forms during the ring closure reaction.
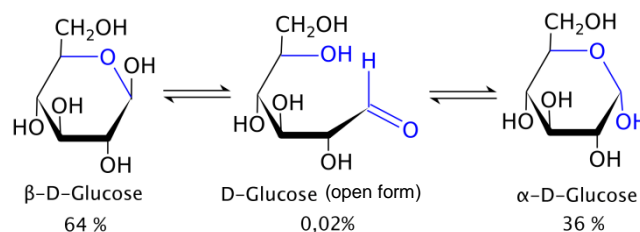

Fig. 2: Equilibrium between open-chained and ring-shaped glucoses in water. During ring closure, a semiacetal is formed.

The open-chained form has either an aldehyde group or a keto group as a head group. This is how sugars are subdivided into aldoses and ketoses. The carbon atom at which the aldehyde or keto group is present in the open-chained form is called the anomeric carbon atom in the ring shape since upon ring closure a new chiral centre arises.

The Fehling test is an indicator for aldehyde. Ketones cannot be oxidised using the Fehling reagent. However, the Fehling test also reacts to ketoses since they exist in chemical equilibrium with their corresponding aldoses in aqueous solutions due to keto-enol tautomerisation (Fig. 3).

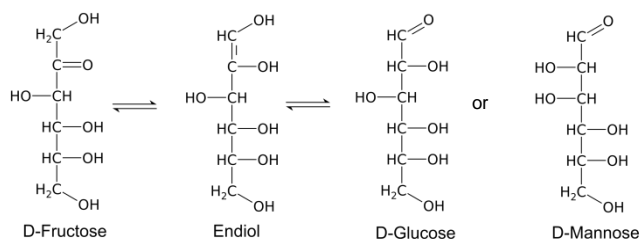**Fig. 1:** Set-up of the experiment.

Fig.3: Keto-enol tautomerisation of fructose in water.

Only the open-chained form of the sugar can oxidise since the ring-shaped form has no aldehyde groups.

Ring-shaped sugars can be linked to dimeric and polymeric sugars through their hydroxide groups in a condensation reaction. In the process, acetals arise if the link takes place at the anomeric carbon atom. The anomeric carbon atom then no longer has any hydroxide groups. This blocks the ring-opening reaction (Fig. 4).
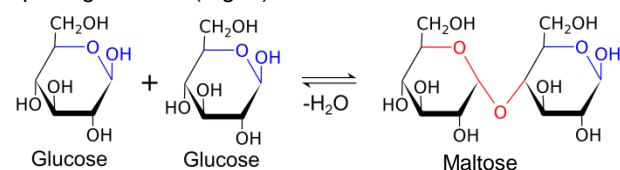

Fig. 4: Dimerisation of galactose. An acetal arises at the anomeric carbon of the left glucose molecule.

In dimeric sugars, there are two different cases of possible linkage. For example, maltose consists of two glucose molecules that are linked together 1,4. Thus, the anomeric carbon atom of one glucose molecule is linked with the fourth carbon atom of the other glucose molecule. The ring opening of the first glucose ring is thus blocked. However, in the second glucose ring, the ring opening can continue to take place so that the open-chained form can be oxidised. Therefore, this is a reducing sugar (Fig. 5).
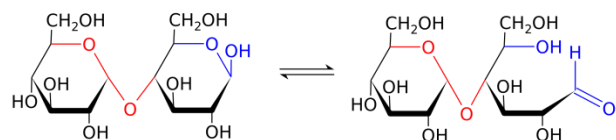

Fig. 5: Ring opening of maltose. The anomeric carbon atom of the left glucose molecule is blocked as acetal. The anomeric carbon atom of the right glucose molecule can be opened as a semiacetal.

