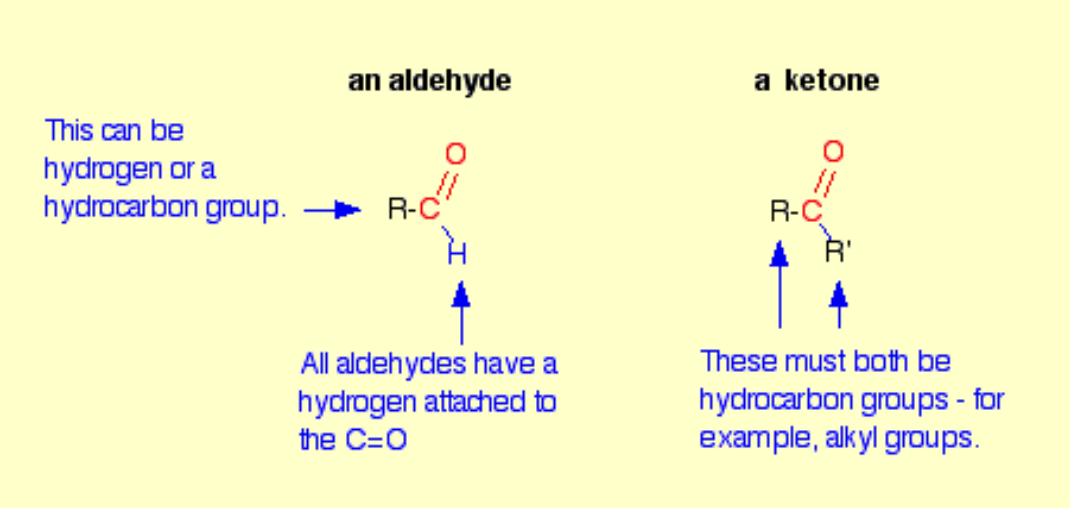
Reduction

This Note is about **Reducing Sugars**. The Chemistry is mine. The Biology comes from other, reputable, sources.

OILRIG

Oxidisation involves the LOSS of electrons, and
Reduction involves the GAIN of electrons.

A REDOX reaction is one where both REDuction and OXidisation are taking place at the same time – one substance is being Reduced (gaining electrons) while the other is simultaneously being Oxidised.
(A **reducing sugar** is thus one that is reducing another substance whilst it is itself becoming oxidised.)



Source: the great Jim Clark at https://www.chemguide.co.uk/organicprops/carbonyls/oxidation.html

- However, before talking about Reduction, let me say first of **all** that **Carbonyl compounds** can be identified by a test using something called "Brady's Reagent".

Brady's Reagent: a mixture of 2,4-DNPH/methanol/and Sulphuric Acid

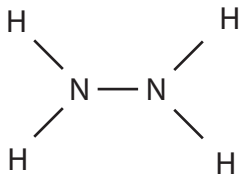
- The element Nitrogen is in Group V and thus needs to form three bonds in order to achieve the stability of the Noble Gas configuration. The most common Nitrogen compound is Ammonia, NH_3, and the N atom has a lone pair of electrons with which it can form dative bonds – and it does precisely that in Ammonium, (NH_4^+).

- The French word for Nitrogen is "azote", and the English word '*azo'* in Chemistry is derived from the French word for Nitrogen. The word "azo" thus indicates that there is an N atom in the compound. "Diazo" means that two N atoms are involved.

- "**Hydrazine**"[1] has the formula $H_2N–NH_2$, (the diagram on the left overleaf), and "*diazine*" has the formula "HN=NH" ("azo" gives you "Nitrogen" and "di" means "two"). Diazine can also be known as "diimine", and the phrase "diazo" (as in diazo dyes) comes from "Diazine".

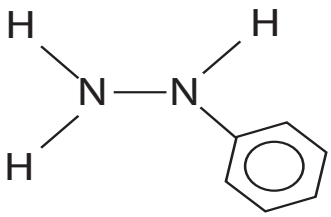
[1] It should really be "dihydrazine".

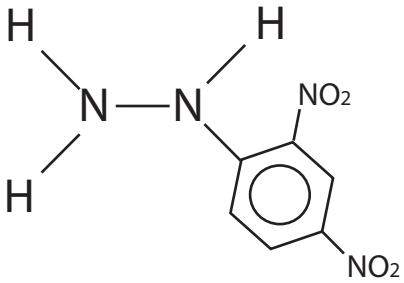
Hydrazine



Diazine

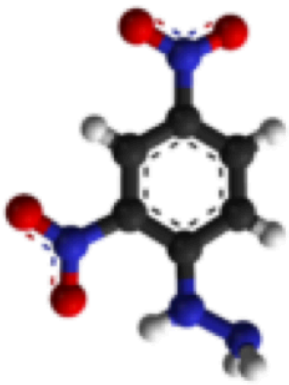
- If you replace one of the H atoms in Hydrazine with a Benzene ring, then you would get **Phenylhydrazine** viz. $H_2N–NH(C_6H_5)$, the molecule on the left below, and if you substituted two "–NO_2" species at positions 2 and 4 on the Benzene ring, then you would get **2,4-DiNitroPhenylHydrazine**, 2,4-DNPH ("phenyl" is the adjective for "–C_6H_5").

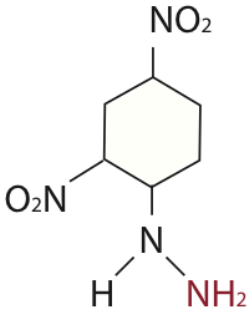




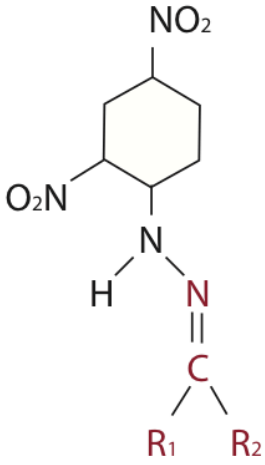
- Without looking at the next diagram, could **YOU** now please draw **2,4-DiNitroPhenylHydrazine** (2,4-DNPH). It will remind you of the rules in the naming of Benzene compounds. Our interest here centres on the Hydrazine bit and not on the Nitro bits.

2,4-DiNitroPhenylHydrazine





2,4-DiNitroPhenylHydrazone

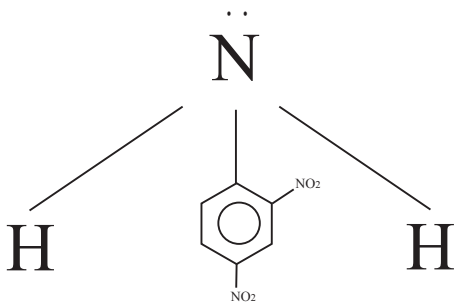


- The only difference between the "**–zine**" and the "–**zone**" is that "zine" has two H atoms attached to the bottom N atom on the right, whilst the "zone" has R_1 and R_2 attached to a C atom which is double bonded to the N atom. (In Chemistry and in Biology, "R" can be any legitimate species, but it is usually an H atom/an alkyl species/or an arene species).

NB In a recent Edexcel exam paper you were expected to know that

- **there is a double bond in the 2,4-DiNitroPhenylHydrazone species "–N=C "**. Please therefore be aware of *both* forms ("–zine" and "–zone") of 2,4-DNPH.
- In another recent exam paper you were expected to know that it is the unbonded/the lone pair of electrons on the 2,4-DNPH that makes it nucleophilic (viz. it is the lone pair of electrons that makes it a nucleophile).

Phenylhydrazine where the emphasis is on the fact that the molecule is an **AMINE**.



- There are other organic compounds besides Aldehydes and Ketones that contain the Carbonyl ">C=O" species (e.g. Esters/Amides/Carboxylic Acids/Acyl Chlorides/etc) and in some circumstances an Ester (but not the other Carbonyl compounds) will test positive for Brady's reagent (by the formation of a bright orangey-yellow precipitate) but **for 'A' Level purposes please assume that it is only Aldehydes and Ketones that will test positive to Brady's reagent – and then proceed from there to distinguish the Aldehyde from the Ketone using the Tollens'/Fehling's/Benedict's tests.** **NB** An Aldehyde will oxidise into a Carboxylic Acid, **but a Ketone will not do so**! (That is the nasis of the tests.)

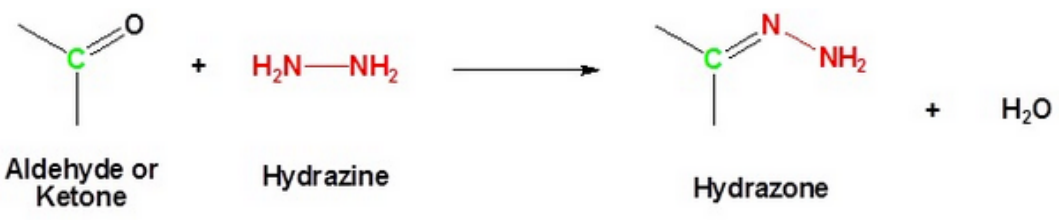
What is Brady's Reagent and what does the test reveal?

- Brady's Reagent is a mixture of 2,4-DNPH / Methanol /and some Sulphuric Acid (and all three together make up Brady's or Borche's Reagent) and it will react with a Carbonyl compound (in the manner shown above) and there will be a colour change (**an orangey-yellow precipitate will appear in the presence of an Aldehyde or a Ketone**) as the "hydrazine" turns into "hydrazone" and a molecule of water is ejected. The equation for the reaction is

$$RR'C{=}O \;+\; H_2N{-}NH(C_6H_3(NO_2)_2) \;\rightarrow\; H[C_6H_3(NO_2)_2]N{-}N{=}CRR' \;+\; H_2O$$

and please note that the C_6H_6 of the Benzene ring here is C_6H_3 (because three of the H species have been replaced by other species).

- Writing out the reaction of an Aldehyde or a Ketone with the Hydrazine can be daunting for many students, and they find it easier to draw the reaction as follows



Source: Chemistry LibreTexts

- Brady's Reagent will thus identify an Aldehyde and a Ketone (by the appearance of the orangey-yellow precipitate), but it will not distinguish the Aldehyde from the Ketone. That requires something like Tollens' (a colourless Silver Ag^{+} complex solution)/Benedict's/or Fehling's solutions (both the latter being Cu^{2+} complexes) which will cause an Aldehyde to convert into a Carboxylic Acid but neither will convert a Ketone because a Ketone does **not** convert into a Carboxylic Acid.

Test to distinguish an Aldehyde from a Ketone

- Let us imagine that we have three test tubes, and in each one there was just one of three Alcohols viz. a 1˚, a 2˚, and a 3˚ Alcohol, and there was a fourth test-tube with conc. sulphuric acid and excess Potassium Dichromate in it – and all four test-tubes were in a bath of hot water. (This would get all the liquids to the *same* temperature, heat speeds up any reaction.).

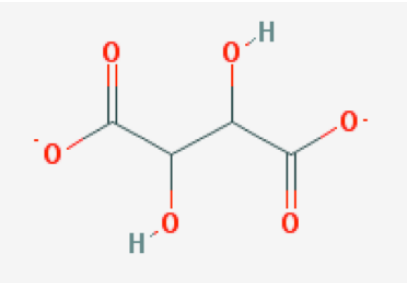
- If one were then to put an equal amount of the acidified Dichromate into each of the test-tubes that contained the Alcohols, then two of the test-tubes would go from orange to green because
- The 1˚ Alcohol was being turned into an Aldehyde (or into a Carboxylic Acid).
- The 2˚ Alcohol was being turned into a Ketone, and
- there would be no colour change in the third, because 3˚ Alcohols are not oxidised under these conditions.
- A lack of a colour change would thus identify the test-tube that had the 3˚ Alcohol in it – *but how would we know which test-tube contained the 2˚ Alcohol and which the 1˚ Alcohol?*

- Luckily there is another simple colour change that we can utilise to identify an Aldehyde from a Ketone (and thereby identify the 1˚ Alcohol from the 2˚ Alcohol). There is a substance called Fehling's **solution** and also another called Benedict's **solution**, and both of these solutions are coloured blue/cyan by the dissolved copper sulphate Cu^{2+} (aq) ions in them.[2] *(Tollens' solution contains Ag^{+} Silver ions rather than Cu^{2+} Copper ions.)*

- Both substances (Fehling's and Benedict's) are oxidising agents and will oxidise the Aldehyde into a Carboxylic Acid **but cannot oxidise the Ketone into anything** – and (by definition) the oxidising agents themselves will thus be reduced from soluble Cu^{2+} sulphate ions to (in this case) insoluble Cu^{+} oxide ions, and thus the Cu^{+} ions precipitate out to give a dirty reddy-brown solution initially (but given time, the copper oxide particles will settle to the bottom of the test-tube to leave a colourless solution). In Tollens', the Ag^{+} ions will gain an electron (they will be **reduced**) to Ag (s) metal.

