



UV/Visible Spectroscopy

Determination of Sugar as Glucose in a Soft Drink Using the LAMBDA PDA UV/Vis Spectrophotometer

Introduction

In this application note, the amount of sugar or carbohydrate in a soft drink was determined using a colorimetric method. The

rapid measurement of the PDA (Photodiode Array) UV/Vis Spectrophotometer allows for the collection of accurate data from the time-dependent reaction. The calibration curve was automatically calculated using the Quantification mode of the UV Lab™ software.

Principle

This method is based on the color which forms when sugar is reduced from 3,5-dinitrosalicylic acid (DNSA) to 3-amino-5-nitrosalicylic acid as shown in Figure 1. The sucrose does not react with DNSA, therefore it must be broken down into simple sugars like glucose first by boiling the sample with hydrochloric acid. In addition, the sugar in soft drinks is too highly concentrated to be used for this method unless it is diluted. The red-brown color of the product is detected at 580 nm.

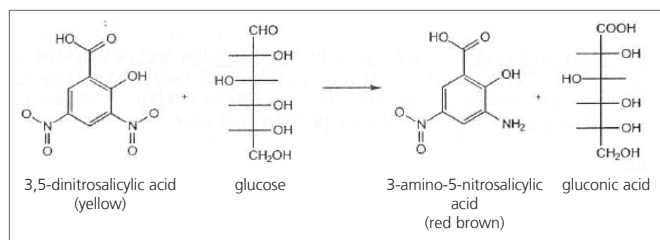


Figure 1. The reaction of 3,5-dinitrosalicylic acid and glucose.

Reagents and Apparatus

1. Sucrose stock solution (1000 mg/dL)
2. Soft drink (non-diet, not dark-colored)
3. 6 M HCl solution
4. 2.5 M NaOH solution
5. 0.05 M 3,5-dinitrosalicylic acid (DNSA) solution
6. D.I water
7. LAMBDA™ 465 UV-Vis Spectrophotometer
8. UV Lab software
9. Cuvettes (10 mm pathlength)

Procedure

1. Prepare five diluted sucrose stock solution samples as standards.
2. Prepare diluted soft drinks as samples
3. Pipet 2 ml of each sucrose standard and samples into a test tube. Also pipet 2 ml of D.I water into a separate test tube for the blank solution.
4. Add 2 ml of 6 M HCl solution.
5. Place in boiling water for 10 minutes.
6. Add 8 ml of 2.5 M NaOH solution.
7. Add 2 ml of 0.05 M DNSA solution then cover the test tube with parafilm and shake well to mix.
8. Place in boiling water for 5 minutes followed by ice water for 10 minutes. (The time between DNSA addition and measurements should be same for all test solutions.)
9. Measure the absorbance of the five standards and references at 580 nm using the Quantification Standard mode
10. Measure the samples using the Quantification Sample mode and calculate its concentrations.

Instrument Parameters

The instrument parameters of the LAMBDA 465 are as follows. Figure 2 shows the experimental setup.

Experiment Setup

Data type: Absorbance
 Sampling: Single cell
 Mode: Spectra no.: 1; Scan no.: 30;
 Integration no.: 1

Experiment method

Use wavelength: 580 nm
 Curve dimension: 1

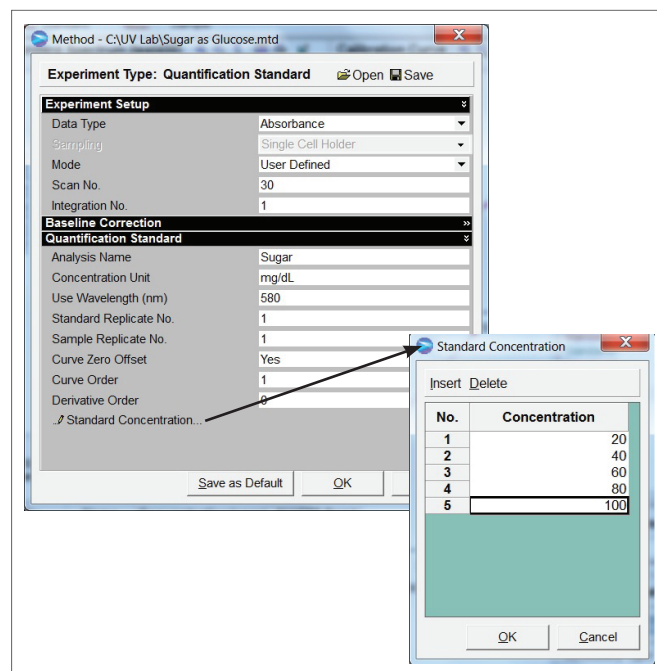


Figure 2. Experiment method.

