Qualitative Carbohydrate Analysis using Alkaline Potassium Ferricyanide

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We present here a qualitative test that distinguishes between monosaccharides, reducing disaccharides, non-reducing disaccharides, aldoses and ketoses using alkaline potassium ferricyanide. This is a simple, quick and unambiguous test to distinguish various carbohydrates from one another and is reliable enough to replace other tests reported in standard laboratory textbooks.

Carbohydrates form a distinct class of organic compounds often identified by their characteristic behaviour towards a host of reagents [1–4]. Based on a kinetic study on the oxidation of carbohydrates with alkaline potassium ferricyanide [5], we had reported, in the April 2007 issue of *Resonance,* an unambiguous test to distinguish between two common carbohydrates, namely, D-glucose and D-fructose. We now report an extension of that test to several more carbohydrates, which include mono- as well as di-saccharides.

The test procedure is as follows. After performing Molisch test initially, only one more test using alkaline potassium ferricyanide reagent can help us to identify whether the given carbohydrate is an aldose, a ketose, a reducing disaccharide or a non-reducing disaccharide. The graphical protocol is presented in *Figure* 1.

Experimental Details

The reagent used for the test is 1% potassium ferricyanide in 20% aqueous NaOH solution and is prepared as described in [6].

A sample of 20 mg of the carbohydrate to be identified is mixed with 1.0 mL of the reagent. The mixture is shaken for 3–5 minutes at ambient temperature, and the colour changes as shown in *Figure* 1 are observed. The sugars used are listed in *Table* 1.

Keywords
Alkaline potassium ferricyanide, qualitative carbohydrate analysis, aldoses, ketoses, disaccharides, monosaccharides.

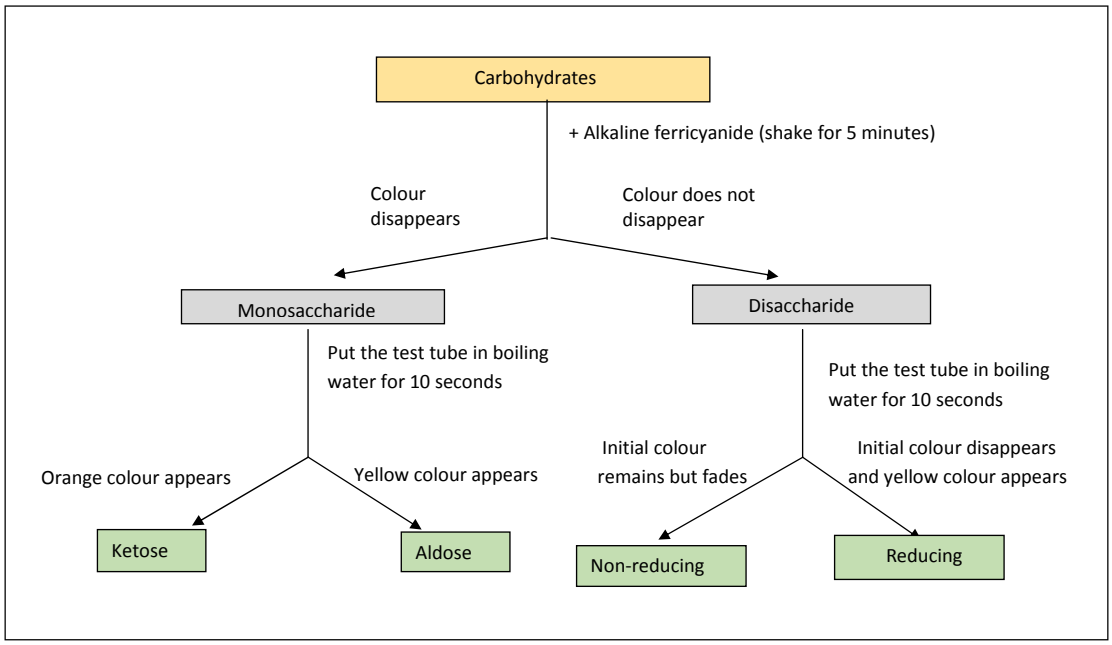

Figure 1. Alkaline ferricyanide test for classification of carbohydrates

The carbohydrates chosen here are those that are usually analyzed by the students who study chemistry as a major subject. Fructose and sorbose are common ketoses; however, tagatose, though expensive, was also included to broaden the validity of the test among ketoses.

