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A rapid and sensitive method for determination of trace amounts of glucose by anthrone-sulfuric acid method

F. Leng[1*], S. Sun[1], Y. Jing[1], F. Wang[1], Q. Wei[1], X. Wang[2*], X. Zhu[2]

[1] *School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou 730050, China*
[2] *Lanzhou Institute of Husbandry and Pharmaceutical Sciences of CAAS, P. R. China*

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Anthrone-sulfuric acid assay, a rapid, sensitive microanalytic method, was modified to quantify carbohydrate in a colorimetric tube. A single test methodology was applied to design and optimize the factors including solvents, heating, anthrone concentration, sulfuric acid concentration, amount of anthrone reagent and reaction duration. The tube with the reaction system of total volume 5.0 mL, which contained 3.0 mL of anthrone-sulfuric acid solution, 1.0 mL of distilled water and 1.0 mL of glucose solution, was kept in a water bath at 93°C for 15 min. Under these conditions, the absorption values at 520 nm for different glucose concentrations were in linear relationship with the fitting equation of linear regression with a correlation coefficient R^2 of 0.997. The linear range was 0 ~ 0.01 mg/mL of glucose. Standard addition test results revealed that the method has a relative deviation of 2.42%, the tagged recoveries were 101.4%. The method developed in this work proved to be simple, valid, stable, and repeatable with little interference based on the precision test and the interference test, and could be applied for total sugar content determination.

Keywords: Anthrone, Sulfuric acid, Glucose, Content determination, Spectrum.

INTRODUCTION

Glucose is a kind of polyhydroxy aldehyde which can react with a variety of compounds. Determination of the carbohydrate content in a variety of samples is a basic and common analytical operation in many biotechnologic processes [1]. It has been reported that many methods can be applied to measure the polysaccharide content [2, 3], while these methods mainly used visible absorption spectra based on the reducibility of polysaccharides and the reaction of furfural condensation, including 3,5-dinitrosalicylic acid colorimetric method (DNS method), Nelson-Somogyi method [2, 3], orcinol -hydrochloric acid (sulfuric acid) method, phenol-sulfuric acid method [3] and anthrone-sulfuric acid method [4-10]. Among the above mentioned colorimetric methods for carbohydrate determination, anthrone-sulfuric acid method is one of the most commonly used techniques. This method has been employed to measure the soluble sugars in different samples [2, 5, 6, 11]. Other methods used to quantify carbohydrate include the phenol-sulfuric acid method, the orcinol method and the resorcinol method. These assays must be first validated to demonstrate that they are useful for their intended purpose. In a previous report [12], the results of a preliminary investigation of the adaptation of anthrone-sulfuric acid method for a 96-well microplate assay were given. However, the detectable range of this method is 50-400 mg/mL and it needs a long reaction period. Here, we described a simple and more sensitive microplate assay to quantify carbohydrate using the same reaction for colorimetric determination of total carbohydrate.

The aim of this study was to establish a rapid and sensitive approach to determine trace amounts of glucose. In this paper we succeeded in standardizing and validating an anthrone-sulfuric acid method adapted to colorimetry. This assay was able to efficiently quantify glucose with the detectable range of 0~0.01 mg/mL. The procedure was confirmed to be the most sensitive and the simplest among the anthrone-sulfuric acid assays reported so far.

EXPERIMENTAL

Apparatus and chemical reagents

An ultraviolet spectrophotometer (Cary50, Varian, USA) equipped with 1.0-cm quartz cells was used to collect all spectral data at room temperature. All reagents were weighed by an analytical balance (0.0001g, Mettler-Toledo Instruments, USA). Digital electronic constant temperature water-bath (HY-4, Guohua Electrical

* To whom all correspondence should be sent:
E-mail: lff0928@sina.com, wangxiaoli6578@sina.com

Instruments, China) was used to control the temperature.

All reagents used were of chemical purity or analytical purity grade. The solutions were prepared with distilled water or deionized water, and working solutions were obtained by appropriate dilution.

Anthrone-sulfuric acid (0.01 mg·mL^{-1}) solution was prepared by dissolving 1.000 mg of anthrone (Damao Chemicals, Tianjin) in 100 mL 80% sulfuric acid (Liangyou Chemicals, Baiyin) (freshly prepared solution should be used). Glucose solution was prepared by dissolving 10.000 g glucose (Beichen Founder chemicals, China) in distilled water, and then diluted to 100 mL in a volumetric flask with distilled water.

