

Biosensor for Determination of Glucose and Sucrose in Fruit Juices by Flow Injection Analysis

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Abstract

Glucose and sucrose were measured with an amperometric method by using the flow injection analysis technique. A carbon paste electrode with a renewable surface containing glucose oxidase, horseradish peroxidase, and ferrocene was used in combination with the soluble enzymes invertase and mutarotase. The effect of invertase, mutarotase, and ascorbic acid on the electrode response was examined. Glucose and sucrose concentrations were determined with <3% errors. The proposed method for glucose and sucrose measurements was validated in real samples of fruit juices. The results were also compared with those obtained with the ultraviolet method.

Index Entries: Biosensors; food analysis; glucose; sucrose; carbon paste electrode.

Introduction

Both glucose and sucrose concentrations are often determined in the clinical field and in the food industry. Various methods such as the ultraviolet (UV) method or high-performance liquid chromatography are used for these measurements. Recently, much attention has been focused on the determination of sugars, particularly glucose and sucrose, using biosensors. Currently, the use of enzymatic reactions coupled with amperometric detection is widely investigated.

Various amperometric methods were proposed for the selective or simultaneous measurements of glucose and sucrose. Detections based on

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various species (e.g., hydrogen peroxide, oxygen, or H^+) have been explored, and it was often shown that the determination of sucrose concentration is affected by the presence of glucose (1–6). Hence, glucose can be removed by using either a layer of catalase and glucose oxidase (GOD) to recover the electrode surface (7) or a glucose eliminating reactor (8). For selective determination of glucose and sucrose, two different measurements are necessary, and both invertase and mutarotase must be added in the sample solution (9) or be immobilized into membranes (10), cells (11), or over solid supports such as enzymatic reactors (12,13). The simultaneous determinations of glucose and sucrose have also been investigated, and various strategies have been suggested, such as the use of enzyme reactors (14,15) and enzyme electrodes combined with invertase-attached cells (16), multienzymes (17), or two enzymes separated by a catalase-modified membrane (18).

In this article, we describe the successive determinations of glucose and sucrose by a GOD/horseradish peroxidase (HRP)/ferrocene (FCH)-modified carbon paste electrode in combination with invertase and mutarotase to be added in the sample solutions. The assays were achieved using the flow injection analysis (FIA) mode. We discuss the analytical characteristics and advantages of this modified electrode combined with hydrolysis enzyme. Owing to the importance of glucose and sucrose determination in agrifood industries, we also report the feasibility of this method for the analysis of fruit juices.

Materials and Methods

Materials

GOD (EC 1.1.3.4) (138.5 U/mg), HRP (EC 1.11.1.7) (934 U/mg), and invertase (EC 3.2.1.26) (392 U/mg) were purchased from Boehringer Mannheim. Mutarotase (EC 5.1.3.3) (1887 U/mg) was obtained from Biozyme. The mediator bis(cyclopentadienyl)iron (FcH, ref. no. 46260) and graphite powder (cat. no. 50870) came from Fluka. The pasting liquid used was the paraffin oil purchased from Merck. Glucose and sucrose were commercialized by Fluka, and solutions were prepared with 0.1 M phosphate buffer, pH 7.2, containing 0.1 M KCl.

Preparation of Electrode

The HRP/GOD/FcH electrode was prepared as previously described (19). HRP (8.6 mg) (7900 U) was dissolved in 3 mL of $NaIO_4$ solution (1.6 mg/mL) and stirred for 20 min at room temperature. The oxidized HRP was ultrafiltrated using a Centricon from Amicon (30-kDa cutoff) and dissolved in 800 μ L of 0.05 M phosphate buffer, pH 8.0. The graphite powder (400 mg) was then added to the resulting HRP mixture and stirred for 20 min at room temperature. GOD (16 mg) was dissolved in the same phosphate buffer (800 μ L), added to the carbon mixture already containing the

oxidized HRP, and stirred for an additional 30 min. The enzymatic carbon mixture was freeze-dried to obtain the bienzymatic modified carbon powder. The HRP/GOD/FcH-modified carbon paste was obtained by mixing in a mortar the resulting HRP/GOD modified carbon powder with a given amount of paraffin oil in which FcH was already dissolved. The final carbon paste was made with 24.24% oil (w/w). A plastic cartridge was filled with this paste and used as a working electrode in a three-electrode electrochemical cell. One extremity of the cartridge was in contact with the solution and the other was connected through a carbon rod to the potentiostat.