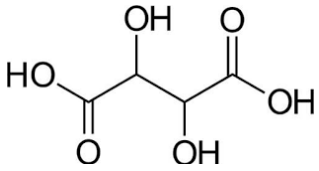
- Having identified the 3˚ Alcohol by the lack of a colour change from orange to green, we can then identify the 1˚ Alcohol by the colour change from blue to dirty reddy-brown, and the 2˚ Alcohol will be in the test-tube where the blue coloured solution has not altered.

- If (when talking about Fehling's solution) an exam question talks about 2,3-dihydroxybutanedioate ions, then this is what such a species looks like (where there is an Ester "–C=O(O–)" bit at each end).



[2] Both solutions involve "complexes" and "ligands" and we will talk about complexes and ligands in Inorganic Chemistry in the Chapter on Transition Metals (Chapter 12). You need $(OH)^{-}$ ions in both the Fehling's and Benedict's complex, and we will talk about how this is achieved.

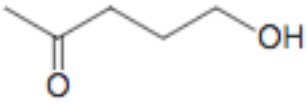
and it comes from 2,3-dihydroxybutanedioic acid, Tartaric Acid, which contains one acid functional group (–COOH) at each end of the molecule (and two Alcohol functional groups in the middle of the molecule).



Source: https://en.wikipedia.org/wiki/Tartaric_acid

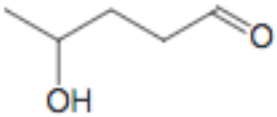
- An OCR paper had a question about how to distinguish one Carbonyl Group from another. You were given the three species/compounds (below) and you were asked to distinguish one from another.

Ketone with a 1˚ Alcohol attached.



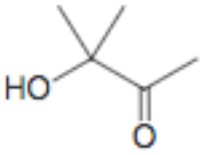
compound C

Aldehyde with a 2˚ Alcohol attached



compound D

Ketone with a 3˚ Alcohol attached.



compound E

- If you examine the three species (and please remember that in skeletal diagrams the "C–H" bonds are not shown) you will see that D is an Aldehyde with a **secondary Alcohol** attached to it. (It is 4-hydroxypentanaldehyde.) C is a Ketone with a **primary Alcohol** attached to it. (It is 5-hydroxypentan-2-one.) E is a Ketone with a **tertiary Alcohol** attached to it because the C atom that is attached to the "–OH" species is attached to three C atoms. (It is 3-hydroxy-3-methylbutan-2-one.)

- Ketones cannot be oxidised further but an Aldehyde (a Carbonyl compound) will react with Tollens' reagent to form a silver precipitate and with either Benedict's or Fehling's solutions to form the brown-red Copper (Cu^{+}) oxide ion. Tollens'/Benedict's/or Fehling's will therefore distinguish **D** from C and E.

- How then can C be distinguished from E? Well, a 2˚ Alcohol will oxidise into a Ketone, but a 3˚ will resist gentle oxidisation, therefore if you react both with concentrated Sulphuric Acid and Sodium or Potassium Dichromate, then the solution will turn from orange to green for C, but nothing will happen with E – and now you have distinguished all three compounds from each other.

Reducing vs. Non-Reducing sugars

https://www.khanacademy.org/test-prep/mcat/chemical-processes/aldehydes-and-ketones/a/cyclic-hemiacetals-and-hemiketals

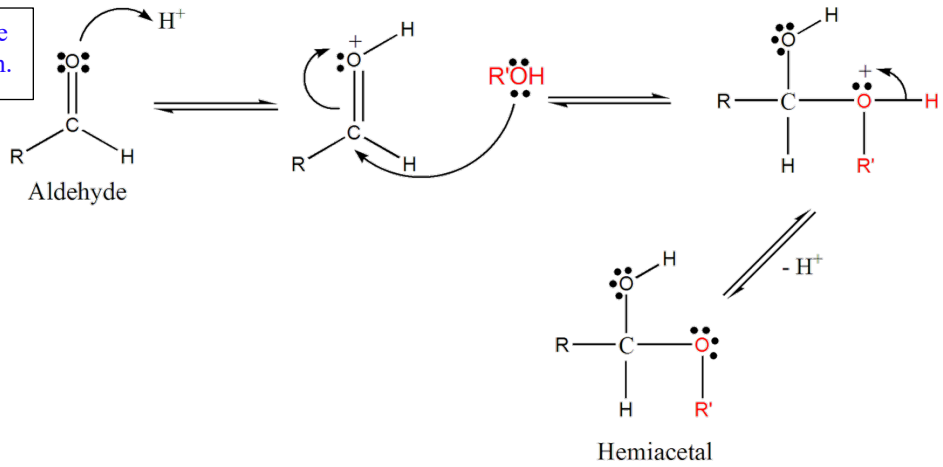
(Source: The Khan Academy, but with occasional editorial modifications by me.)

A sugar without a hemiacetal is a **non-reducing** sugar. An important and simple test for identifying blood glucose is where an aldehyde reduces a Cu^{2+} ion (as in Benedict's solution) and a colour change occurs.

A **hemiacetal** (from an Adehyde) or a **hemiketal** (from a Ketone) is a compound that results from the addition of an alcohol to an aldehyde or to a ketone respectively. (Alcohols add reversibly to aldehydes and ketones to form hemiacetals or hemiketals (h*emi,* Greek, half). This reaction can continue by adding another alcohol to form an acetal or ketal. These are important functional groups because they appear in sugars.)

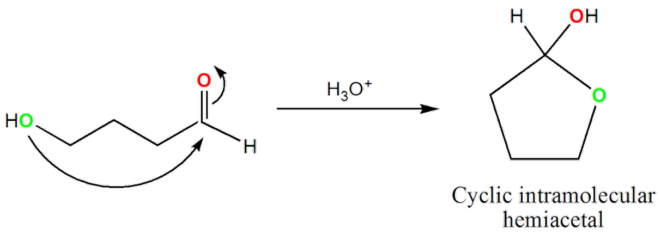
Before we get into the discussion of cyclic hemiacetals and hemiketals, let us quickly recollect how they are formed. They are formed when an alcohol (–OH) oxygen atom adds to the carbonyl (C=O) carbon of an aldehyde or a ketone. This happens through the nucleophilic attack of the hydroxyl group (where the O atom is heavily rich in electron density) at the electrophilic carbonyl group (the $C^{\delta+}$ in the "C=O"). Since alcohols are weak nucleophiles, the attack on the carbonyl carbon is usually promoted by protonation of the carbonyl oxygen. When this reaction takes place with an aldehyde, the product is called a '**hemiacetal'**; and when this reaction takes place with a ketone, the product is referred to as a '**hemiketal'**.

Protonation of the Carbonyl Oxygen.



The above reaction exemplifies the formation of an intermolecular hemiacetal. These are intrinsically unstable and tend to favour the parent aldehyde.

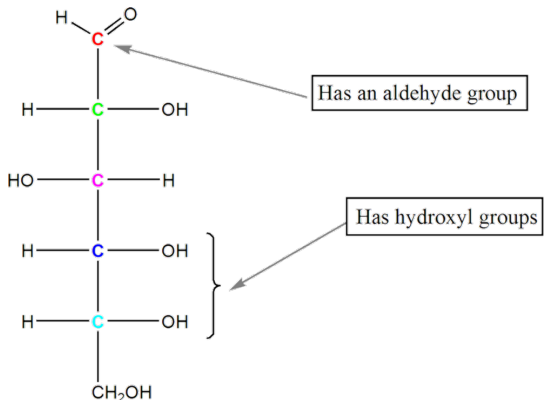
Molecules (aldehyde or ketone), which contain both an alcohol and a carbonyl group, can instead undergo an intramolecular reaction to form a cyclic hemiacetal/or hemiketal. These, on the contrary, are more stable as compared to the intermolecular hemiacetals/hemiketals. Stability of cyclic hemiacetals/hemiketals is highly dependent on the size of the ring, where 5 & 6 membered rings are generally favoured.



Intramolecular hemiacetal and hemiketal formation is commonly encountered in sugar chemistry. Just to give you an example: in solution: 99% of glucose exists in the **cyclic hemiacetal form** and only 1% of glucose exists in the open form.

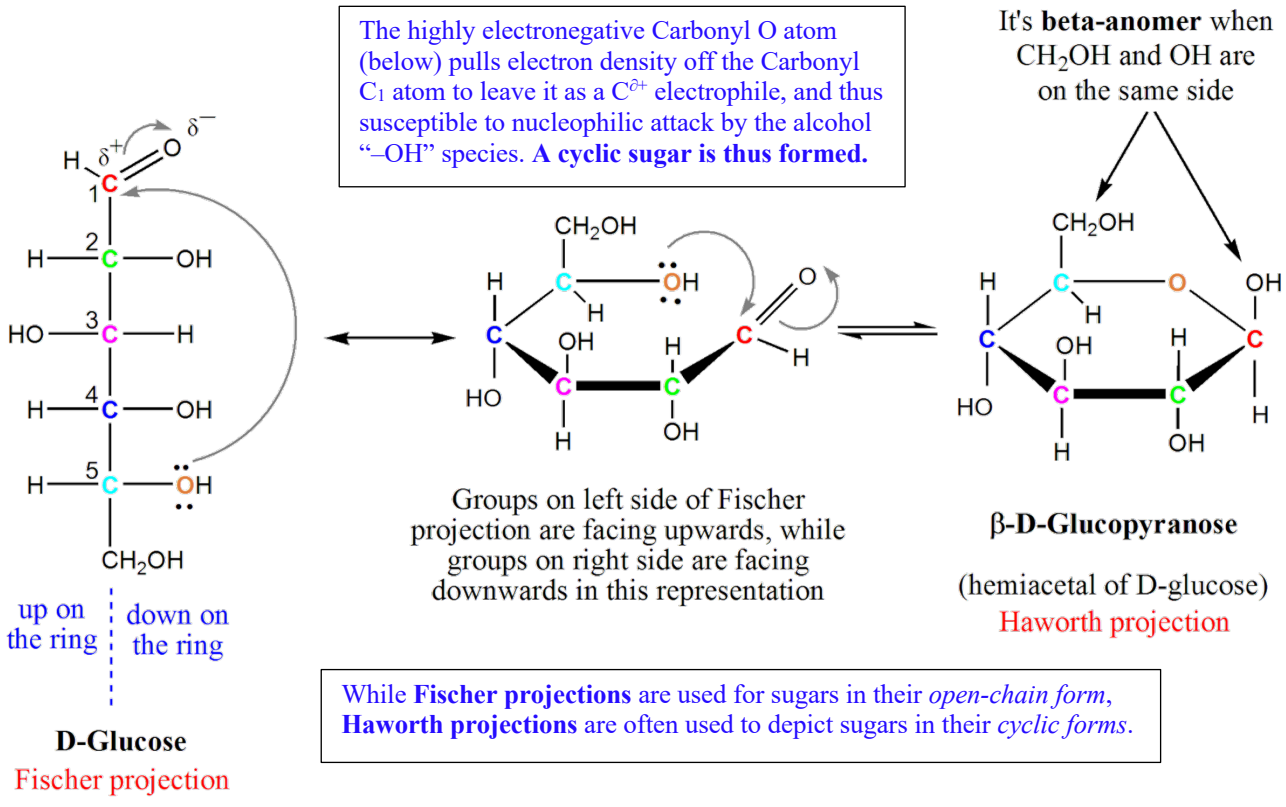
Cyclisation of glucose to its hemiacetal form

Let us first draw a molecule of glucose. The simplest way to do so is by using the Fischer Projection as shown below. (*Wikipedia says that* The *Fischer projection*, is a two-dimensional representation of a three-dimensional organic molecule by projection. Fischer projections were originally proposed for the depiction of carbohydrates and used by chemists, particularly in organic chemistry and biochemistry.)



