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## **Rapid screening of plasma volume expanders using Benedict's solution**

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### **Abstract**

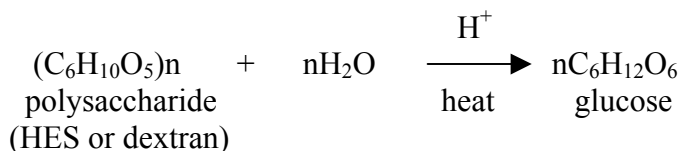
Both hydroxyethyl starch (HES) and dextran are plasma volume expanders used in the management of hypovolaemic shock. However, the life-saving properties of these substances have not been used for medicinal purposes alone but have also found their way among athletes in endurance sports. Thus, the detection of HES and dextran in human urine is now of great interest to WADA accredited laboratories; these masking agents have now been included in the list of prohibited substances, under the World Anti Doping Code.

A quick and simple qualitative method by way of acid hydrolysis to obtain the monosaccharides of glucose and hydroxyethyl glucose from the polysaccharide-based plasma volume expanders is described. Elimination urine samples obtained from Cologne, blank urine and spiked urine samples were hydrolysed with hydrochloric acid and heat. Upon cooling, the excess acid was neutralized and the samples were further adjusted to alkaline pH. Using Benedict's solution as a reagent to test for the presence of reducing sugars in urine which include glucose, suspect samples were identified. The colour of the final solution ranged from yellow to brick-red; depending on the amount of glucose formed during hydrolysis, varying colours of the precipitate were produced in the samples.

### **Introduction**

Carbohydrates are polyhydroxy aldehydes or polyhydroxy ketones or substances which, when hydrolysed, produce glucose as products<sup>1</sup>. Carbohydrates are classified as mono-, di-, and polysaccharides with the general formula of  $C_n(H_2O)_m$ . The important functional groups in carbohydrates are the hydroxyl (-OH) group and the carbonyl (-C=O) group<sup>6</sup>. Carbohydrates are also divided into 2 main classes namely, the sugars (mono- and disaccharides) and the polysaccharides.

Polysaccharides contain many monosaccharide units linked together into long chains<sup>1</sup> and therefore have very high relative molecular weight. Polysaccharides such as hydroxyethyl starch (average molecular weight of about 450 000)<sup>3</sup> and dextran 40 (average molecular weight of about 40 000)<sup>3</sup> yield high amounts of glucose subunits when hydrolysed by mineral acids.



Glucose is a monosaccharide sugar and its properties as an aldehyde are due to the fact that in addition to its normal ring form, it can exist as an open-chain structure. Aldehydes are strong reducing agents and can be oxidized easily to form carboxylic acids. A monosaccharide sugar that can donate electrons to other molecules and can therefore act as a reducing agent is called a reducing sugar.

Benedict's solution is a reagent used as a test for the presence of reducing sugars in urine samples. The reagent is an alkaline solution which is prepared by mixing solutions of copper sulphate, sodium carbonate and sodium citrate (pH 10.5). The citrate will form soluble complex ions with  $\text{Cu}^{2+}$ , preventing the precipitation of  $\text{CuCO}_3$  in alkaline solutions. When an aldehyde is heated with an alkaline solution of a copper(II) complex (Benedict's solution), a reddish-brown or brick-red precipitate of copper(I) oxide is produced and the aldehyde is oxidized to carboxylic acid. Benedict's test is considered as one of the classical tests for determining the presence of an aldehyde functional group<sup>1,2</sup>.

## Method

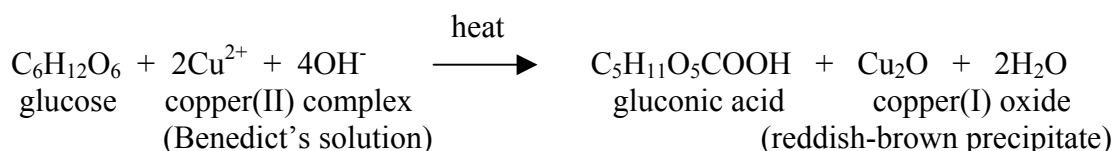
The hydrolysis of hydroxyethyl starch (HES) or dextran (6000  $\mu\text{g}/\text{mL}$ ) spiked in 250  $\mu\text{L}$  of urine samples, using 100 mL of 3M hydrochloric acid and heated at 100°C in a heating block for an hour,<sup>4</sup> yielded monosaccharides of glucose isomers and its hydroxyethyl-glucose derivatives. The analysis was carried out together with a reagent blank, urine blank and HES elimination urine. Upon cooling to room temperature, 110 mL of 3M sodium hydroxide was added to neutralise the excess hydrochloric acid and to adjust the pH of the samples to alkaline (pH 10 to 11). If the solution remains acidic when tested with pH paper, a drop or two of the sodium hydroxide was added until the solution is just basic.

100 mL of Benedict's solution was then added to the samples and heating was repeated at 100°C in a heating block. The tubes were removed from heat after 45 minutes and allowed to cool in a rack without disturbance. The different colours of the precipitate which has settled at the bottom of the tubes, if any, can be observed and were noted immediately. Evaluation of results can also be done within 3-4 hours later.

### Reaction

Alkaline solutions of copper were reduced by glucose providing a free aldehyde group, resulting in a formation of coloured copper(I) oxide (Cu<sub>2</sub>O). Glucose also known as an aldose, has 5 hydroxyl (-OH) groups and an aldehyde group at the C1 position<sup>6</sup>.

In solution, the ring forms of glucose are in equilibrium with the open-chain forms, which bear the aldehyde groups. Carbonyl groups (-C=O) in aldehydes act as reducing agents and are oxidised to carboxylic acid (-COOH)<sup>6</sup>. In this case, glucose is the strong reducing agent.



### Results

The results were recorded as follows:

<u>Vol. of spiked</u>	<u>Observation</u>	<u>Evaluation</u>	<u>Concentration</u>
			[µg/mL]
<u>HES or dextran</u>			
None	No precipitate	negative	
2.5 µL	Green precipitate	a trace	600
5.0 µL	Yellowish green precipitate	+	1,200
7.5 µL	Yellow precipitate	++	1,800
10.0 µL	Brown precipitate	+++	2,400
12.5 µL	Reddish-brown precipitate	++++	3,000
25.0 µL	Reddish-brown precipitate	+++++	6,000
50.0 µL	Reddish-brown precipitate	+++++	12,000
Elimination urine	Reddish-brown precipitate	+++++	>12,000

A reddish-brown (brick-red), brown or yellow precipitate<sup>1,2</sup> depending on the amount of copper(II) ions present, gives a positive test for a reducing sugar, indicating the presence of

glucose. A precipitate must form in order to constitute a positive test<sup>1,2,5</sup>, irrespective of the change in colour of the solution because the copper(I) oxide is insoluble in water.

The colour of the precipitate formed is stable and visible for many hours or overnight, provided that the precipitate remains undisturbed.

Samples tested positive for glucose were treated as suspect positive for HES or dextran. The identified samples were checked for the presence of glucose before screening by referring to the initial measurement data which were done by glucose tests strip. Subsequently, the samples were subjected to further analysis using GC/MS to confirm the presence of the plasma volume expander.

### **Conclusion**

Plasma volume expanders such as hydroxyethyl starch (HES) and dextran can be easily hydrolysed in acid solution to their composing parts of glucose units, which can then be tested with Benedict's solution. This method is simple, quick and economical and Benedict's solution is easily available.

### **References**

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