In saccharose, on the other hand, one glucose molecule is linked with a fructose molecule 1,2. The linkage is done through the two anomeric carbon atoms. In this case, the ring opening is blocked at both rings. The sugar can not be oxidised. Therefore, this is a non-reducing sugar (Fig. 6).
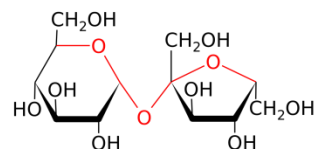

Fig. 6: Structure of saccharose. Both anomeric carbon atoms are linked together as acetals.

In the experiment presented here, the Fehling test will be carried out with the reducing sugars glucose and fructose and with the non-reducing sugar saccharose, for example. Saccharose can be split into glucose and fructose in the acidic range; these two sugars can in turn react as reducing sugars. This will also be shown in this experiment.

Risk assessment

Exercise particular care when handling sodium hydroxide and hydrochloric acid since they are corrosive materials. When carrying out the experiment, wear goggles, an apron and gloves.

Copper (II) sulfate	
Signal word: Caution	**Hazard statements** H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H410: Very toxic to aquatic life with long-lasting effects. **Safety statements** P273: Avoid release to the environment. P305+P351+P338: IF IN EYES: Rinse carefully with water for several minutes. Remove contact lenses if present and easy to do so. Continue rinsing. P302+P352: IN CASE OF CONTACT WITH SKIN: Wash with plenty of water and soap.

Hydrochloric acid, 25 %	
Signal word: Hazard	**Hazard statements** H314: Causes severe skin burns and severe eye damage. H335: May cause respiratory irritation. H290: May be corrosive to metals. **Safety statements** P234: Keep only in original container. P260: Do not breathe dust/fume/gas/mist/vapours/spray. P305+P351+P338: IF IN EYES: Rinse carefully with water for several minutes. Remove contact lenses if present and easy to do so. Continue rinsing. P303+P361+P353: IF ON SKIN (or hair): Remove/take off all contaminated clothing immediately. Rinse skin with water/shower. P304+P340: IF INHALED: Take person to fresh air and keep at rest in a position comfortable for breathing. P309+P311: If exposed or you feel ill: Call a POISON CENTER or doctor/physician. P501: Dispose of contents/container at a recognised waste disposal facility.

Sodium hydroxide	
Signal word: Hazard	**Hazard statements**
	H290: May be corrosive to metals.
	H314: Causes severe skin burns and severe eye damage.
	Safety statements
	P280: Wear protective gloves/protective clothing/eye protection/face protection.
	P301+P330+P331: IF SWALLOWED: Rinse mouth. Do NOT induce vomiting.
	P305+P351+P338: IF IN EYES: Rinse carefully with water for several minutes. Remove contact lenses if present and easy to do so. Continue rinsing.
	P308+P310: If exposed or concerned: Call a POISON CENTER or doctor/physician immediately.

Equipment and chemicals

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2   Volumetric flask Boro 3.3, 100 ml ............... 665 793
1   Glass stirring rod 200 mm x 8 mm dia.,10 .. 665 212ET10
1   Beaker, Boro 3.3, 150 ml, tall ..................... 602 010
1   Spatula, micro double ended, 185 mm ....... 666 961
1   Electronic balance 440-3N, 200 g : 0.01 g.. 667 7977
1   Measuring cylinder 100 ml, with plastic b. .. 665 754
4   Test tubes, 30 x 200 mm ............................ 664 045
1   Test tube rack, 32 mm diam. ...................... 667 054
1   Dropping pipette, 7 x 150 mm, 10 pcs. ....... 665 953
1   Rubber bulbs ............................................. 665 954
1   Magnetic stirrer with hotplate..................... 666 8471
1   Thermometer, -10...+150 °C/1 K................. 382 33
1   Beaker, Boro 3.3, 600 ml, squat ................. 664 132
1   Graduated pipette 10 ml ............................. 665 997
1   Pipetting ball ............................................. 666 003
1   Watch glass dish, 100 mm diam. ................ 664 155
1   Wash bottle, PE, 500 ml ............................. 661 243
1   D(+) Glucose, 100 g ................................... 672 1100
1   D(+) Fructose, 50 g.................................... 672 0700
1   D(+) Saccharose, 100 g.............................. 674 6050
1   Sodium potassium tartrate, 250 g............... 672 6710
1   Sodium hydroxide, pellets, 100 g............... 673 6800
1   Copper(II) sulfate-5-hydrate, 100 g............. 672 9600
1   Hydrochloric acid, conc. 25%, 250 ml......... 674 6750
1   Boiling stones 250 g ................................... 670 9160
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Set-up and preparation of the experiment

The Fehling reagent consists of the two Fehling solutions I and II.

