Analysis of carbohydrates is a significant part of qualitative organic analysis at the undergraduate level. A qualitative test that distinguishes them from other classes of organic compounds is the Molisch test. Depending on their structure, sugars are classified as reducing or non-reducing. Reducing carbohydrates give positive Tollens’, Fehling’s and Benedict’s tests [1,2].

D-glucose and D-fructose are two commonly used carbohydrates in teaching labs. While both compounds respond easily to all the above tests, students frequently find it difficult to establish their identity when given as unknowns, because:

- Both D-glucose and D-fructose form the same osazone derivative (3). Other derivatives prepared for characterization (e.g., acetates) require knowledge of advanced experimental methods and are usually not attempted by undergraduates.

- Melting points tend to be of little help in identification as carbohydrates generally show wide melting ranges [2].

We present here a simple test that makes a clear distinction between D-glucose and D-fructose. It is the easiest to perform among all the distinguishing tests available in the literature [2,3] for these two sugars, gives unambiguous results and uses ordinary chemicals such as NaOH and potassium ferricyanide.

**Preparation of the Reagent**

The alkaline potassium ferricyanide reagent is prepared by dissolving potassium ferricyanide (1g) in 100 mL of 20% aqueous NaOH solution in distilled water, and cooled to ambient temperature. The reagent has a greenish-yellow colour and a shelf life of two weeks.
Procedure for Test

A typical test is performed by adding 1mL of the reagent to 20 mg of the solid compound in a test tube at room temperature. The contents are gently shaken and colour changes are observed. The greenish-yellow colour of the solution begins to fade and eventually disappears completely. The disappearance of colour looks spectacular when viewed against a white background (Figure 1).

Discussion

The results presented in Table 1 show that D-fructose reacts faster than D-glucose, making distinction between the two amply clear. Since the alkaline ferricyanide oxidation of reducing sugars is known to be zero order with respect to ferricyanide and first order with respect to the hydroxyl ion and the reducing sugar [4], concentration of the reagent and sugar used in the test are important. We found that 20% NaOH and 20 mg of the sugar gave optimum results in terms of time taken for decolourization.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Amount (mg)</th>
<th>Time (min:sec) taken for colour to:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Commence fading</td>
</tr>
<tr>
<td>D-Fructose</td>
<td>20</td>
<td>0:54</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>20</td>
<td>1:40</td>
</tr>
</tbody>
</table>
Fructose is easily identified by its following characteristic behaviour under test conditions:

- Greenish-yellow colour of reagent disappears in about a minute at ambient temperature.

- The time taken for complete disappearance of the colour after its fading commences is very short.

- Soon after the colour disappears, a faint reddish-yellow colour slowly develops, deepens and remains so for about an hour.

In the case of D-glucose, the fading of colour is gradual, taking about 5 minutes to disappear completely and the solution remains colourless (1 hour). Galactose, lactose and sucrose behave like glucose but take 15, 13 and 35 minutes respectively for complete disappearance of colour. If the colour does not disappear within 5 minutes under test conditions, the presence of glucose or fructose is ruled out.

We observed that only carbohydrates cause complete disappearance of colour. Phenols and amines give dark red colour, while carboxylic acids, ketones, esters, etc., show no observable change.

**Suggested Reading**