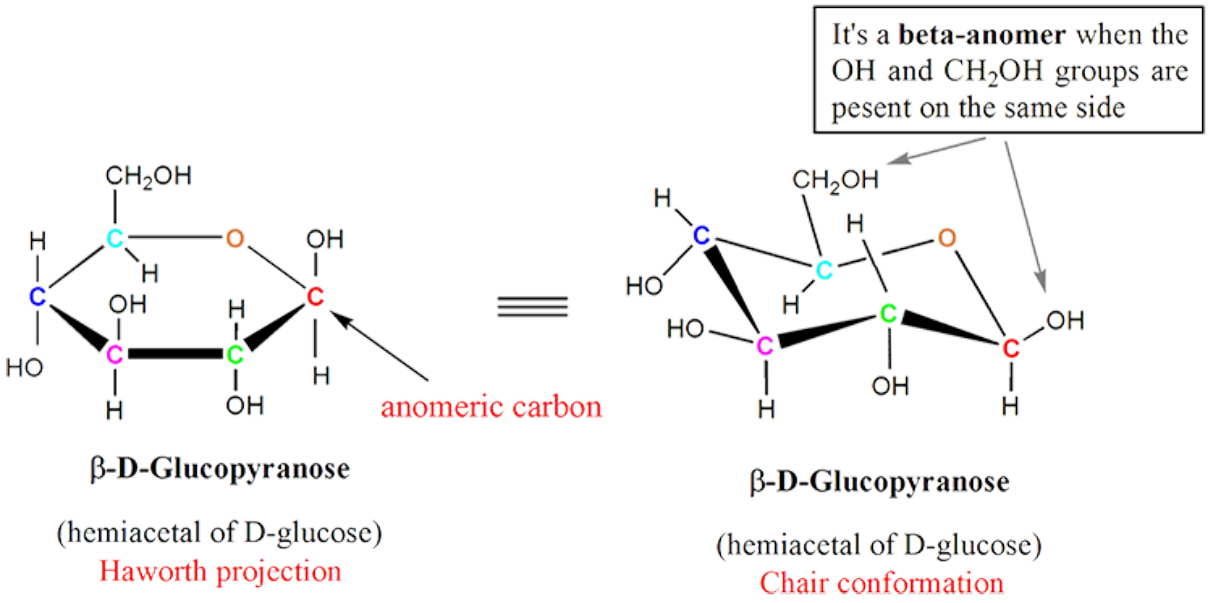
Glucose has an aldehyde group and five hydroxyl groups. Does that ring a bell? Yes, glucose can form an **intramolecular cyclic hemiacetal**. Let us now show the formation of hemiacetal of glucose starting from its open structure (*Fischer projection*).

Anomers are cyclic monosaccharides or glycosides that are epimers, differing from each other in the configuration of C-1 if they are aldoses or in the configuration at C-2 if they are ketoses. (An **aldose** is a monosaccharide, a simple sugar, with a carbon backbone chain with a carbonyl group on the endmost carbon atom, making it an aldehyde, and hydroxyl groups connected to all the other carbon atoms. *Source: Wikipedia.*) The epimeric carbon in **anomers** are known as the **anomeric** carbon or the **anomeric** center.



So why does not the hydroxyl attached to **C**-4 react with the carbonyl group? Why does the carbonyl group react with the hydroxyl attached to **C**-5? **C**-4 hydroxyl attacking the carbonyl group will lead to the formation of a 5-membered ring, while the attack of **C**-5 hydroxyl at the carbonyl group will generate a 6-membered ring (as shown in the above figure). In the case of glucose, a 6-membered ring is **thermodynamically more stable** than a 5-membered ring, thus favouring the formation of a 6-membered ring over a 5-membered ring.

Now let us shift our focus to the hemiacetal of glucose (H*aworth projection*). If you notice this cyclisation process creates a new stereogenic center, C-1, which is referred to as the anomeric carbon. Glucose can exist as an α or a β isomer, depending on whether the OH group attached to the anomeric carbon (C-1) is on the same side as the CH_2OH group or is on the opposite side. These two forms are referred to as **anomers** of glucose.



β-D-Glucopyranose

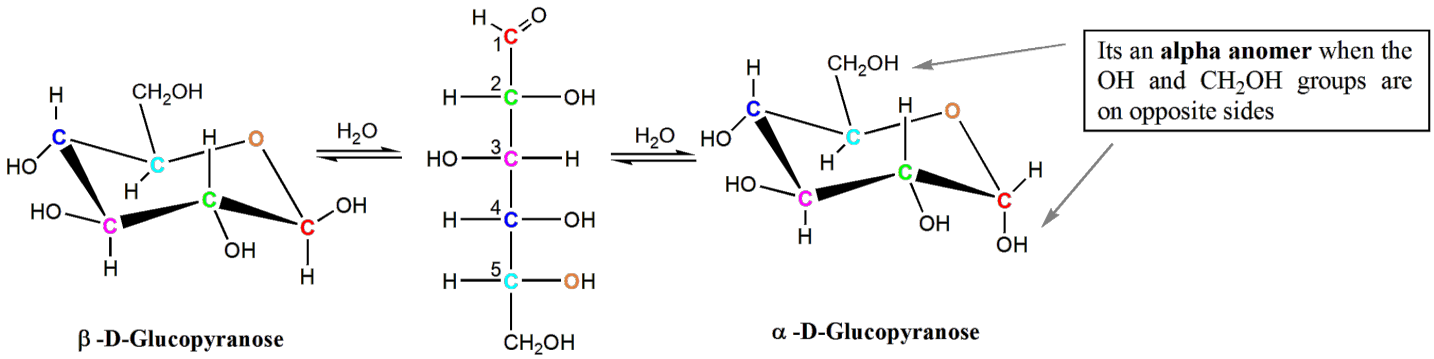
(hemiacetal of D-glucose)
Haworth projection

β-D-Glucopyranose

(hemiacetal of D-glucose)
Chair conformation

NB When you move from a Haworth projection to a chair conformation, the groups pointing upwards in the former become equatorial and the groups pointing downwards become axial respectively in the latter.

In aqueous solution, glucose exists in both the open and closed forms. These two forms always exist in equilibrium. In the process of converting from closed to open form and then back to closed form, the C-1→ C-2 bond rotates. This rotation produces either one of the two anomers. We term this phenomenon of opening of the ring, rotation of the C-1→ C-2 bond and the subsequent closing of the ring as mutarotation. So as a result of mutarotation, both the α and β anomers are present in equilibrium in solution. In the case of glucose, the β anomer is more predominant than the α anomer. This may not be the case with all the monosaccharides.



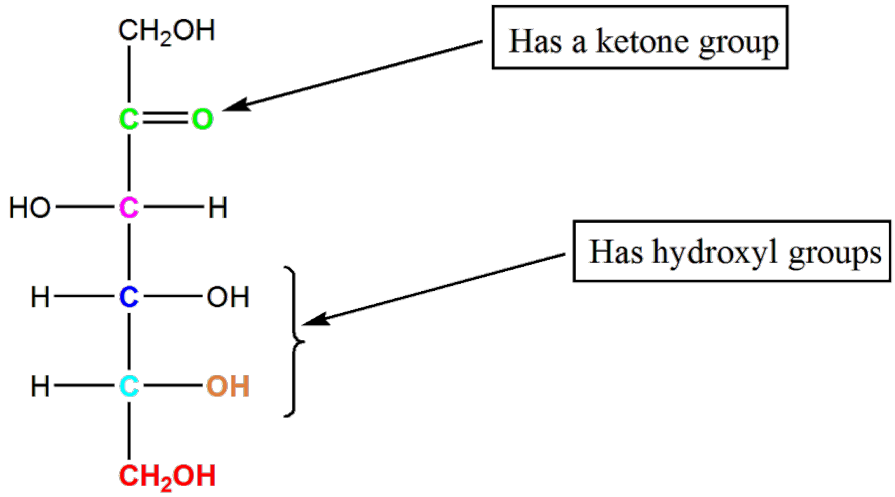
β -D-Glucopyranose
63%

D-Glucose
1%

α -D-Glucopyranose
36%

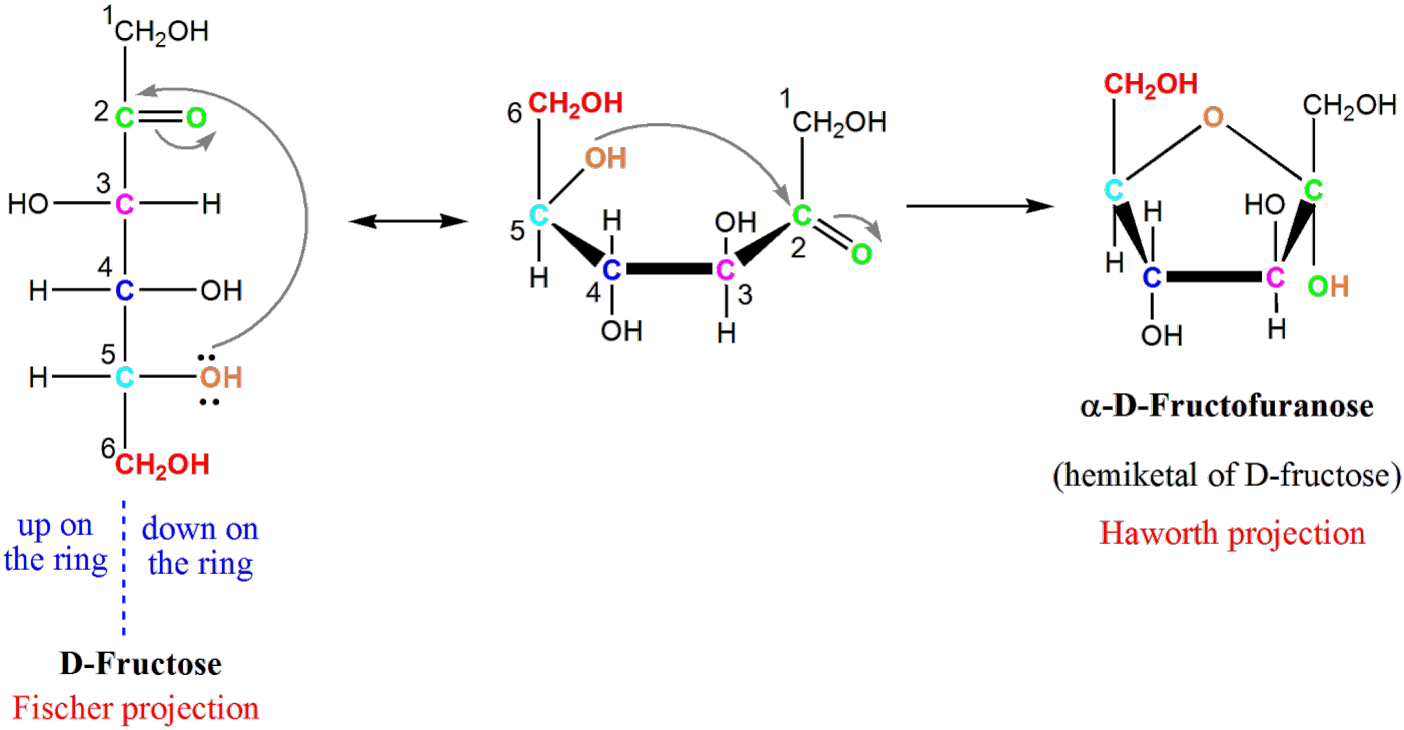
Cyclisation of fructose to its hemiketal form

Now let us change gears and apply the same principles (as applied to glucose) to a molecule of fructose. Fructose has a ketone group and five hydroxyl groups. So, fructose should also be able to cyclise to form an intramolecular hemiketal.