Experimental Methods

Spectral characteristics: According to the method [10], the absorption spectra of the reaction between anthrone-sulfuric acid and glucose were recorded by the spectrometer, and the maximum absorption wavelength was determined.

Effect of different factors on the absorption at 625 nm: the main factors studied were type of solvent, heating temperature, anthrone concentration, sulfuric acid concentration, amount of anthrone reagent and reaction duration. The interference study without glucose, precision experiments and recovery test were performed to evaluate the method developed in this paper.

RESULTS AND DISCUSSION

Spectral characteristics of the reaction between anthrone-sulfuric acid and glucose

Full wavelength scan curve is shown in Figure 1. The maximum absorption was determined at 625 nm.

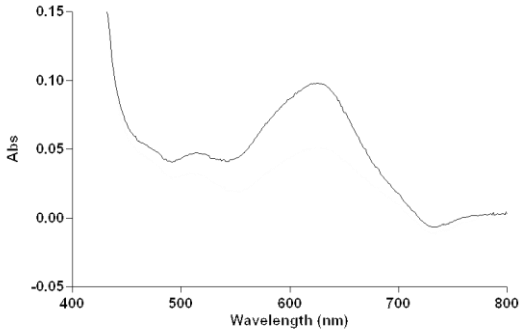

Fig. 1. Absorption spectrum of anthrone-sulfuric acid method.

Optimization of the method for glucose determination

Effect of solvent on the absorbance

From the two solvents (distilled water and PBS) tested in the reaction system, distilled water was found to be the better one for the selected system. The absorbance value in the PBS reaction system was somewhat lower (shown in Figure 2).

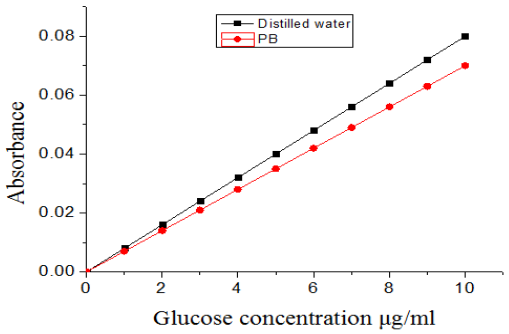

Fig. 2. The effect of two different solvents on the absorbance.

Effect of heating on the absorbance

Two temperature conditions, i.e., with heating or without heating, were investigated. One of the sets was heated for 10 min and cooled for 5 min with running water (shown in Figure 3), while the other set was let to stay for 15 min without heating (shown in Figure 4). The results showed that heating is a critical step in the experiment, and could significantly promote the reaction and accelerate the reaction rate.

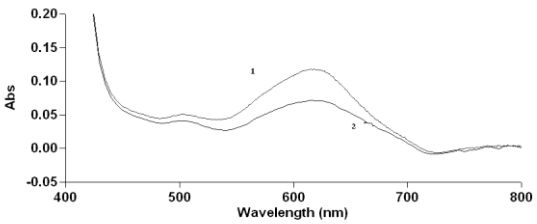

Fig. 3. Effect of heating on the absorbance. (1) Experimental set; (2) Reagent blank.

Effect of anthrone concentration on the absorbance

In this work, anthrone concentration range was selected from 0.5% to 2.5% (0.5, 1.0, 1.5, 2.0 and 2.5%). As shown in Figure 5, the highest absorbance value was registered with anthrone concentration of 1.0% and slowly decreased when anthrone concentration exceeded 1.0%.

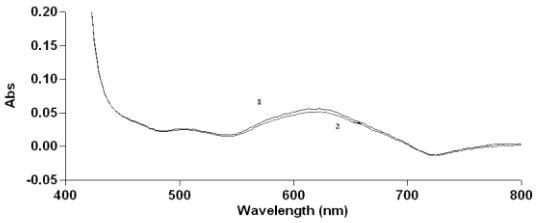

Fig. 4. Absorbance without heating in the experiment. (1) Experimental set; (2) Reagent blank.

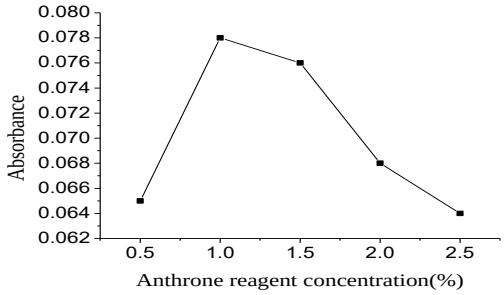

Fig. 5. Effect of anthrone concentration on the absorbance.

