Classroom

In this section of *Resonance*, we invite readers to pose questions likely to be raised in a classroom situation. We may suggest strategies for dealing with them, or invite responses, or both. "Classroom" is equally a forum for raising broader issues and sharing personal experiences and viewpoints on matters related to teaching and learning science.

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Distinguishing between Monosaccharides and Disaccharides

Identification of carbohydrates is a part of qualitative analysis in undergraduate chemistry laboratory. As a class of organic compounds, carbohydrates are detected by the Molisch test. Tollens', Fehling's and Benedict's tests [1] help to further classify them as reducing and non-reducing carbohydrates. In order to identify an unknown carbohydrate as a monosaccharide or a disaccharide, the Barfoed's test is commonly performed [1,2]. However, students seldom report satisfactory results from this test. We have observed that a test using ceric ammonium nitrate reagent gives more satisfactory results. The performance and results of these reactions are reported here.

Ceric Ammonium Nitrate Reaction with Carbohydrates

Ceric ammonium nitrate (CAN) is a versatile single electron oxidant used for oxidation of a variety of organic substrates [3]. The commonly known CAN reagent is a yellow solution of diammonium hexanitratocerate(IV) in dilute nitric acid. It forms distinct red complexes with compounds that contain alcoholic hydroxyl groups (-OH), e.g., alcohols, diols, carbohydrates, etc. The red complex has been shown to be an intermediate in the

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oxidation of alcohols by Ce(IV). In the second phase of the test the red colour disappears due to the reduction of coloured Ce(IV) to the colourless Ce(III). The overall sequence of reactions may be formulated as given below.

$$\underset{\text{yellow}}{(NH_4)_2Ce(NO_3)_6} + RCH_2OH \longrightarrow \text{[red complex]} \qquad (1)$$

$$\text{[red complex]} \longrightarrow RCH_2O^{\cdot} + \underset{\text{colourless}}{(NH_4)_2Ce(NO_3)_5} + HNO_3 \qquad (2)$$

$$RCH_2O^{\cdot} + \underset{\text{yellow}}{(NH_4)_2Ce(NO_3)_6} \longrightarrow RCHO + \underset{\text{colourless}}{(NH_4)_2Ce(NO_3)_5} + HNO_3 \qquad (3)$$

The rates of the oxidation steps shown in (2) and (3) depend on the structure of the hydroxyl compound. Primary, secondary and tertiary aliphatic alcohols give red colour [2] which is stable for a long time (6 minutes to several hours) and is easy to observe. Carbohydrates, however, give an intense red colour that is discharged quickly. This makes it difficult to observe and is often missed by students. Since there is an inherent difference in the kinetics of CAN oxidation of carbohydrates [4] and simple alcohols, we studied this reaction with several carbohydrates at different temperatures and varying masses of sugars with a view to distinguish between mono- and di-saccharides. A test conducted at 25 ^{o}C using 20 mg mass of carbohydrate works the best during a typical laboratory session.

Preparation of the Reagent

Dilute nitric acid is prepared by carefully adding 13 mL of concentrated HNO_3 to 400 mL of distilled water. To this are added 109.6 g of ceric ammonium nitrate with stirring to dissolve the solid completely. The solution is made upto 500 mL. The resulting reagent solution is yellow in colour and stays good for about a month. This method of preparation is based on the procedure described in Shriner's *The Systematic Identification of Organic Compounds* [2].

The Typical Test Procedure

The carbohydrate (20 mg) is taken in a test tube, 1 mL of the reagent is added and the stopwatch is started immediately. A deep red colour forms instantly and begins to fade soon thereafter. The time taken for complete disappearance of red colour is recorded.

Results

The results are presented in *Table* 1.

The monosaccharides presented in *Table* 1 (entries 1–10), are reducing in nature and cause the red colour to form and disappear in seconds**.** The disaccharides studied fall into two groups, namely,

Table 1. Duration of colour reactions of common carbohydrates (20 mg) with ceric ammonium nitrate reagent (1 mL) at 25 °C and 29 °C.