D-Fructose

There are in fact two ways in which a molecule of fructose can cyclise. The first is as illustrated below



D-Fructose

Fischer projection

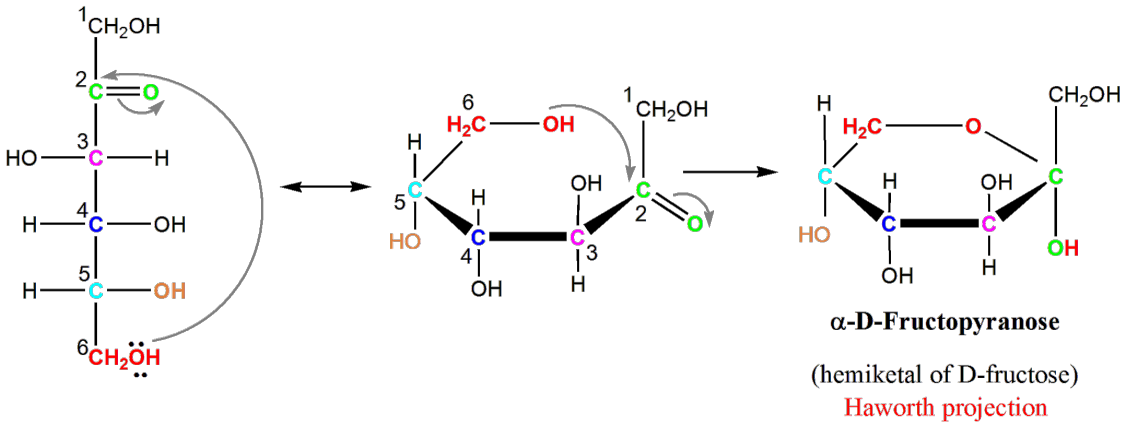
α-D-Fructofuranose

(hemiketal of D-fructose)

Haworth projection

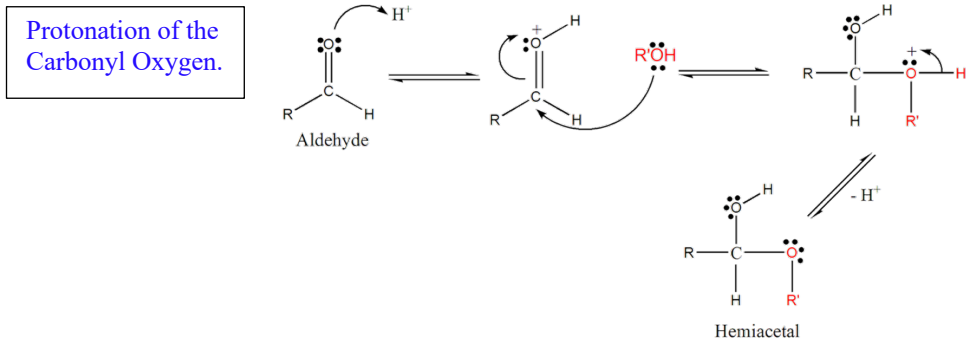
Here, as you can see, the hydroxyl attached to C-5 attacks the carbonyl group, yielding a 5-membered ring (furanose form).

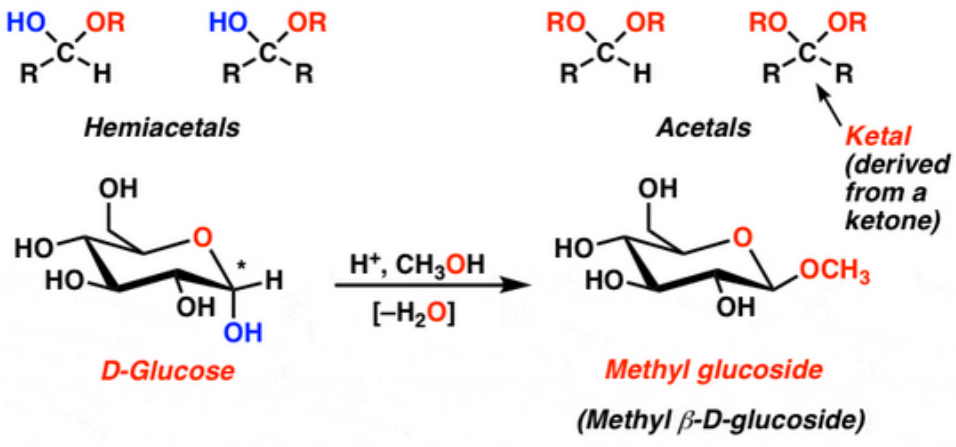
In the second scenario (as shown below), the hydroxyl attached to C-6 attacks the carbonyl group, resulting in a 6-membered ring (pyranose form).



reaction creating alpha-D-fructopyranose

The next extract (page 11) on Reducing Sugars is from *Master Organic Chemistry*, and please remember that a **hemiacetal** (from an Adehyde) or a **hemiketal** (from a Ketone) is a compound that results from the addition of an alcohol to an aldehyde or to a ketone respectively (*cf. page 6*). The main difference between **acetals** and **hemiacetals** is that **acetals** contain two –**OR** groups whereas **hemiacetals** contain one –**OR and** one –OH group.





Reducing Sugars by masterorganicchemistry

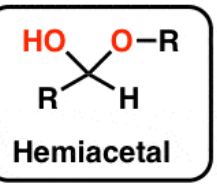
(https://www.masterorganicchemistry.com/2017/09/12/reducing-sugars/)

Summary

Any sugar* containing a hemiacetal is a reducing sugar.

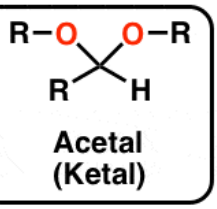
**for our purposes here, "sugar" refers to mono- and di-saccharides, not complex carbohydrates like starch, glycogen, and cellulose*

Recall the difference between hemiacetals and acetals:



Hemiacetal

- one OH group and one O-R group attached to same carbon
- in equilibrium with aldehyde/ketone

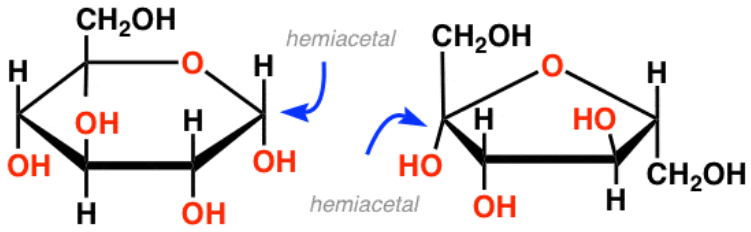


Acetal (Ketal)

- two O-R groups attached to same carbon
- not in equilibrium with aldehyde/ketone ("locked")

Sugars with a hemiacetal are in equilibrium with a ring-opened form containing an aldehyde and will react as a **reducing agent** toward certain (oxidizing) metal salts such as Cu^{2+} (Benedict's test, Fehlings aolution) and Ag^{+} (Tollens test)

Examples:



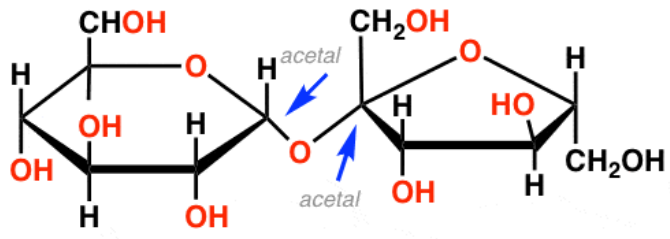
α-D-glucose D-Fructose

- Other monosaccharides such as galactose, mannose, ribose...
- Certain disaccharides such as lactose and maltose

A sugar without a hemiacetal is a non-reducing sugar

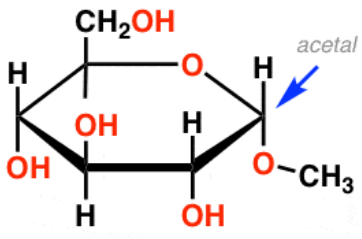
Acetals are "locked" and **not** in equilibrium with a ring-opened form with an aldehyde. They will therefore not react as a reducing agent.

Examples:



Sucrose

(a disaccharide; no hemiacetal present!)



α-D-glucose methyl glucoside

no hemiacetal

Why is this important?

An important test for quantifying blood glucose (Benedict's solution) relies on a reaction where Cu^{2+} is reduced by an aldehyde, resulting in a color change.

1. Before We Talk About Reducing Sugars: The Chemistry Of "Peeing On The Stick"

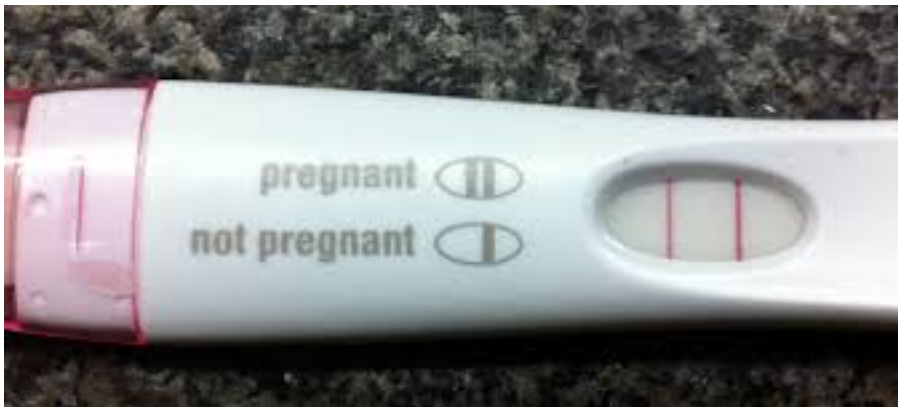
Q. Can you think of a situation where it might be useful to be able to measure the concentration of glucose in a solution (especially in blood or urine) ?

A. **Diabetes.** Once you have a way of quickly and easily measuring the concentration of sugar, then you can determine how much insulin is needed to counteract it.

Next question. What would be an easy, visual way to detect the presence of glucose? Especially something that doesn't require you to be an expert chemist?

Ideally, you'd like a chemical reaction that results in a **color change**.

Think about pregnancy tests: you just pee on a stick and know within a few minutes whether you're pregnant. You don't need to know any chemistry. It's brainless.



99.999% of people who use this do not know the chemistry behind how it works. And that's OK!

A test for blood sugar suitable for diabetics should have a similar ease of use.

This brings us (via aldehydes) to the topic of **reducing sugars**, since they are the basis of an historically important colour-based test for blood glucose.

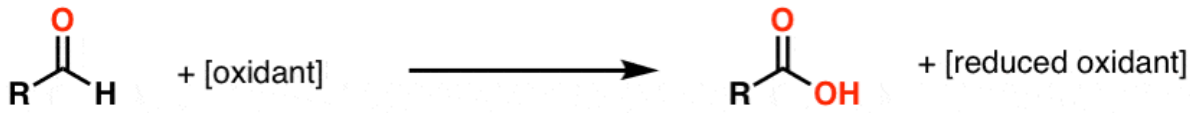
2. Three Visual "Tests" For The Presence of Aldehydes: Benedict's, Fehling's, and Tollens' Tests

Before we get to sugars, let's talk about the oxidation of aldehydes.

We've seen previously that aldehydes are a functional group that can be oxidized relatively easily to carboxylic acids. For example, oxidation of alcohols with a "strong" oxidant like chromic acid (H_2CrO_4) results in an aldehyde that is quickly oxidized further to a carboxylic acid.

During this process, the aldehyde is oxidized (i.e. it loses electrons) and the oxidizing agent is reduced (it gains electrons and e.g. blue Cu^{2+} ions become reddy-brown Cu^{+} ions). The Aldehyde is oxidised, therefore another way of describing this is to say that **the aldehyde is the *reducing* agent in this process**.