Apparatus and Measurements

The flow injection system consisted of a potentiostat (Amel model 472), a homemade flow cell equipped with a three-electrode system, a peristaltic pump (Ismatec), and a six-way automatic injection valve (Rheodyne). Enzyme-modified carbon paste was used as the working electrode, and Ag/AgCl and stainless steel wire were used as the reference and counter-electrode, respectively. An xt recorder was connected to the potentiostat to record the output currents.

The surface of the working electrode was polished and smoothed on clean paper before use. The working potential was set at 0.0 V vs Ag/AgCl and 10 min was necessary for the stabilization of the baseline current. A loop of 100 μ L was used for the injection of the sample and phosphate buffer as a carrier with a flow rate of 0.7 mL/min.

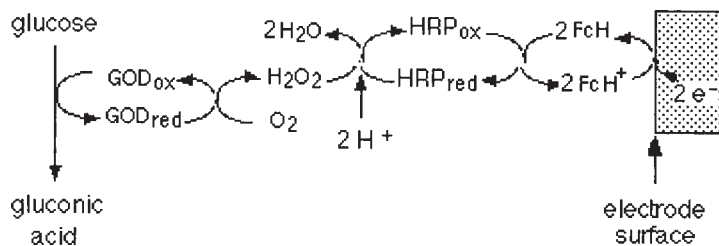
For glucose and sucrose assays, two injections were achieved in the absence and presence of hydrolysis enzymes. Two minutes was necessary for the total hydrolysis of sucrose in the sample. Sucrose determination was obtained by subtraction of the current before hydrolysis from the one obtained after hydrolysis.

For the UV method, a spectrophotometer from Bioblock (Spectronic 401) was used. Measurements of glucose and sucrose were performed using Test-Combination from Boehringer Mannheim following exactly the advised instructions (20).

Results and Discussion

The optimization of the electrode modified with HRP/GOD/FcH was investigated in detail and the effect of each component (GOD, HRP, FcH, oil) evaluated. The optimized and final electrode contained 4 U of GOD and 14.5 U of HRP per milligram of carbon paste, 24.24% paraffin oil (w/w), and 0.32% FcH (w/w). This electrode operated at 0.0 V vs Ag/AgCl using the flow injection technique and allowed the determination of glucose according to the reactions shown in Scheme 1.

The repeatability and the operational stability of the enzyme-modified electrode were examined. The experiments were achieved by injecting a standard solution of glucose and recording the resulting peaks in function of time (Fig. 1). The GOD/HRP/FcH-modified carbon paste electrode



Scheme 1. Detection of glucose at the electrode/solution interface.

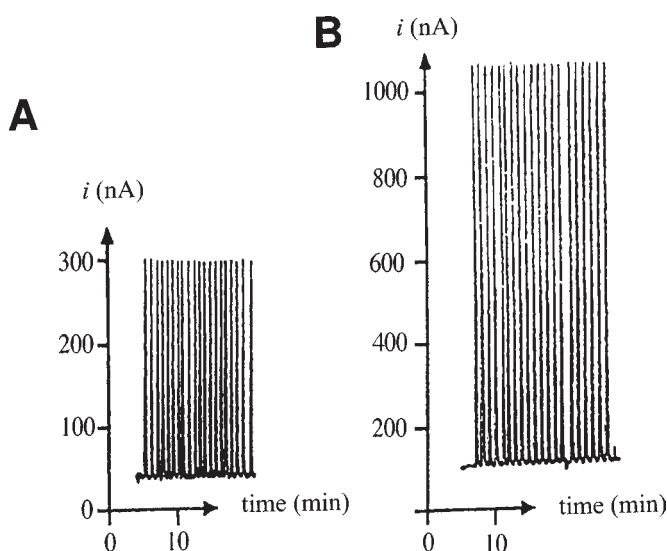


Fig. 1. Repeatability of the amperometric response of the GOD/HRP/FcH-modified carbon paste electrode. Assays were made with standard glucose solutions: (A) 20 mg/L; (B) 80 mg/L.

