SPECTROPHOTOMETERS Application note: A09-008A

Quantitative determination of glucose in an energy drink, apple juice and wine

Introduction

The rate of an enzyme reaction varies with substrate concentration; therefore this can be used to determine the amount of substrate present. Measurement of such a reaction requires the determination of the amount of product produced or the disappearance of substrate consumed. With many assays this cannot always be done directly, therefore the reaction may be coupled to a second enzyme that can convert one of the products into a measurable substance. One such example is the coupling of reactions to dehydrogenases that use nicotinamide adenine dinucleotide (NAD^+) or the reduced form, NADH, as coenzymes. NADH can be readily measured in a spectrophotometer at 340nm or by fluorimetry with excitation at 340nm and emission at 460nm.

Many enzyme reactions are used as analytical tools. In this application note we demonstrate the use of the Glucose (HK) Assay Kit from Sigma (product code GAHK-20) to measure the amount of glucose in three different types of drink. The principle of the kit is shown below:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{Hexokinase}} \text{Glucose-6-phosphate} + \text{ADP}$$

$$\text{G6P} + \text{NAD}^+ \xrightarrow{\text{G6PDH}} \text{6-Phosphogluconate} + \text{NADH}$$

Glucose is first phosphorylated by hexokinase in a reaction with ATP. The product, glucose-6-phosphate (G6P), is then oxidised to 6-phosphogluconate in the presence of NAD^+ in a reaction catalysed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD^+ is reduced to NADH. Therefore the reaction can be monitored by measuring the increase in absorbance at 340nm and this increase is directly proportional to the original glucose concentration.

Methods

The Glucose (HK) Assay Reagent was reconstituted according to the manufacturers' instructions[1] in 20ml deionised water. The three drinks tested were Lucozade Energy Original (GlaxoSmithKline plc, UK), Pressed Apple Juice (Wm Morrison Supermarkets plc, UK) and a sweet white dessert wine.

Lucozade Energy was diluted 1 in 100 and the apple juice and wine were diluted 1 in 50 with deionised water to bring them into the range of approximately 0.05 to 5mg glucose/ml. 100µl of these solutions were added to 1.0ml of the Glucose Assay Reagent in a cuvette and incubated at room temperature for 15 minutes. A sample blank consisting of 100µl of sample and 1.0ml of water and a reagent blank consisting of 1.0ml of Glucose Assay Reagent and 100µl of water were also prepared. After 15 minutes the absorbance at 340nm was measured against a deionised water blank. The cuvettes were measured in four different spectrophotometers for comparison: models 6300, 6315, Genova and 6505.

Results

The concentration of glucose in the samples can be calculated using the millimolar extinction coefficient for NADH at 340nm using the equation shown below as given in the manufacturers' instructions.

$$A_{\text{Total Blank}} = A_{\text{Sample Blank}} + A_{\text{Reagent Blank}}$$

$$\text{mg glucose/ml} = \frac{(\Delta A)(TV)(\text{Glucose MW})(F)}{(\varepsilon)(d)(SV)(\text{Conversion Factor µg to mg})}$$

Where:

ΔA	=	$A_{\text{Test}} - A_{\text{Total Blank}}$
TV	=	Total Assay Volume (ml)
SV	=	Sample Volume (ml)
Glucose MW	=	180.2g/mole
F	=	Dilution Factor from sample preparation
ε	=	Millimolar extinction coefficient for NADH at 340nm (6.22)
d	=	Light path (cm)
1000	=	Conversion Factor for µg to mg

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Using this calculation, the glucose concentration measured in each of the drinks is shown in Table 1. The results obtained from each model of spectrophotometer are in good agreement.

	Glucose (mg/ml)		
	Lucozade Energy	Apple Juice	Wine
6300	41.84	15.92	22.00
6315	41.94	16.05	21.94
Genova	42.07	16.00	22.08
6505	42.89	16.01	22.56
Average	**42.19**	**15.99**	**22.15**
SD	**0.48**	**0.05**	**0.28**

Table 1: Measured glucose content of three different drinks.

Lucozade Energy is marketed as a high energy drink to rapidly provide an energy boost after intense physical activity. This is reflected in the high level of glucose which provides a ready source of carbohydrate that can be easily metabolised. The bottle label states that the drink contains 21.8g sugars per 250ml which equates to 87.2mg/ml. The measured amount of glucose obtained by the assay was found to be only about half of this, therefore sugars other than glucose would also appear to be present in the drink.

The sugar content of apple juice consists mainly of glucose, fructose and sucrose and the profile of each of these depends strongly on the cultivar and region in which the fruit is grown. The glucose content of apples can range from less than 10mg/ml to greater than 30mg/ml[(2)]. The glucose content of the juice measured in this experiment (16mg/ml) falls well within this range.

Glucose and fructose are the main fermentable sugars in grape juice. At the end of fermentation any sugars remaining in the wine are known as residual sugars and the amount of these defines the level of sweetness of the wine. The residual sugar concentration is expressed in g/l and includes all reducing sugars including glucose, fructose and other unfermentable sugars such as pentoses. The assay used in this experiment will measure only the residual glucose present in the wine and this was found to be around 22g/l. Since glucose is fermented at a faster rate than fructose, this may represent only a fraction of the total residual sugar in the wine sample. Wines from around 18g/l up to 45g/l are classified as medium sweet[(3)] and those with greater than 45g/l are classified as sweet. The wine used in this experiment was a sweet white dessert wine so the results are in agreement of this classification.

Conclusions

Glucose phosphorylation by hexokinase is one of many assays that can be coupled to dehydrogenases that use NAD^+ or NADH as coenzymes. Dehydrogenases that use these coenzymes or the phosphorylated forms $NADP^+$ and NADPH can also be assayed directly by measuring the changes in absorbance at 340nm. Since NADH has a well define extinction coefficient the use of standards is not required thus avoiding the necessity of constructing a calibration curve, as with other types of colorimetric assay. All of the Jenway spectrophotometers used gave comparable results and are therefore suitable for these types of assay.

References

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