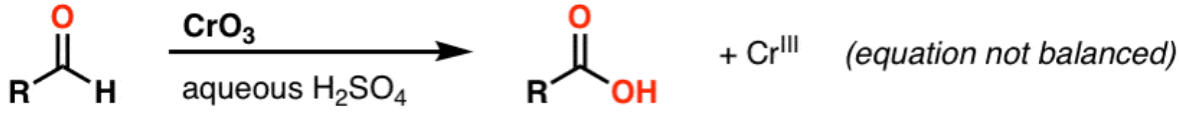
Oxidation of Aldehydes to Carboxylic Acids



Aldehyde **Carboxylic acid**

- the aldehyde is **oxidized** to a carboxylic acid (break C-H, form C-O)
- the oxidant is **reduced** by the aldehyde; therefore the **aldehyde is the reducing agent** here.

Example: Jones Oxidation



- Aldehyde oxidized to carboxylic acid
- Chromium (VI) is reduced to Chromium (III)

The list of reagents that can be used to oxidize aldehydes to carboxylic acids is very long. Of these, a few methods stand out in providing a particularly clear visual indication that the reaction has proceeded to completion. *[**Note 1**]*

Three "visual" tests for aldehydes that you might encounter in an introductory organic chemistry lab are the following:

- Fehling's solution, where an aldehyde changes the colour of a blue Cu(II) solution to red Cu(I) *[as Cu_2O]*.
- Benedict's solution a slightly modified version of Fehling's solution
- Tollens' test, where aldehyde oxidation results in a beautiful "mirror" of silver metal to precipitate on the reaction vessel.

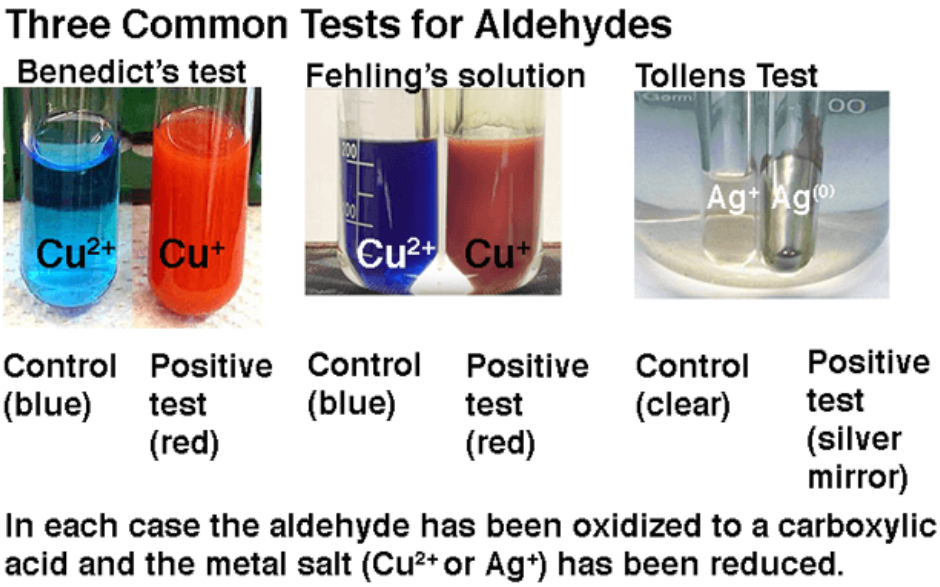
The "Tollens Test" For Aldehydes

Aldehyde **Carboxylic acid**

This is a redox reaction:

- The aldehyde is oxidized by silver(I) to the carboxylic acid
- Silver(I) is reduced by the aldehyde, forming atomic silver, which forms a mirror on the reaction flask

[details on the actual chemistry of these tests at the bottom of the post [jump]



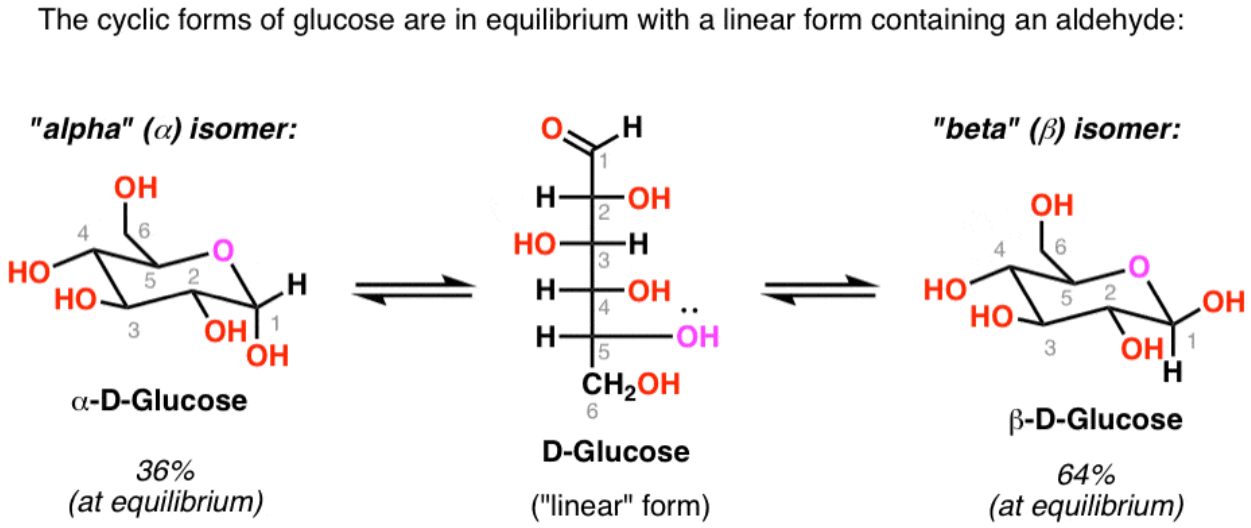
[image sources]

Importantly, **ketones do not react under any of these conditions.** The above tests were also a useful way of distinguishing aldehydes from ketones in the dark days before IR and NMR spectroscopy made this routine.

So what does this have to do with sugars?

3. Reducing Sugars: Sugars With A Hemiacetal Functional Group Give Positive Tests Since They Are In Equilibrium With An Open-Chain Aldehyde

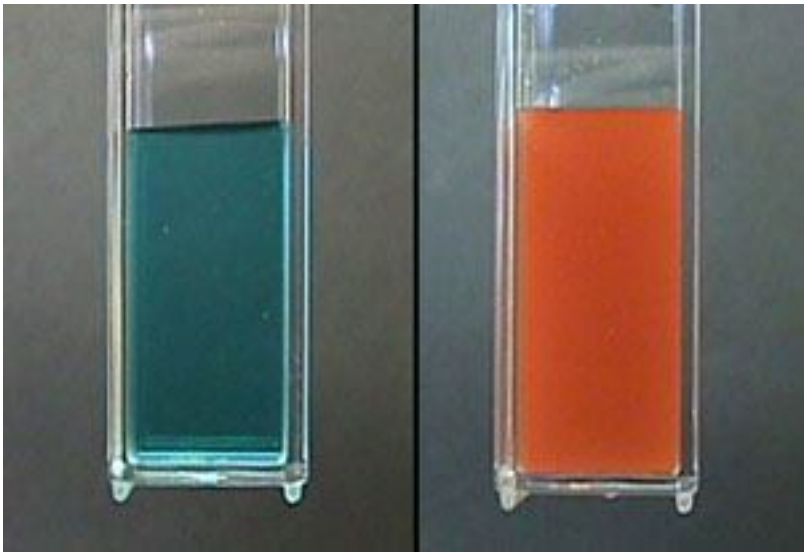
As we've seen, glucose is in equilibrium with an open-chain (or "linear") form containing an aldehyde.



The concentration of aldehyde at any given time is small (<1%), but long-lived enough to be trapped with the right reagent.

This means that glucose will give a positive test with Benedicts' reagent, Fehling's solution, or the Tollens' test, and the aldehyde will be oxidized to a carboxylic acid (and a colour change will occur)

Voila! A simple colour change tells you whether glucose is present!



Negative (left) and positive (right) tests for glucose using Benedict's reagent

image source

What about quantification?

It's nice to have a quick visual test for glucose. But what if we want to determine the exact concentration of glucose in a solution of, say, urine or blood?

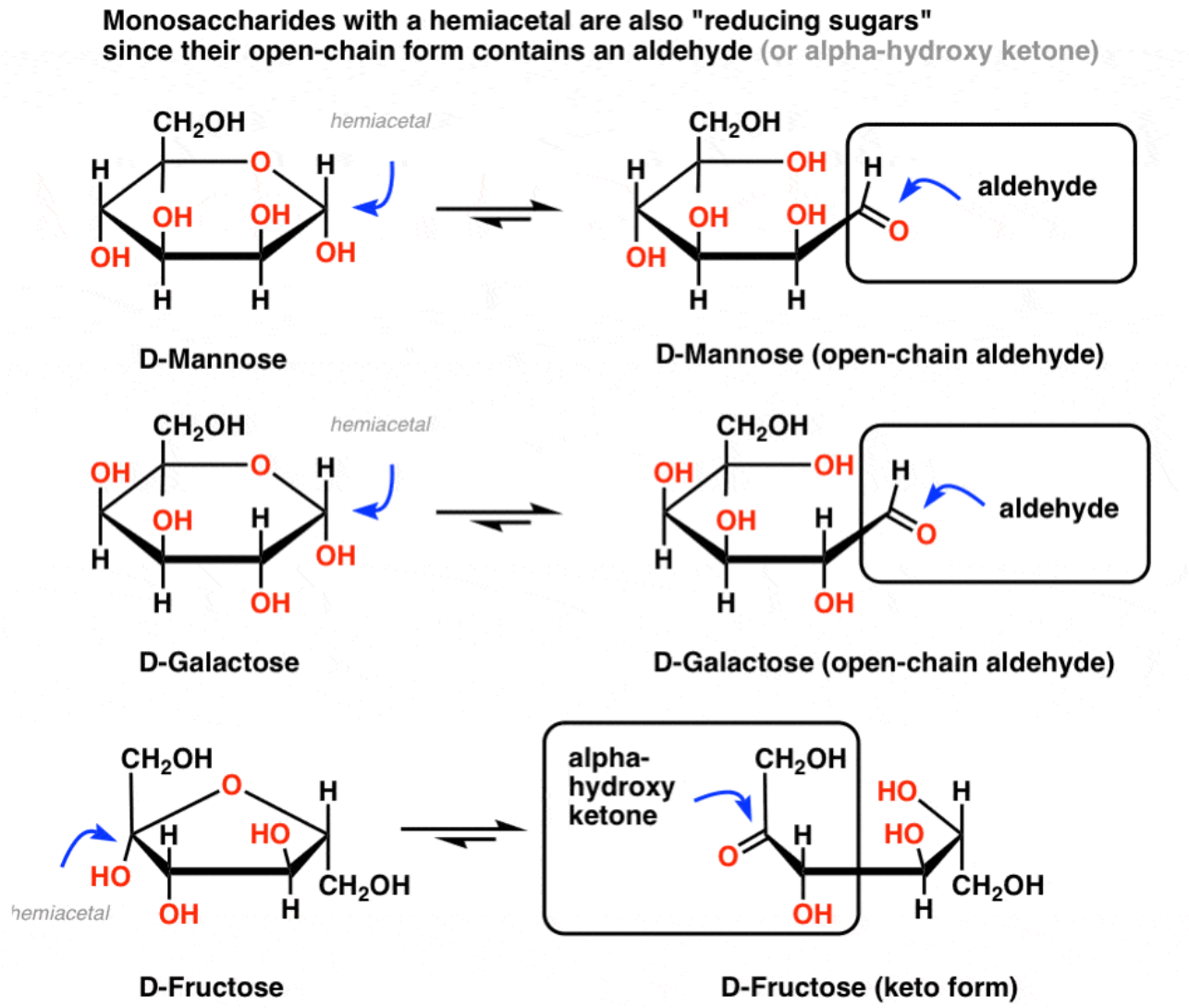
In this case, a slightly different formulation of Benedicts solution is used [Note 2] which results in a colourless precipitate rather than a red color. A solution of the sample to be analysed is added, via a burette, to a flask containing a known amount of Benedict's solution until the blue colour of the Cu(II) disappears. The unknown sample is then calibrated using a 1% solution of glucose. *[Full details here]*

Benedict's assay was the method of choice for quantifying glucose for over 50 years. One researcher recalls that all inductees into the U.S. army during World War II had their urine tested for sugar with Benedict's Solution.

