

Simple Potentiometric Determination of Reducing Sugars

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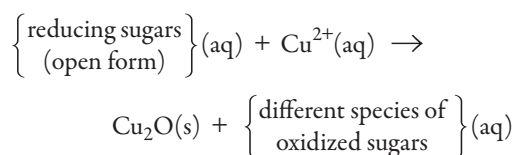
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The determination of reducing sugars is a widespread analytical tool with applications in different fields, ranging from food analysis to biology. Various methods available for such determination have been discussed in Browne and Zerban (1), who pointed out that the Bertrand and Lane–Eynon methods are remarkable because of their wide use and accuracy. Usually, the Lane–Eynon method is employed in both academic and industrial settings (2), even though it presents several disadvantages, namely, working with boiling solutions and long experimental times. On the other hand, in the Bertrand method a gravimetric assay is required. As a consequence, both methods have tedious and time-consuming protocols, yet they have been used for more than eighty years (3).

For the analysis of reducing sugars in aqueous solutions, the most commonly used methods are chromatography (both GC and LC) and Lane–Eynon. The latter, and all its variants, is based on the reaction between copper(II) ion and reducing sugar:



This interaction is particularly complex. It is a quantitative but non-stoichiometric reaction, where the sugar is oxidized to a number of different species, and copper(II) ion is reduced to copper(I) ion, which forms a red-colored precipitate. The sugar's reducing power depends mainly on four factors—nature of the sugar, reactant concentration, pH of the medium, and temperature—that also regulate the equilibrium between the open and closed forms of the sugar (Figure 1). In these methods, an alkaline tartrate solution is used to provide the basic medium that ensures that the sugar is no longer in the hemiketal form but in the open form and keeps the copper(II) ion in solution by forming a copper(II)–tartrate complex.

Also, in these methods the reaction is performed at the boiling point of the solution in order to achieve the sugar's highest reducing power. Such conditions can be hazardous for students, resulting in occasional burnings. Over the years, some modifications have been introduced to improve either the safety or the experimental procedure of the original Lane–Eynon protocol. One of the most successful variants consists of a potentiometric titration of the remaining copper(II) ion. To this end, several electrodes have been used, such as platinum (4), trinitrobenzenesulfonate ion-selective (5), sulfide-selective (6), and copper-selective electrodes (7–9). However, except for the expensive platinum electrode, all of these electrodes are difficult to fabricate and not commercially available.

In this article a potentiometric determination of copper(II) ion using copper wire as the copper-selective electrode is described. This simple and inexpensive method was applied to the determination of reducing sugars in aqueous solution, under Lane–Eynon conditions. This constitutes a novel procedure, since the potentiometric determination is done at room temperature, favorably affecting the safety and ease of the manipulation.

Procedure

The quantification of reducing sugars is performed in three, four-hour laboratory periods by students in the second-year analytical chemistry course. In the proposed method, standard Lane–Eynon conditions are maintained throughout, but the excess of copper(II) ion is determined by potentiometry. It is a simple method that involves routine laboratory operations and an easy copper(II) ion determination at room temperature using a simple system: a copper wire and a voltmeter (details available in the online supplement). Since the sugar's reducing power depends on the reaction conditions, a calibration curve using standard glucose solutions should be prepared and the sample handled exactly in the same way.

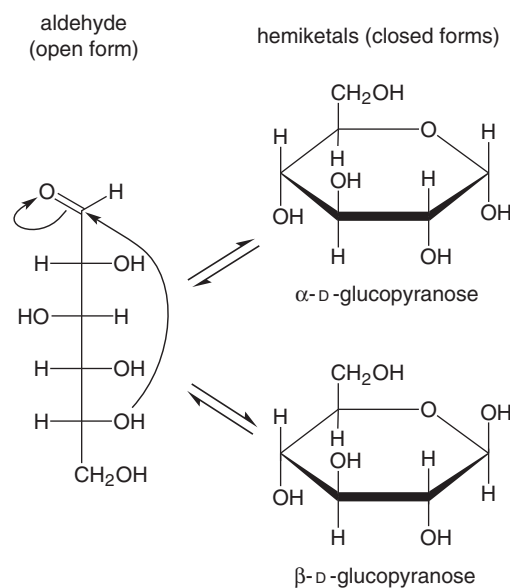


Figure 1. Open and closed forms of glucose.