1. For Fehling solution I, 3.5 g of copper (II) sulfate-5-hydrate are dissolved in 50 ml of water in a 100 ml volumetric flask.

2. In another 100 ml volumetric flask, 17.5 g of potassium sodium tartrate and 10 g of sodium hydroxide are also dissolved in 50 ml of water to produce Fehling solution II.

3. Now, if Fehling solution II is poured into Fehling solution I, it would be observed that the copper sulfate solution, previously bright blue, becomes dark blue in colour.

4. From the 3 sugars (glucose, fructose, saccharose) 0.5 g each are weighed out, added to a test tube and the tubes labelled correspondingly (prepare two test tubes with saccharose). 50 ml each of distilled water are added. In one of the two test tubes with saccharose solution, 10 drops of concentrated hydrochloric acid are added.

Tip: The solution batches must be heated somewhat on the hotplate. Place test tubes into a 150 ml beaker to weigh them.

Performing the experiment

1. The 600 ml beaker is filled with water and placed on the magnetic stirrer as a water bath with heating plate.

2. The water bath is heated up to 80 °C with boiling stones. The temperature is tracked using the thermometer. When the temperature is reached, the water bath is removed from the heating plate and the three test tubes with the non-acidified sugar solutions are placed in it.

3. To each of the solutions, 10 ml of Fehling reagent is added using the graduated pipette. An orange-red precipitate should immediately occur in the test tubes with the fructose and the glucose. The saccharose should not indicate any changes.

4. After some time, the two test tubes with the reducing sugars are removed from the water bath and the test tube prepared with the acidified saccharose solution is added.

Note: If the temperature of the water bath has dropped too far, it should be heated back up before proceeding.

10 ml of Fehling reagent are added as before.

A red precipitate also appears in the acidified saccharose solution, whereas the unacidified saccharose solution indicates no colour change.

Observation

1. Fehling solution I is initially a bright blue colour. Fehling solution II is colourless. When the two solutions are mixed, the mixture becomes dark blue.

2. If a bit of Fehling reagent is added to the solutions of the two reducing sugars and heated, a red precipitate drops out.

3. Saccharose exhibits no reaction with the Fehling reagent as it is a non-reducing sugar.

4. The acidified saccharose solution on the other hand does exhibit a reaction with the Fehling solution. It also gives a red precipitate.

Result of the experiment

The bright blue colour of Fehling solution I arises due to fact that copper ions in the aqueous solution form a blue complex compound with water. The copper therefore does not exist as Cu^{2+}, but as $[Cu(H_2O)_4]^{2+}$ or $[Cu(H_2O)_6]^{2+}$. The complex $[Cu(H_2O)_4]^{2+}$ is also present in the solid copper(II) sulfate-5-hydrate. Therefore, it is also blue.

After adding Fehling solution II, the solution becomes dark blue since the copper reacts with the tartrate ions. The water ligands are replaced by the stronger tartrate ligands. The dark blue copper tartrate complex forms (Fig.7).
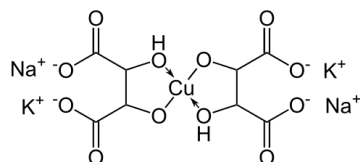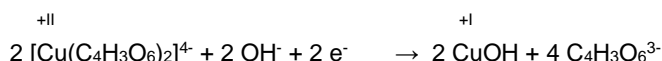
$$[Cu(H_2O)_4]^{2+} + 2\ C_4H_4O_6^{2-} + 2\ OH^- \rightarrow [Cu(C_4H_3O_6)_2]^{4-} + 6\ H_2O$$
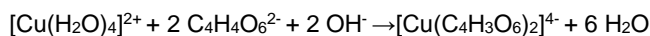
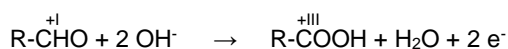**Fig.7**: Copper tartrate complex.