Entry	Carbohydrate	Time taken for disappearance of red colour (min:sec) at	
		25 °C	29 °C
1	D-(+)-Xylose	00:27	00:17
2	D-(-)-Lyxose	00:16	00:12
3	D-Ribose	00:12	00:11
4	L-(+)-Arabinose	00:12	00:12
5	D-(-)-Fructose	00:32	00:17
6	D-(+)-Glucose	01:01	01:00
7	L-(-)- Sorbose	00:51	00:23
8	D-Mannitol	00:52	00:32
9	D-(+)-Galactose	00:38	00:22
10	D-(+)-Mannose	01:05	00:51
11	D-(+)-Maltose	03:38	03:05
12	D-(+)-Mellibiose	03:47	03:01
13	D-(+)-Cellobiose	05:56	03:30
14	D-(+)-Lactose	06:30	03:40
15	Sucrose	19:29	11:55
16	D-(+)-Trehalose	Red colour does not disappear till 2 hours	

- Reducing: maltose, cellobiose, mellibiose and lactose (entries 11–14) take 3–7 minutes to cause complete disappearance of red colour.
- Non-reducing: sucrose and trehalose (entries 15–16) take about 20 minutes and longer.

The data in *Table* 1 show that increase in the temperature increases the rate of disappearance of the red colour. Although the test results at higher temperature are accomplished in lesser time for all the carbohydrates studied, yet a clear distinction is maintained between monosaccharides and disaccharides.

It should also be noted that since the reaction is first order in carbohydrate [4], the time taken for the disappearance of red color directly depends on the mass of the compound.

The flow chart given in *Figure* 1 gives the essential outlines of the test.

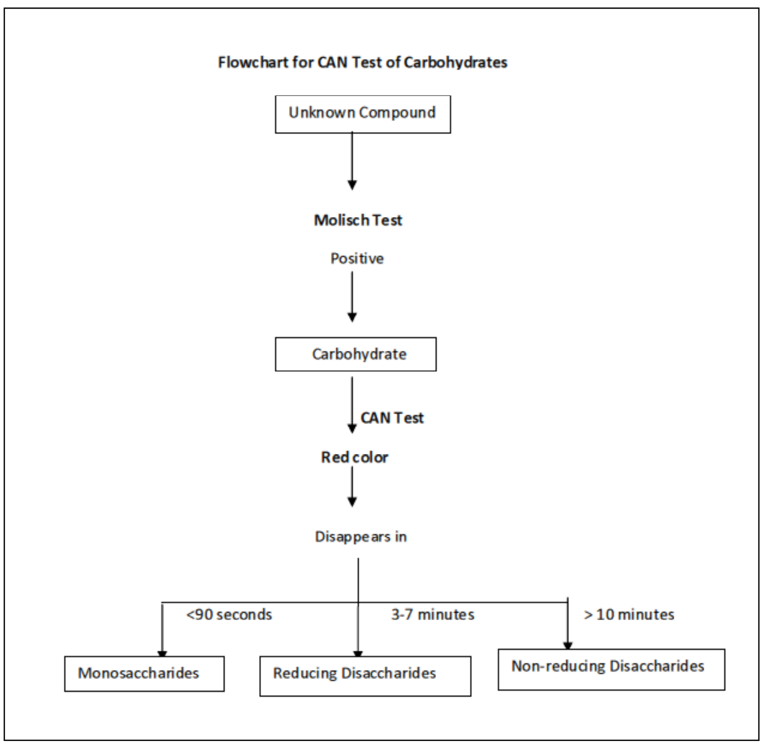

Figure 1. Flow chart for CAN test of carbohydrates.

Conclusion

Ceric ammonium nitrate reagent is easy to prepare and use. Based on its ability to oxidize different carbohydrates at different rates, it can be used to distinguish

a) monosaccharides from disaccharides, and

b) reducing disaccharides from non-reducing disaccharides.

The CAN test carried out with 20 mg of a carbohydrate at 25 ^{o}C can eliminate the need for performing Tollens' test, which uses expensive silver nitrate and requires heating, for knowing the reducing or non-reducing nature of the carbohydrate.

Suggested Reading

[1] A I Vogel, *A Text Book of Practical Organic Chemistry*, Addison-Wesley Pub Co., 1989.

[2] R L Shriner, R C Fuson, D Y Curtin and T C Morrill, *The Systematic Identification of Organic Compounds*, 6th ed. John Wiley & Sons, p.146, 1980.

[3] J R Hwu and K Y King, *Current Science*, Vol.81, No.8, pp.1043–1053, 2001.

[4] P O I Virtanen, R Lindaroos and E Oikarinen, *Carbohydrate Research*, Vol. 167, pp.29–37, 1987.