Safety Instructions

Sodium hydroxide solution is corrosive. Potassium ferricyanide in alkaline medium has very low toxicity and is a mild irritant to eyes and skin. The experiment should be performed with usual safety precautions, i.e., wearing lab coat, gloves and goggles.

Table 1. Carbohydrates used for the test

Carbohydrate Type	*Examples Used*
Aldoses	Glucose, galactose, lyxose, mannose, arabinose, xylose
Ketoses	Fructose, sorbose, tagatose
Reducing Disaccharides	Lactose, maltose, cellobiose, melibiose
Non-reducing Disaccharides	Trehalose, sucrose

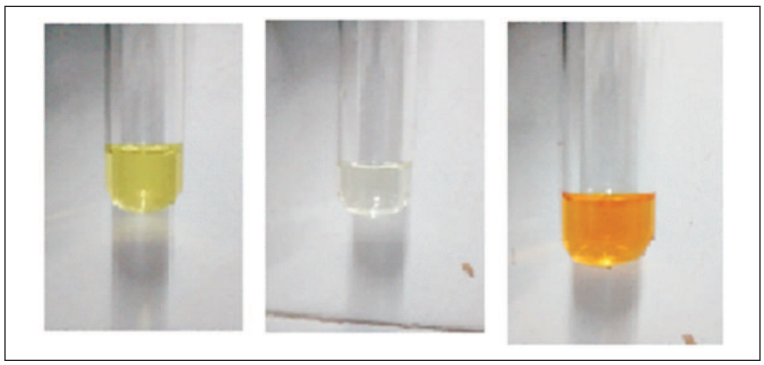

Figure 2. Left to right: Colour changes in case of ketoses.

Results and Discussion

For ketoses, the standard test performed is the Seliwanoff test. This test uses HCl and resorcinol. While it is reported [4] that ketoses are dehydrated faster to hydroxymethyl furfural which then gives a reddish coloured condensate with resorcinol; aldoses are also dehydrated similarly but somewhat slowly. Thus, there is a chance of development of a colour in the case of aldoses if the duration of the test is not properly monitored. This can lead to ambiguity, which needs to be carefully avoided.

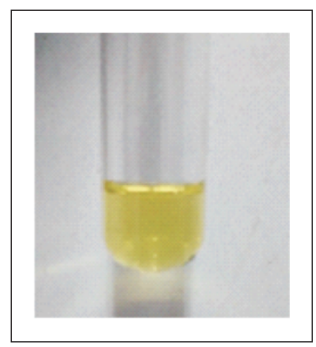

Figure 3. Final colour in case of aldoses and reducing disaccharides. The colourless stage is observed in both.

The alkaline ferricyanide test presented here is, however, free from ambiguity as the pale orange colour produced by ketoses is distinctive and is clearly distinguishable from the colour formed by aldoses and disaccharides (*Figures* 2 and 3).

Conclusion

The alkaline ferricyanide test presented in this article can classify an unknown carbohydrate as monosaccharide, reducing disaccharide or non-reducing disaccharide and make a clear distinction between aldoses and ketoses. Thus, after initial indication by the Molisch test, only one more test with alkaline potassium ferricyanide will be enough to provide the information that is obtained by performing the Tollens' test, Barfoed's test and Seliwanoff's test together. The overall protocol for carbohydrate analysis is thus simplified. The test exposes the students to an interesting aspect of carbohydrate chemistry, i.e.,

the reaction of carbohydrates with alkali which forms the basis of Lobry de Bruyn van Ekenstein rearrangement [3, 4]. They will also be introduced to the kinetics of this oxidation [5].

Acknowledgement

We express our gratitude to our college authorities for the necessary laboratory facilities.

Suggested Reading

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