Results

1. Calibration Curve

Figure 3 shows the spectra and Table 1 shows the calibration data of the sucrose standards. The calibration curve of five standards is shown in Figure 4. The correlation coefficient R^2 is 0.99962

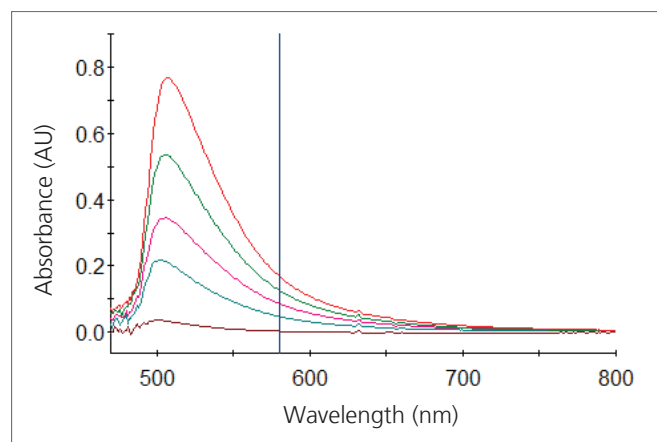


Figure 3. The spectra of Sucrose standards.

Table 1. Calibration data of Sucrose standards.

$R^2 = 0.99962$
 Function : $Y = 0.0002X + -0.0365$

Name	Concentration(mg/dL)	AU(580nm)
Standard1	20	0.0034
Standard2	40	0.0474
Standard3	60	0.086
Standard4	80	0.1264
Standard5	100	0.1687

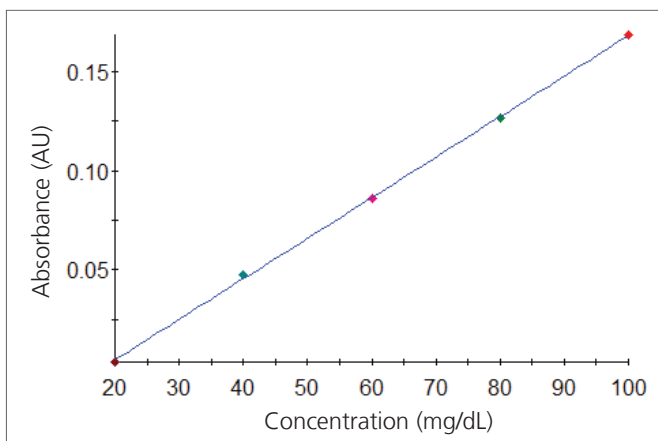


Figure 4. The Calibration curve of Sucrose standards.

2. Concentration of Sugar in a Soft Drink

The concentrations of sugar in the unknown samples was acquired using the calibration curve shown in Figure 4. The original concentrations of sugar in the soft drinks is calculated by the following equation:

$$\text{Original concentration} = \text{Diluted sample concentration} * \text{Dilution factor}$$

The concentrations of sugar in the unknown samples of soft drinks are shown in Table 2.

Table 2. Concentrations of sugar in soft drink samples.

Name	Conc. (mg/dL)	Dilution Factor	AU (580 nm)	Original Conc. (mg/dL)
Beverage1	59.911	200	0.0862	11982.2
Beverage2	70.36	200	0.1076	14072
Beverage3	71.239	200	0.1094	14247.8

Conclusion

The determination of sugar in soft drinks was performed using the LAMBDA 465 UV/Vis spectrophotometer and the UV Lab software. Using this method the amount of color is highly time-dependent, yet a good calibration curve with an R^2 value of 0.99962 was obtained due to the fast data acquisition and high sensitivity of the LAMBDA 465. Quantification mode in the UV Lab software enables the operator to efficiently and conveniently collect data for the calibration standards and to perform quantitative analysis of unknown samples.