

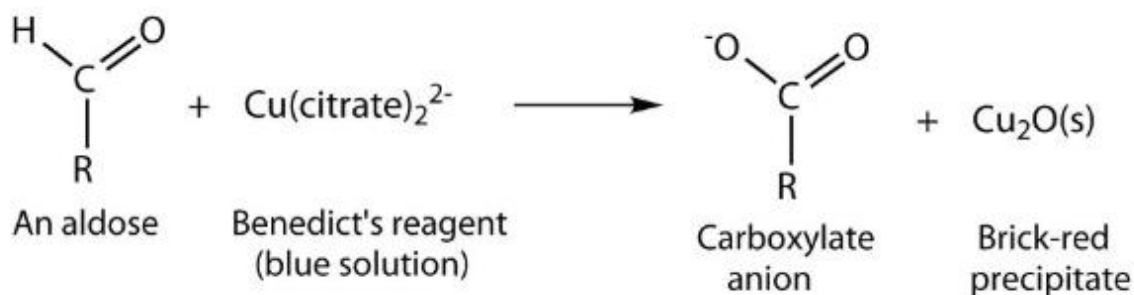
## Benedict's test

Benedict's test is a chemical test that can be used to check for the presence of reducing sugars in a given sample. Therefore, simple carbohydrates containing a free ketone or aldehyde functional group can be identified with this test. The test is based on Benedict's reagent (also known as Benedict's solution), which is a complex mixture of sodium citrate, sodium carbonate, and the pentahydrate of copper(II) sulfate.

When exposed to reducing sugars, the reactions undergone by Benedict's reagent result in the formation of a brick-red precipitate, which indicates a positive Benedict's test.

### Principle

When a reducing sugar is subjected to heat in the presence of an alkali, it gets converted into an enediol (which is a relatively powerful reducing agent). Therefore, when reducing sugars are present in the analyte, the cupric ions ( $\text{Cu}^{2+}$ ) in Benedict's reagent are reduced to cuprous ions ( $\text{Cu}^+$ ). These cuprous ions form copper(I) oxide with the reaction mixture and precipitate out as a brick-red coloured compound.



### Procedure

#### *Preparation of Benedict's Reagent*

**One litre** of Benedict's reagent can be prepared by mixing 17.3 grams of copper sulfate pentahydrate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 100 grams of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), and 173 grams of sodium citrate in distilled water (required quantity). Here, the copper(II) sulfate acts as a source of  $\text{Cu}^{2+}$  ions, the sodium carbonate provides an alkaline medium, and the sodium citrate forms complexes with the  $\text{Cu}^{2+}$  ions. Distilled water is used as a solvent.

The purity of Benedict's reagent can be checked by heating it in a test tube. No changes in the blue colour of the solution upon heating is an implication that the reagent is pure.

## Testing for Reducing Sugars

One ml of the analyte sample must be mixed with 2 ml of Benedict's reagent and heated in a bath of boiling water for 3 to 5 minutes. The development of a brick-red coloured precipitate of cuprous oxide confirms the presence of reducing sugars in the analyte.

## Interpreting the Results

Colour of the Precipitate	g% of Reducing Sugar
Green	0.5%
Yellow	1%
Orange	1.5%
Red	2%

An illustration detailing the reaction between an aldose and Benedict's reagent that results in the formation of cuprous oxide is provided. The changes in the colour of Benedict's reagent (from clear blue to brick-red) that are triggered by exposure to reducing sugars.



## Formation of a Brick-Red Precipitate is a Positive Indicator of Benedict's Test

It can be noted that Benedict's test can also be used to check for the presence of glucose in a urine sample. Since this test detects any aldehydes and  $\alpha$ -hydroxy ketones and glucose is an aldose whose open-chain forms an aldehyde group, the test yields a positive result when glucose is present in the analyte. However, a positive reaction can also be given by the presence of ascorbic acid, homogentisic acid, and other reducing substances urine. Therefore, a positive Benedict's test does not necessarily imply that the test subject is diabetic.

---

### **Factors affecting Benedict's Test**

---

**False positive** reactions may also be obtained if certain drugs are present, e.g. salicylates, penicillin, streptomycin, isoniazid, and p-aminosalicylic acid.

**Chemicals** present in a concentrated urine which may reduce Benedict's reaction include creatinine, urate, and ascorbic acid (reduction is slight).