Effect of sulfuric acid concentration on the absorbance

The effect of different sulfuric acid concentrations (75, 80, 85, 90, and 95%, respectively) on the absorbance was tested. The anthrone concentration was 1%. With 80% sulfuric acid as a solvent for anthrone the optimal absorbance was registered (shown in Figure 6).

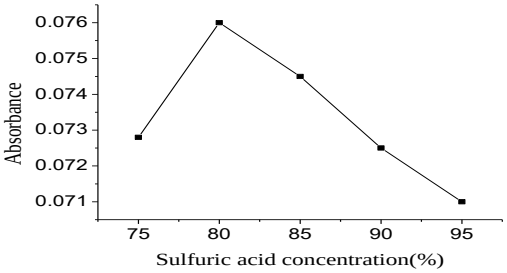

Fig. 6. Effect of different concentrations of sulfuric acid on the absorbance.

Effect of the amount of anthrone reagent on the absorbance

Different volumes of 1.0 g/L anthrone-80% sulfuric acid solution such as 3, 4, 5, 6, 7, 8, 9, 10 mL were added to the reaction system and the absorbance was measured at 625 nm. The highest absorbance at the wavelength of 625 nm was registered when 3 mL of the anthrone sulfuric acid solution was added to the reaction system (shown in Figure 7). When the anthrone reagent was less than 3 mL, some precipitates were formed.

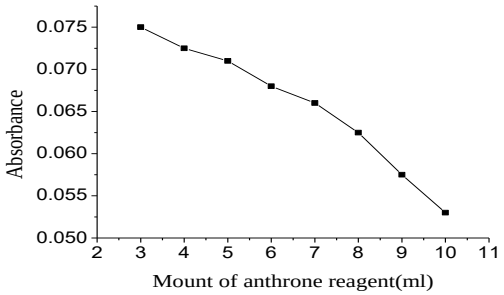

Fig. 7. Effect of different amounts of anthrone reagent on the absorbance.

Effect of reaction duration on the absorbance

The maximum absorbance of anthrone-glucose reaction system was attained after 15 min (10 min of heating and 5 min of cooling), which remained stable for 2 h. A longer period of reaction duration will cause a hypochromic effect.

Glucose determination assay (standard method)

The glucose solutions were with concentrations of 10, 15, 20, 25, 30, 35, and 40 μg/mL. Glucose solutions up to 1 mL were pipetted into 12×100 mm test tubes and the volume in the test tube was adjusted to 1 mL with distilled water. Three mL of anthrone reagent was added to each test tube and the content was mixed by oscillation. After heating for 10 min and cooling for 5 min with running water the absorbance was measured in 3-mL cuvettes against a reagent blank (1 ml distilled water). The weight of glucose was plotted against the absorbance resulting in a calibration curve used to determine the glucose content in unknown samples. Figure 8 shows the calibration curve obtained by the standard method. The resulting regression equation was $y=12.13x-0.002$, $R^2 = 0.999$.

Glucose microdeterminaion assay

Glucose solutions containing 1 to 10 μg glucose were pipetted into 12×100 mm test tubes. The volume of the test tube was adjusted to 1 mL with distilled water. 3 mL of anthrone sulfuric acid and 1 mL of glucose solution were added to the test tube and the contents were mixed as in the standard method. The absorbance at 625 nm was measured as in the standard method using 1 mL cuvettes against a reagent blank (1 mL distilled water). Calibration curves (shown in Figure 9) were prepared as in the standard method. The resulting regression equation was $y = 7.495x + 0.002$, $R^2= 0.997$.

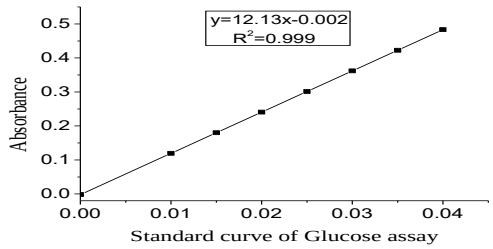

Fig. 8. Calibration curve of glucose assay.