As mentioned at the start, the indication reaction is a redox reaction.

Here, the aldehyde group of the sugar is oxidised to acid and the Cu^{2+} bound in the copper tartrate complex reduces to copper(I) oxide. The reduction occurs via copper(I) hydroxide as the intermediate step.
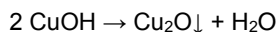
Reduction:

$$2\ \overset{+II}{[Cu(C_4H_3O_6)_2]^{4-}} + 2\ OH^- + 2\ e^- \rightarrow 2\ \overset{+I}{CuOH} + 4\ C_4H_3O_6{}^{3-}$$

Oxidation:

$$R\text{-}\overset{+I}{C}HO + 2\ OH^- \rightarrow R\text{-}\overset{+III}{C}OOH + H_2O + 2\ e^-$$
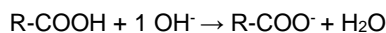
The subsequent dehydration leads to solid copper(I) oxide which drops out as a red precipitate. The resultant carboxylic acid is immediately deprotonated in the alkaline solution.
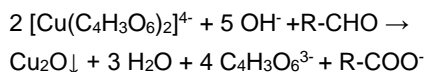
Dehydration:

$$2\ CuOH \rightarrow Cu_2O\downarrow + H_2O$$

Deprotonation of the carboxylic acid:

$$R\text{-}COOH + 1\ OH^- \rightarrow R\text{-}COO^- + H_2O$$

Overall reaction:

$$2\ [Cu(C_4H_3O_6)_2]^{4-} + 5\ OH^- + R\text{-}CHO \rightarrow$$

$$Cu_2O\downarrow + 3\ H_2O + 4\ C_4H_3O_6{}^{3-} + R\text{-}COO^-$$

Although the open-chained form of the sugar only exists at less than 0.1% in the aqueous solution, the sugar reacts completely to form gluconate, the anion of gluconic acid. The reason is because the reaction products copper(I) oxide and gluconic acid are both continuously removed from the reaction equilibrium. Copper-II-oxide precipitates as a solid and the gluconic acid is deprotonated in the base. Since both reaction equilibria are coupled together, sugar is converted from the ring form to the open-chained form until the reaction has gone to completion.

As can be seen from the reaction equation, the reaction proceeds as a function of concentration of the hydroxide ions. The indicating reaction therefore does not function in the acidic region.

The complexing of the Cu^{2+} as a copper tartrate complex has the function of preventing the formation of insoluble copper (II) hydroxide in the base. This would not be reduced by the aldehyde.

The saccharose initially exhibits no reaction since it is a non-reducing sugar. The linking of the two anomeric carbon atoms prevents the ring from opening. Therefore, there are no open-chained sugar molecules that could be oxidised.

The acidification of the saccharose causes splitting to the monomeric sugars glucose and fructose (Fig. 8). These sugars in turn can be oxidised by the Fehling reagent.
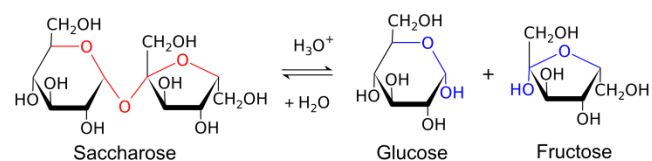

Fig.8: Splitting of the saccharose. The reducing sugars glucose and fructose arise.

Cleaning and disposal

The remaining Fehling reagent can be kept in a sealed vessel for further analyses. All other solutions are placed in the waste container for inorganic solvent waste as they contain copper. The red precipitate of copper -(I)- oxide can be dissolved in concentrated hydrochloric acid.