In recent times, however, the use of Benedict's solution has been supplanted by enzymatic methods such as glucose oxidase. Why?

The Benedict test is not *specific* for glucose; it just tells you whether or not an *aldehyde* is present, and it will also give a positive test for other reducing sugars.

In short, *any* sugar* *(*mono- or disaccharide)* with a hemiacetal will also give a positive test, since these sugars are in equilibrium with an open-chain aldehyde. So if the blood/urine contains common monosaccharides like mannose, galactose, or fructose, these will deliver a positive test. In other words, those sugars **are also reducing sugars.**



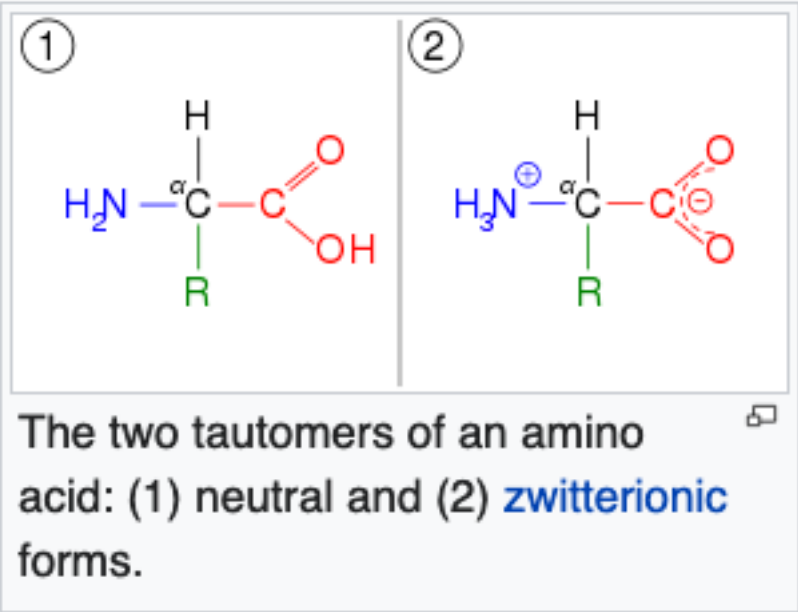
Hold on for a second. *Et tu,* fructose?

Ketones are not supposed to oxidise under these conditions! So why does fructose give a positive test?

Good question. Although fructose is a keto sugar, and ketones generally give a negative test with Benedict's test, there is an exception. If the carbon adjacent to the ketone carbon (the "alpha carbon") contains a hydroxyl group, the ketone will be in equilibrium with an aldehyde through tautomerisation *(just for the record, this is called an "enediol rearrangement"). [Note 3]*

Wikipedia says that **Tautomers** are structural isomers (constitutional isomers) of chemical compounds that readily interconvert. This reaction commonly results in the relocation of a proton. Tautomerism is for example relevant to the behaviour of amino acids and nucleic acids, two of the fundamental building blocks of life.

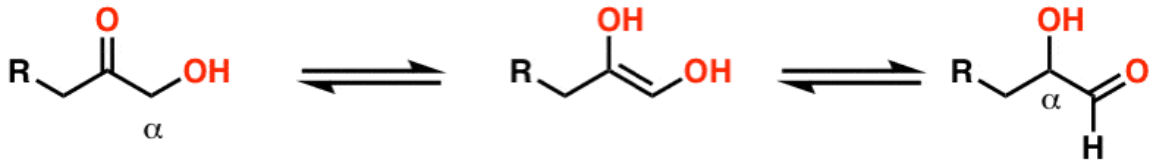
The concept of tautomerizations is called **tautomerism**. Tautomerism is also called desmotropism. The chemical reaction interconverting the two is called **tautomerisation**.



The two tautomers of an amino acid: (1) neutral and (2) zwitterionic forms.

***Et tu,* Fructose?**

Alpha-hydroxy ketones are in equilibrium with aldehydes through tautomerization. So fructose also counts as a "reducing sugar"



α-hydroxy ketone

"enediol" intermediate

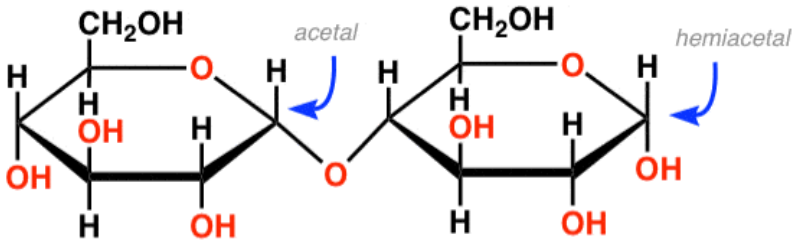
α-hydroxy aldehyde

gives positive Tollens, Benedict, Fehlings' test

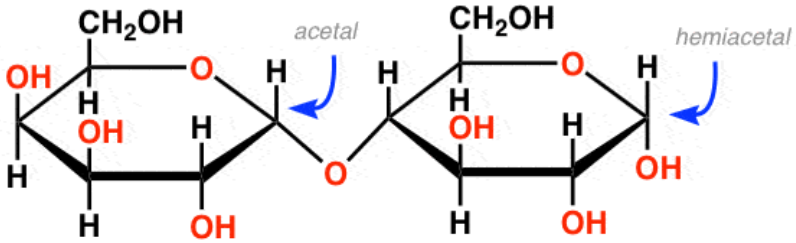
Likewise, some disaccharides such as maltose and lactose contain a hemiacetal. They also are reducing sugars that give a positive Fehling's, Benedict's, or Tollens' test (picture of lactose positive test follows).

Lactose and Maltose are also reducing sugars and give a positive Benedict test

Maltose:



Lactose:



The bottom line is that Benedicts' reagent quantifies *reducing sugars* which includes not just glucose but also mannose, lactose, maltose, fructose, and others. That means that the test isn't as specific as we'd like!

4. So what *isn't* a reducing sugar?

So far, it seems like every sugar we've encountered is a reducing sugar. So it's fair to ask: when is a sugar *not* a reducing sugar?

Two main cases:

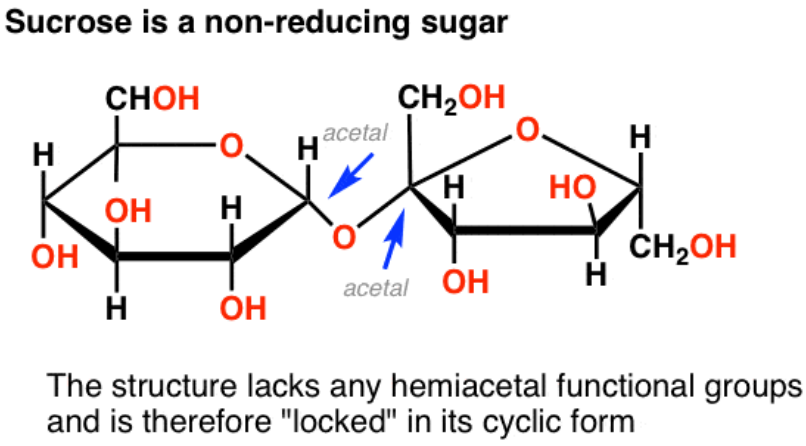
- mono and di-saccharides which lack a hemiacetal
- polysaccharides where the ratio of hemiacetals to acetal linkages is very low (e.g. starch)

5. Saccharides That Lack A Hemiacetal Are Not Reducing Sugars

We saw at the top of the post that hemiacetals are in equilibrium with an aldehyde or ketone. In contrast, acetals and ketals are **locked** in place and can be converted back to the aldehyde or ketone only with aqueous acid. That's why they make great protecting groups for aldehydes/ketones.

The poster child for a non-reducing sugar is **sucrose**, a.k.a. table sugar.

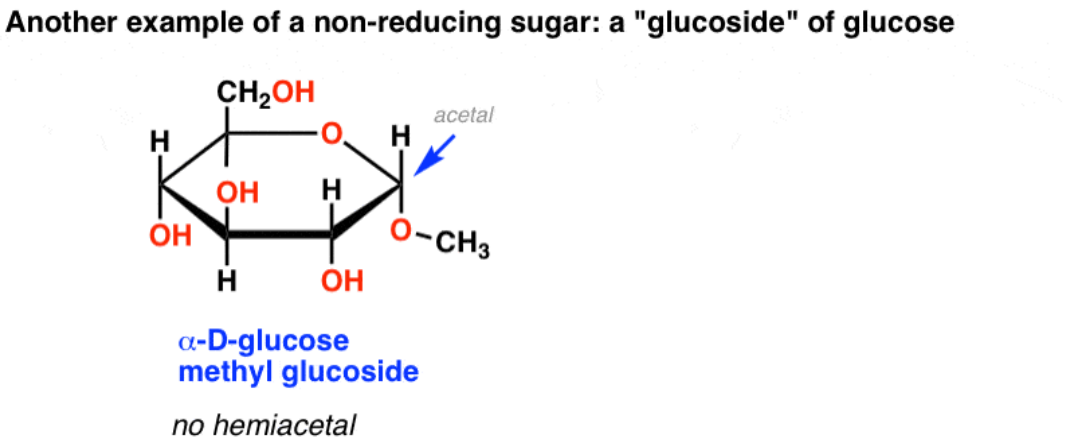
Sucrose is a disaccharide of glucose and fructose. See if you can find a hemiacetal in its structure, below:



There isn't one! Sucrose has only acetal groups, and since acetals do **not** open up to aldehydes under the basic conditions present in the Benedict test, sucrose is **not** a reducing sugar. [Note 4]

Sucrose gives a negative test (the colour changes from blue to a reddy-brown) with Benedict's solution.

Another example of non-reducing sugars are the so-called "glucosides" of common sugars, such as glucose methyl glucoside (below). This is obtained by heating glucose in acidic methanol.



Lacking a hemiacetal which could open to an aldehyde, this methyl glucoside also gives a negative Benedict's test.

6. Complex Polysaccharides (e.g. Starch) Which Have Only A Single Hemiacetal Unit Do not Count As Reducing Sugars

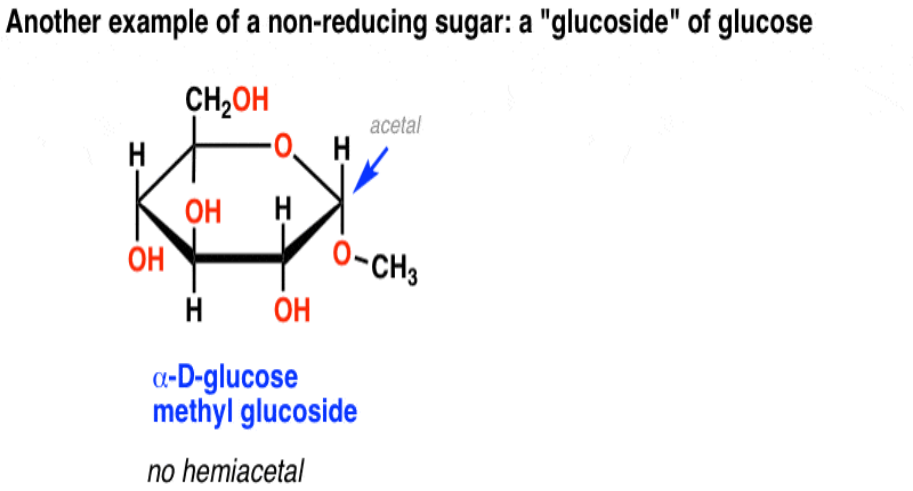
Sugars are able to form long chains with each other in arrangements known as polysaccharides. Common examples of polysaccharides are starch, cellulose, and glycogen.

The vast majority of individual sugar units in these polysaccharides are joined to each other via acetal ("glycosidic") linkages. Hemiacetals are present, but only at the termini of the polymer.