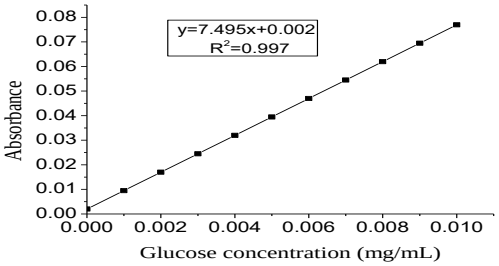

Fig. 9. Calibration curve of microglucose assay.

Interference by non-glucose compounds

Four kinds of foreign compounds were individually studied for their effect on the determination of 1 to 10 μg glucose. The criterion for interference was an absorbance value exceeding by more than 5% the expected value for glucose alone. The results are shown in Table 1. There was no interference from the following: 1M NaCl, 1M $MgCl_2$, 1M KCl and 0.1%Tris.

Table 1. Interference by non-glucose compounds.

Substance	Change in OD 625 nm	(μg) Equivalent Glucose
1M NaCl	0.0000	0
1M $MgCl_2$	0.0000	0
1M KCl	0.0000	0
0.1% Tris	0.0015	0

Precision experiments

Quintuplicate standard assay of glucose was tested as a standard result. Statistical analysis gives a relative standard deviation of 2.42% of the mean value for the assay. The relative standard deviation of less than 3% meets the demand for experimental determination.

Recovery test

A standard solution was added to the experimental set and the absorbance was measured. The statistical treatment of the results (n = 3) gave a recovery of 101.4% of the mean value. Recoveries should be in the 90-110% range to meet the requirements.

Three sets of parallel tests were conducted. The absorbance of the standard method ranged from 0.10 to 0.51 OD. The interference of the reagent itself was excluded by spectrophotometer zeroing. This method showed a good linear relationship in the concentration range detected. The weight of glucose gives an absorbance change of 0 vs 0.035 OD in the microassay. The experimental results indicate that the proposed method is easy for operation with high selectivity, reproducibility and a broad working range.

CONCLUSIONS

The results show that the appropriate use of anthrone sulfuric acid allows the exact determination of trace amounts of glucose. To ensure satisfactory results, some conditions such as heating the solution, concentration of anthrone and sulfuric acid, amount of anthrone reagent, reaction time and effect of foreign ions on the reaction system were optimized. Results showed that the optimum conditions are: total volume of 5.0 mL of the reaction system containing 3.0 mL of anthrone-sulfuric acid solution, 1.0 mL distilled water and 1.0 mL glucose solution. The tube with the solution mixture was kept in a water bath at 93°C for 15 min. The absorption values at 625 nm of different glucose concentrations are in linear relationship with the fitting equation of linear regression with a correlation coefficient R^2 of 0.997, the linear range was 0 ~ 0.01 mg/mL. Standard addition test results revealed that the method has a relative deviation of 2.42%, the tagged recoveries were 101.4%. The application of spectrophotometry provides a very simple and relatively rapid determination of glucose. The method recommended is precise, easy to operate and sensitive. It may be a new test tool for determination of microglucose.

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БЪРЗО ОПРЕДЕЛЯНЕ НА СЛЕДИ ОТ ГЛЮКОЗА ПО АНТРОН-СЕРНОКИСЕЛИЯ МЕТОД

Ф. Ленг[1*], С. Сун[1], И. Джинг[1], Ф. Уанг[1], К. Уей[1], Кс. Уанг[2*], Кс. Жу[2]

[1] *Училище за науки за живота и инженерство, Технологичен университет Ланжоу, Ланжоу 730050, Китай*
[2] *Институт по стопанство и фармацевтични науки в Ланжоу, Китай*

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(Резюме)

Антрон-сернокиселият метод е бърз и микроаналитичен. Тук той е модифициран за определянето на въглехидрати в колориметрична кювета. Разработена е методология за еднократно определяне за оптимизация на факторите, като избор на разтворител, нагряване, концентрация на антрона, на сярната киселина и времетраенето. Реакционната система е с общ обем 5.0 mL, съдържаща 3.0 mL разтвор на антрон в сярна киселина, 1.0 mL дестилирана вода е се държи на водна баня при 93°C за 15 мин. При тези условия абсорбцията на пробата при 520 nm е линейна спрямо различни концентрации на глюкозата (в интервала 0 ~ 0.01 mg/mL) при коефициент на корелация 0.997. Тестът със стандартна добавка показва, че този метод е с относително отклонение от 2.42% с добив от 101.4%. Разработеният метод е прост, стабилен, възпроизводим и може да бъде използван за определянето на общо количество захари.