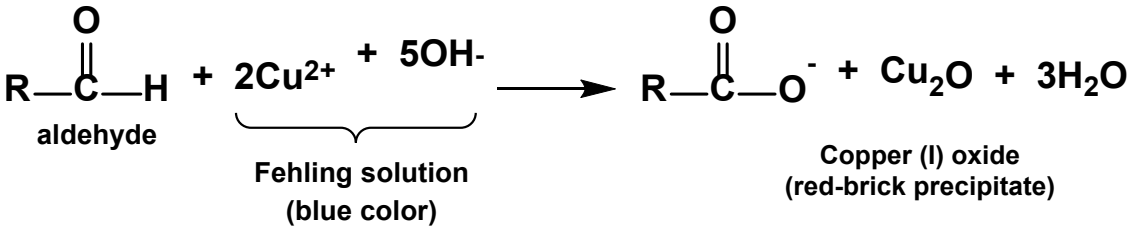
QUALITATIVE ANALYSIS OF WHEY CONSTITUENTS

1) Detection of sugars: Fehling's reaction

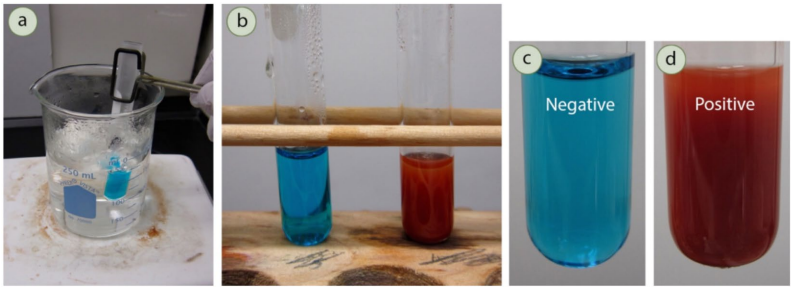
This test reveals the presence of reducing sugars (aldoses such as glucose, ribose, and erythrose, etc.). It is a redox reaction in which the aldehyde (reducing) group of sugars is oxidized to an acid group by Cu^{2+}, which is reduced to Cu^{+}. Both monosaccharides and reducing disaccharides react with Cu^{2+} to produce a red cuprous oxide precipitate. Since the reaction takes place in a basic medium, sodium potassium tartrate must be introduced into the reaction to prevent the precipitation of cuprous hydroxide.

Fehling's reagent A is a solution of 7% $CuSO_4 \cdot 5H_2O$ in water while Fehling's reagent B is 35% sodium potassium sodium tartrate in 10% NaOH in water.

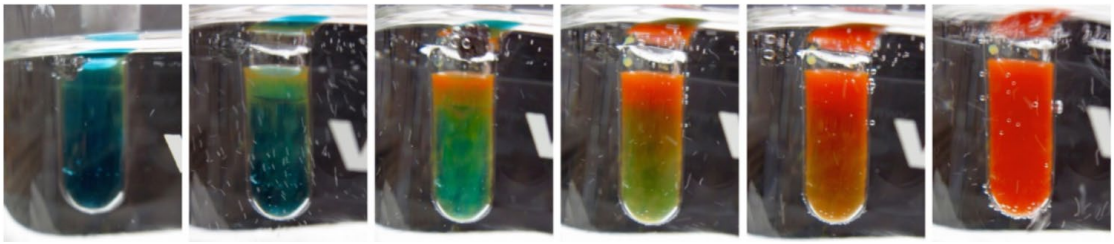
The redox reaction that takes place when reducing sugars are present in the solution is as follows:



The test tube should be heated in a water bath for 10 minutes to observe the change in color.



https://www.youtube.com/watch?v=yjLB_ntM324

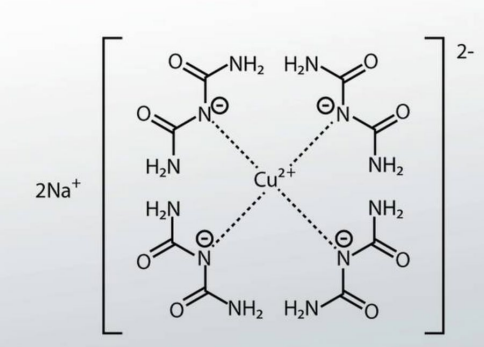


2) **Detection of proteins: Biuret reaction**

The presence of proteins in a mixture can be determined by the Biuret reaction, since this test indicates the presence of peptide bonds. The Biuret reagent contains $CuSO_4$ in aqueous solution at basic pH (since NaOH or KOH is also added). The reaction is based on the formation of a grey-violet compound due to the formation of a coordination complex between the Cu^{2+} ions and the unshared electron pairs of the nitrogen that forms part of the peptide bonds with an absorption maximum at 540 nm.

Biuret reagent A is a 20% sodium hydroxide solution in water and Biuret reagent B is a 1% copper sulphate solution.

The complex formed is shown in the figure below:



Wait five minutes to observe a change in color to grey-violet if the test result is positive. If it is negative, the solution remains blue.

These colors are also considered to provide a positive result.

3) **Detection of chloride**

Chloride ion (Cl^-) is one of the main inorganic anions in water and many foods. We will determine its presence by direct reaction with silver nitrate through the formation of silver chloride, a highly insoluble compound that appears as a precipitate.

The reaction that takes place is as follows:

$$Cl^-(aq) + AgNO_3(aq) \rightarrow AgCl(s) + NO_3^-(aq)$$

The presence of chlorides in solution is considered positive when a white, milky precipitate (AgCl) appears.

4) **Detection of phosphate**

Orthophosphate ions (PO_4^{3-}) form molybdophosphoric acid with molybdate ions in sulphuric solution. The molybdophosphoric acid is reduced to phosphomolybdenum blue to produce a blue solution, which confirms a positive test result.

The method is based on reaction of the phosphate ion with the molybdate ion (MoO_4^{2-}), thus leading to phosphomolybdate ($[PO_4 12MoO_3]^{3-}$). By reduction, the latter gives rise to a compound, the exact structure of which is not known, called “molybdenum blue”.

Phosphate Reagent Test 1 contains a solution of ammonium molybdate in an acidic medium. Phosphate reagent Test 2 contains a solution of ascorbic (reducing) acid.

The appearance of the blue color indicates the presence of phosphates. See the photograph on the next page.

5) **Detection of calcium**

Alkalinizing a solution containing calcium and oxalic acid (or a soluble oxalate such as sodium or ammonium oxalate) with ammonia above pH 4 leads to the precipitation of calcium in the form of calcium oxalate monohydrate. This is a fine, white powder that, depending on the reaction, is not very soluble in cold water but is quite soluble in hot water:

$$Ca^{2+}(ac) + C_2O_4^{2-}(ac) \rightarrow CaC_2O_4 \cdot H_2O\ (s)$$

The reagent used in this test is 1% ammonium oxalate.

The appearance of a white precipitate indicates the presence of calcium in the milk sample.