Starch, for example, generally has about 300-600 individual units of glucose, but only one unit (the terminus) has a hemiacetal.

One hemiacetal “needle” in a haystack of “acetals” is not enough to give a positive test for reducing sugars. Therefore these polysaccharides are not considered reducing sugars. For example, starch gives a negative test (see below).

Here is an example of Benedict’s test with lactose, starch, glucose, fructose, and sucrose



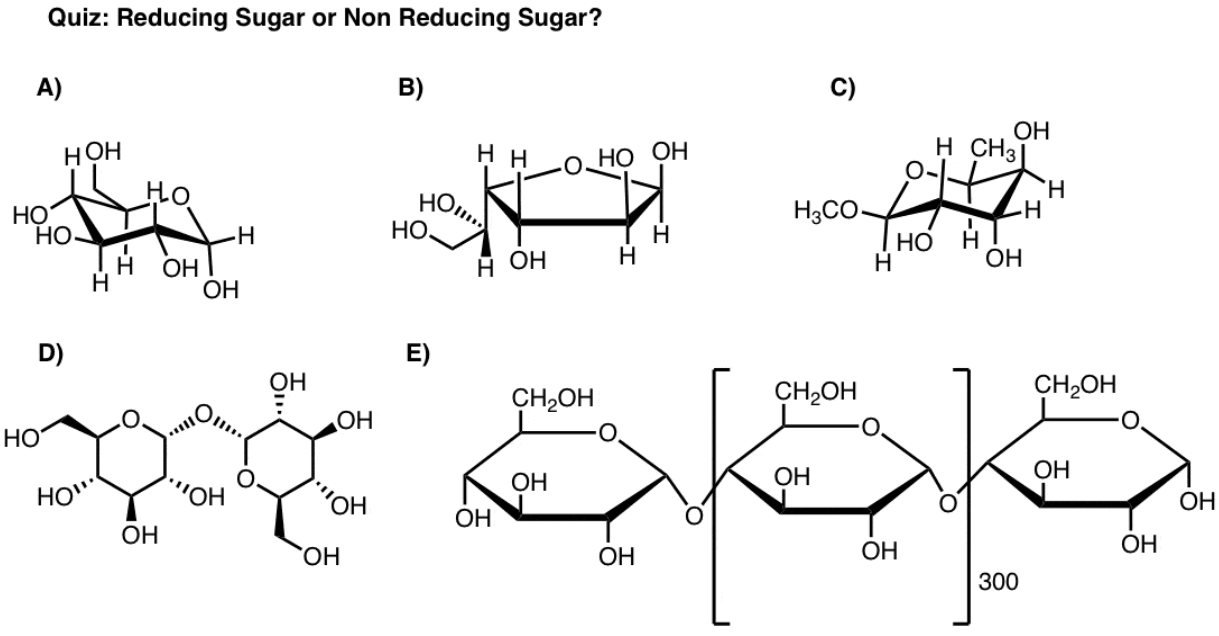
Note that starch and sucrose are blue, classifying them as non-reducing sugars.

That’s enough about what classifies a “reducing sugar” from a “non-reducing sugar”.

Here’s the last step. Test yourself. What’s a reducing sugar and what is not?

7. Test Yourself On Reducing Sugars

Make sense? Quiz yourself on whether the following sugars are reducing sugars or non-reducing sugars.



If you don't need to know anything else other than "what's a reducing sugar", you're done here.

But if you want to go further down the rabbit hole, I invite you to read further to learn about…

8. The Chemistry of the Benedict's, Fehling's, and Tollens' tests

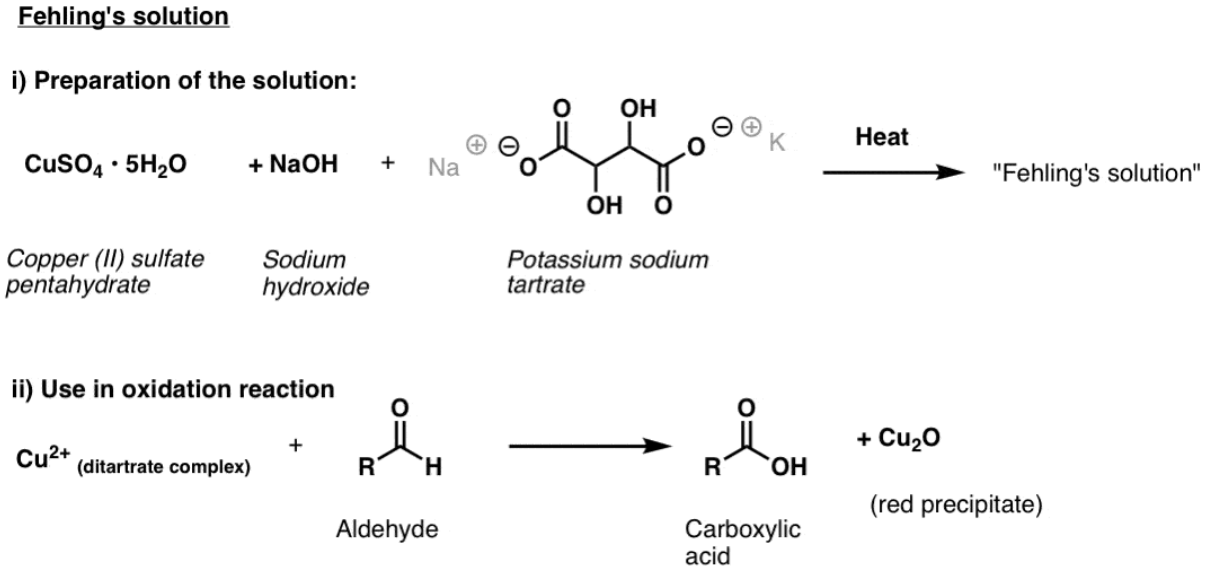
So what's actually going on in the Benedict's, Fehling's and Tollens' tests? Let's discuss the details of the chemistry.

One thing about all three tests is that the active reagent is not particularly bench stable and has to be freshly prepared.

Fehling's Solution

For Fehling's solution, one starts with bright blue copper(II) sulfate, sodium hydroxide, and potassium sodium tartrate (otherwise known as Rochelle's salt). The purpose behind using the tartrate is that it coordinates to the copper(II) and helps prevent it from crashing out of solution.

Once prepared, the substance to be analyzsed is added, and the mixture is heated for a brief period.



This results in a carboxylic acid and red Cu(I) which precipitates out as copper(I) oxide.

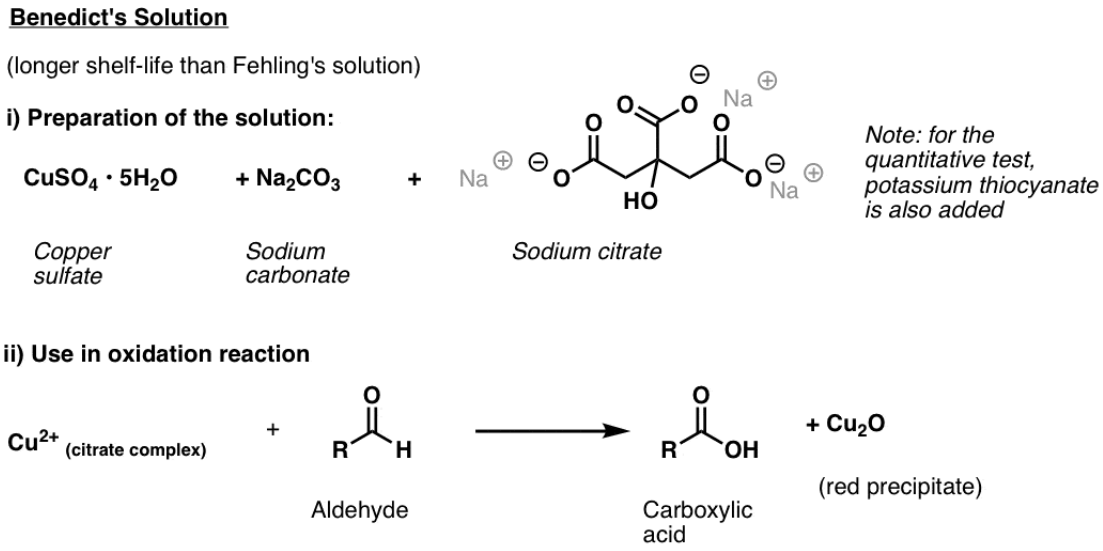
The structure of the active species in Fehling's solution has been determined; it's a square-planar copper complex attached to two tartrate ligands.

Benedict's Solution

Benedict's solution is a slight variation of Fehling's solution that uses citrate instead of tartrate, which provides better stability for the copper(II).

Like Fehling's solution, it is best made fresh. The ingredients are copper(II) sulphate, sodium carbonate (note: hydroxide is also needed! – see reference), and sodium citrate. *(Note: in the quantitative test, potassium thiocyanate is added, which results in a colourless white precipitate)*.

The test is performed by adding the substance to be analysed and heating briefly.

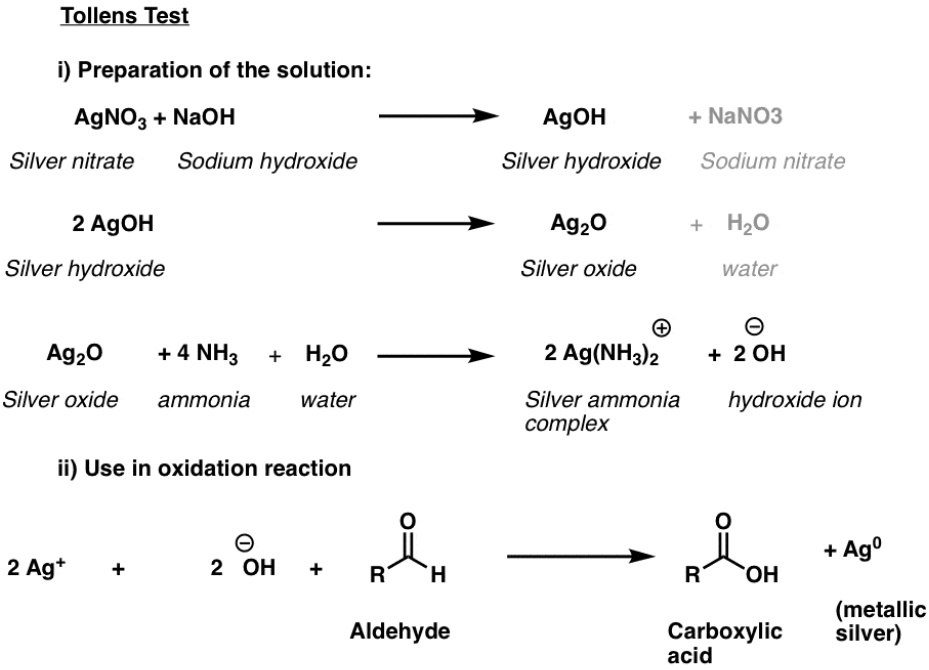


Tollens' Solution

The active ingredient in the Tollens' test, $[Ag(NH_3)_2]^+$, does not have a long shelf life and like the Fehling's and Benedict's solutions, it is best prepared fresh.

Silver nitrate is converted to silver hydroxide, which forms silver (I) oxide, Ag_2O. Then, addition of aqueous ammonia (NH_3) results in formation of the silver-ammonia complex which is the active oxidant.

The sample to be tested is then added to the freshly prepared active oxidant in a basic solution. A positive test results in a beautiful mirror of silver metal being precipitated out on the reaction vessel. (A variant of this procedure is used for the preparation of mirrors).