Table 1. Data and Results for a Student Calibration Curve

| Glucose Solution Volume/mL | Glucose Mass/mg | Voltmeter Response/mV |
|----------------------------|-----------------|-----------------------|
| 0.5 | 7 | 223 |
| 1.0 | 14 | 206 |
| 2.0 | 28 | 172 |
| 4.0 | 56 | 102 |

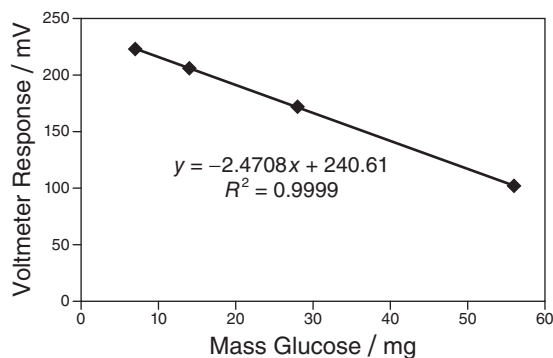


Figure 2. Calibration curve from data on Table 1.

Before performing the experiment, students are required to have knowledge of the fundamentals of electrochemical reactions (10, 11), chemical properties of carbohydrates (12), and calibration curves (13).

From a stock glucose standard solution, each student prepares his or her own solutions to make an individual calibration curve and measures the unknown aqueous sample. Since the sugar's reducing power depends on the reaction conditions, each calibration curve may be different from the others and must be used only for the measurement of the corresponding sample that was subjected to the same conditions.

Typical student results for a calibration curve are shown in Table 1 and Figure 2. The standard deviation was below 0.75% for the series of four readings used to make the calibration curve. The linearity between glucose mass and instrument's response was excellent over the range studied ($R^2 = 0.9999$).

If time is available, students can be assigned to test the reducing values of different hexoses (fructose, galactose), and even to determine the reducing sugar content of a natural product such as honey.

Hazards

Copper(II) sulfate pentahydrate is a strong irritant. Potassium sodium tartrate may cause irritation to skin and eyes. Sodium hydroxide must be handled with precaution. It is corrosive to all tissues and swallowing may cause severe burns of mouth, throat, and stomach (may be fatal if swallowed). Inhalation of the dust may cause damage to respiratory tract.

Discussion

After the students have completed their work, all the results are compiled and presented for discussion to the class. It is interesting to note that the dispersion of the class readings of the unknown sample (expressed as mV) is greater than the final concentration value obtained (expressed as mg of glucose). This allows for further discussion on the advantages and drawbacks of using a calibration curve, such as less dependence on the analytical performance of the instrument (voltmeter), better fit to the actual analytical conditions, dynamic range, and time required to produce the calibration graph.

The reaction between glucose and copper(II) ion is quantitative although non stoichiometric, and hence, the excess of copper(II) ion and the glucose mass do not necessarily have a linear relationship. It has been our experience that the proportionality between the potentiometric reading and glucose mass is linear over the range 3.5–56 mg of glucose, losing linearity above and below this interval. Therefore, the reducing sugar concentration of the sample should be brought into this range. The quantity of both copper(II) and tartaric solutions has also been optimized to 1.0 and 0.5 mL, respectively.

Another topic for discussion is related to the nature of the reducing sugar. In our experience, there are not statistically valid differences between the reduction values of glucose and fructose during the reaction. This can be easily extrapolated to other aldohexoses and ketohexoses. Students are encouraged to propose hypotheses and develop experiments to test them, which can be effectively carried out if enough time is available. The determination of the reducing values of different hexoses (fructose, galactose), and even of a natural product such as honey, constitutes a useful extension of this experiment.

Summary

Some advantages of the proposed method are its simplicity, low cost, and the fact that it can be carried out even in colored and turbid samples. The pedagogical goals of this experiment include the possibility of using a copper wire as a Cu-selective electrode during potentiometric determinations, the use of calibration curves to solve otherwise difficult analytical situations (in this example the reaction studied is quantitative but non-stoichiometric), and the study of the dependence of the reducing power to the nature of the sugar involved. Also, this experiment introduces mathematical, physical, analytical, and organic chemistry concepts to the students.

To our knowledge, the use of a simple copper wire as indicator electrode has not been reported previously for this type of determination, leading to a big simplification of the experimental equipment.

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