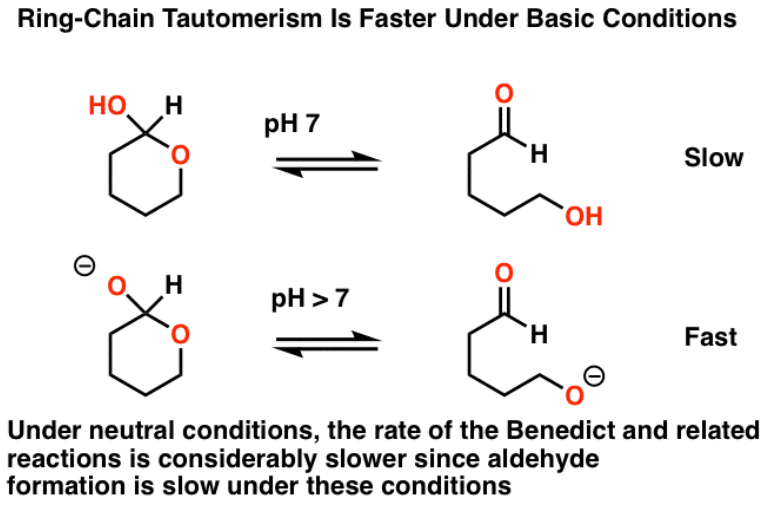


How Does It Work?

The first thing to note is that all of these procedures occur in basic solution.

Why? There are at least two good reasons for this that we can talk about.

- First, acidic conditions might hydrolyse any acetals present to hemiacetals, giving a false positive test.
- Secondly, bases considerably speed up the rate of ring-chain tautomerism (i.e. interconversion between the cyclic hemiacetal form and the linear aldehyde form).



Bottom line here is that adding base has the effect of increasing the concentration of the starting aldehyde.

The Mechanistic Details Are Murky And You Will Not Find Them In Any Introductory Textbook

I can't find a single instance of the mechanism for the Fehling's or Benedict's solutions being elucidated conclusively online. *If I am wrong, please tell me (leave a comment).*

There is a third reason for the use of base, although I'm not very keen on talking about it. You might notice we haven't mentioned the mechanisms of any of these reactions. That's because the exact mechanisms have been tricky to elucidate. One of the key steps involved in the mechanism of each reaction seems to be a process called, "single-electron transfer" which is essentially when the metal salt slurps a single electron off of the substrate, creating a free-radical and/or carbocation.

One of the access points for the initiation of a single-electron transfer reaction is a carbon-metal bond, which can be achieved through base-promoted formation of an enolate.

That requires the aldehyde to have a proton on the alpha carbon (i.e. be "enolisable"). It turns out that Fehling's solution does a poor job in testing for benzaldehyde, which lacks any protons on the alpha-carbon and cannot be enolised. It would thus appear that the reaction needs to proceed through an enol.

However, Fehling's solution also oxidises formaldehyde to formic acid and thereon to carbon dioxide, and this process cannot possibly proceed through an enol/enolate intermediate.

So it is likely that a variety of mechanistic pathways can be in operation.

What might a mechanism look like?

Maybe, possibly, something like this?

Hover here for a pop-up image or [**click for image of a hypothetical mechanism**]

[The key step here would be generation of a carbocation on the alpha-position of the aldehyde, which accepts a hydride from a deprotonated hydrate-like intermediate, leading to formation of a carboxylic acid.]

If anyone else has a better idea, please feel free to comment below.

Notes

Image sources: Benedict's solution. Fehling's solution. Tollens' test.

Note 1. This isn't to say that they're the most *practical* methods for preparing carboxylic acids from aldehydes. When chemists want to prepare a carboxylic acid from an aldehyde in good yield, they don't use any of these three processes. The standard way to do it is the Pinnick oxidation.

Note 2. The quantitative test apparently employs potassium isocyanate, which results in a colourless precipitate.

Note 3: It's likely that the enediol intermediate is actually the species that reacts with Cu^{2+} in the initial step of the mechanism that leads to the aldehyde. See the mechanism section.

Note 4. One thing to note: if sucrose is heated with aqueous acid before a Fehling's/Benedict's/Tollens' test, a positive test will result. That's because the acetal linkages will be hydrolysed by aqueous acid to produce the two constituent sugars of sucrose (glucose and fructose) which are themselves reducing sugars.

Reducing sugars

From Wikipedia, the free encyclopedia

A **reducing sugar** is any sugar that is capable of acting as a reducing agent because it has a free aldehyde group or a free ketone group.[1] All monosaccharides are reducing sugars, along with some disaccharides, some oligosaccharides, and some polysaccharides. The monosaccharides can be divided into two groups: the aldoses, which have an aldehyde group, and the ketoses, which have a ketone group. Ketoses must first tautomerize to aldoses before they can act as reducing sugars. The common dietary monosaccharides galactose, glucose and fructose are all reducing sugars.

Disaccharides are formed from two monosaccharides and can be classified as either reducing or nonreducing. Nonreducing disaccharides like sucrose and trehalose have glycosidic bonds between their anomeric carbons and thus cannot convert to an open-chain form with an aldehyde group; they are stuck in the cyclic form. Reducing disaccharides like lactose and maltose have only one of their two anomeric carbons involved in the glycosidic bond, while the other is free and can convert to an open-chain form with an aldehyde group.

The aldehyde functional group allows the sugar to act as a reducing agent, for example, in the Tollens' test or Benedict's test. The cyclic hemiacetal forms of aldoses can open to reveal an aldehyde, and certain ketoses can undergo tautomerisation to become aldoses. However, acetals, including those found in polysaccharide linkages, cannot easily become free aldehydes.

Reducing sugars react with amino acids in the Maillard reaction, a series of reactions that occurs while cooking food at high temperatures and that is important in determining the flavour of food. Also, the levels of reducing sugars in wine, juice, and sugarcane are indicative of the quality of these food products.

Contents

Terminology

Oxidation-reduction

A *reducing sugar* is one that *reduces* another compound and is itself *oxidized*; that is, the carbonyl carbon of the sugar is oxidized to a carboxyl group.[2]

A sugar is classified as a reducing sugar only if it has an open-chain form with an aldehyde group or a free hemiacetal group.[3]

Aldoses and ketoses

Monosaccharides which contain an aldehyde group are known as aldoses, and those with a ketone group are known as ketoses. The aldehyde can be oxidized via a redox reaction in which another compound is reduced. Thus, aldoses are reducing sugars. Sugars with ketone groups in their open chain form are capable of isomerizing via a series of tautomeric shifts to produce an aldehyde group in solution. Therefore, ketones like fructose are considered reducing sugars but it is the isomer containing an aldehyde group which is reducing since ketones cannot be oxidized without decomposition of the sugar. This type of isomerization is catalyzed by the base present in solutions which test for the presence of reducing sugars.[3]

Reducing end

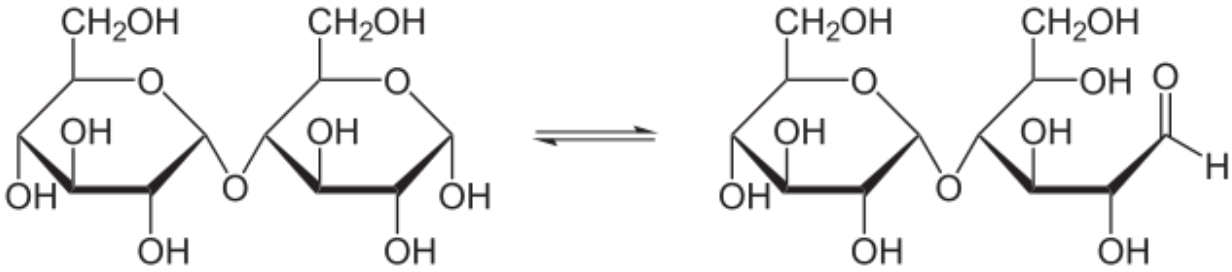
Disaccharides consist of two monosaccharides and may be either reducing or nonreducing. Even a reducing disaccharide will only have one reducing end, as disaccharides are held together by glycosidic bonds, which consist of at least one anomeric carbon. With one anomeric carbon unable to convert to the open-chain form, only the free anomeric carbon is available to reduce another compound, and it is called the *reducing end* of the disaccharide. A nonreducing disaccharide is that which has both anomeric carbons tied up in the glycosidic bond.[4]

Similarly, most polysaccharides have only one reducing end.

Examples

All monosaccharides are reducing sugars because they either have an aldehyde group (if they are aldoses) or can tautomerize in solution to form an aldehyde group (if they are ketoses).[5] This includes common monosaccharides like galactose, glucose, glyceraldehyde, fructose, ribose, and xylose.

Many disaccharides, like cellobiose, lactose, and maltose, also have a reducing form, as one of the two units may have an open-chain form with an aldehyde group.[6] However, sucrose and trehalose, in which the anomeric carbons of the two units are linked together, are nonreducing disaccharides since neither of the rings is capable of opening.[5]



Equilibrium between cyclic and open-chain form in one ring of maltose

In glucose polymers such as starch and starch-derivatives like glucose syrup, maltodextrin and dextrin the macromolecule begins with a reducing sugar, a free aldehyde. When starch has been partially hydrolyzed the chains have been split and hence it contains more reducing sugars per gram. The percentage of reducing sugars present in these starch derivatives is called dextrose equivalent (DE).

Glycogen is a highly branched polymer of glucose that serves as the main form of carbohydrate storage in animals. It is a reducing sugar with only one reducing end, no matter how large the glycogen molecule is or how many branches it has (note, however, that the unique reducing end is usually covalently linked to glycogenin and will therefore not be reducing). Each branch ends in a nonreducing sugar residue. When glycogen is broken down to be used as an energy source, glucose units are removed one at a time from the nonreducing ends by enzymes.[2]

Characterisation

Several qualitative tests are used to detect the presence of reducing sugars. Two of them use solutions of copper(II) ions: Benedict's reagent (Cu^{2+} in aqueous sodium citrate) and Fehling's solution (Cu^{2+} in aqueous sodium tartrate).[7] The reducing sugar reduces the copper(II) ions in these test solutions to copper(I), which then forms a brick red copper(I) oxide precipitate. Reducing sugars can also be detected with the addition of Tollen's reagent, which consist of silver ions (Ag^{+}) in aqueous ammonia.[7] When Tollens' reagent is added to an aldehyde, it precipitates silver metal, often forming a silver mirror on clean glassware.[3]

3,5-dinitrosalicylic acid is another test reagent, one that allows quantitative detection. It reacts with a reducing sugar to form 3-amino-5-nitrosalicylic acid, which can be measured by spectrophotometry to determine the amount of reducing sugar that was present.[8]

Sugars having acetal or ketal linkages are not reducing sugars, as they do not have free aldehyde chains. They therefore do not react with any of the reducing-sugar test solutions. However, a non-reducing sugar can be hydrolyzed using dilute hydrochloric acid. After hydrolysis and neutralization of the acid, the product may be a reducing sugar that gives normal reactions with the test solutions.

All carbohydrates respond positively to Molisch's reagent but the test has a faster rate when it comes to monosaccharides.

Importance in medicine

Fehling's solution was used for many years as a diagnostic test for diabetes, a disease in which blood glucose levels are dangerously elevated by a failure to produce enough insulin (type 1 diabetes) or by an inability to respond to insulin (type 2 diabetes). Measuring the amount of oxidizing agent (in this case, Fehling's solution) reduced by glucose makes it possible to determine the concentration of glucose in the blood or urine. This then enables the right amount of insulin to be injected to bring blood glucose levels back into the normal range.[2]

Importance in food chemistry

Maillard reaction

Main article: Maillard reaction

The carbonyl groups of reducing sugars react with the amino groups of amino acids in the Maillard reaction, a complex series of reactions that occurs when cooking food.[9] Maillard reaction products (MRPs) are diverse; some are beneficial to human health, while others are toxic. However, the overall effect of the Maillard reaction is to decrease the nutritional value of food.[10] One example of a toxic product of the Mailard reaction is acrylamide, a neurotoxin and possible carcinogen that is formed from free asparagine and reducing sugars when cooking starchy foods at high temperatures (above 120°C).[11]

Food quality

The level of reducing sugars in wine, juice, and sugarcane are indicative of the quality of these food products, and monitoring the levels of reducing sugars during food production has improved market quality. The conventional method for doing so is the Lane-Eynon method, which involves titrating the reducing sugar with copper(II) in Fehling's solution in the presence of methylene blue, a common redox indicator. However, it is inaccurate, expensive, and sensitive to impurities.[12]