showed no significant loss of signal during a series of 20 successive injections of the standard glucose solution, indicating good stability of the electrode surface during a relatively long measurement of time. The repeatability was estimated by calculating the relative standard deviations for two different glucose concentrations (20 and 80 mg/L); the resulting relative standard deviations (RSDs) were <1%.

Figure 2 displays the calibration curve of standard glucose solutions obtained under previous experimental conditions. We considered the electrode response to be linear if the differences between measured concentrations and the concentration of the standard solutions were <3%. The validation of the calibration curve was achieved by calculating the percentage of error using the following relationship:

$$\% \text{ error} = (C_{\text{mes}} - C_{\text{std}}) \times 100 / C_{\text{std}}$$

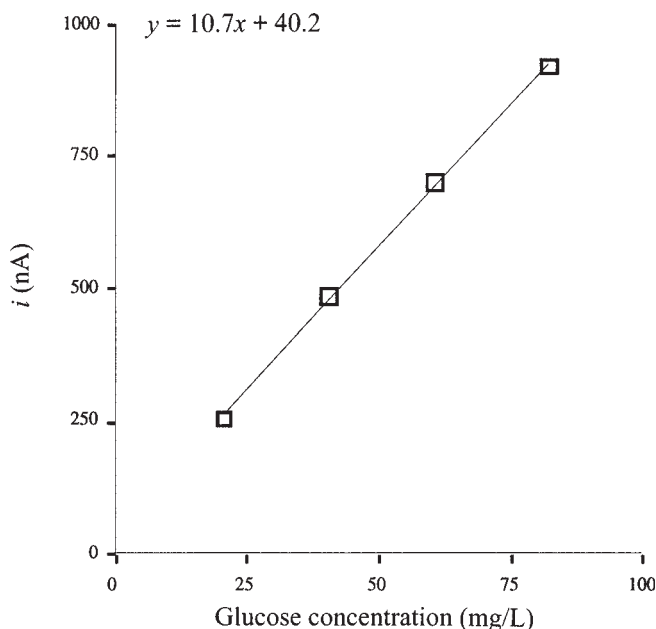
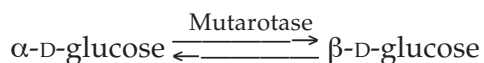
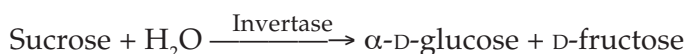


Fig. 2. Calibration curve. Peak intensity, at GOD/HRP/FcH-modified carbon paste electrode, in function of the injected glucose concentration.

in which C_{mes} is the calculated concentration by using the linear regression equation, and C_{std} is the concentration of injected standard solution. Table 1 gives the errors for different standard solutions used for the calibration curve. We note that even for the lowest concentration, the measured value does not vary more than 3% from the standard value.

The GOD/HRP/FcH-modified carbon paste electrode was thereafter used for the measurement of sucrose. Given amounts of both invertase and mutarotase had to be added to the sample in order to hydrolyze sucrose as shown in the following reactions:



To determine whether either of the two enzymes would interfere in the electrochemical detection process, we previously examined whether soluble mutarotase and invertase do affect the electrode response. No significant differences in the intensity peaks were observed when glucose standard solution was injected in the presence and absence of the two enzymes. Neither invertase nor mutarotase reacted with the modifiers of the electrode, and no specific adsorption phenomenon was observed on the electrode surface.

Because the rate of the total sucrose inversion increases with the increase in the amount of invertase and because the total hydrolysis of

Table 1
Validation of Glucose Calibration Curve

Standard glucose concentration (mg/L)	Measured current (nA) ^a	Error (%)
20.6	255	-2.6
40.6	485	2.4
60.8	700	1.4
82.3	920	-0.1

^aMean of two measurements.

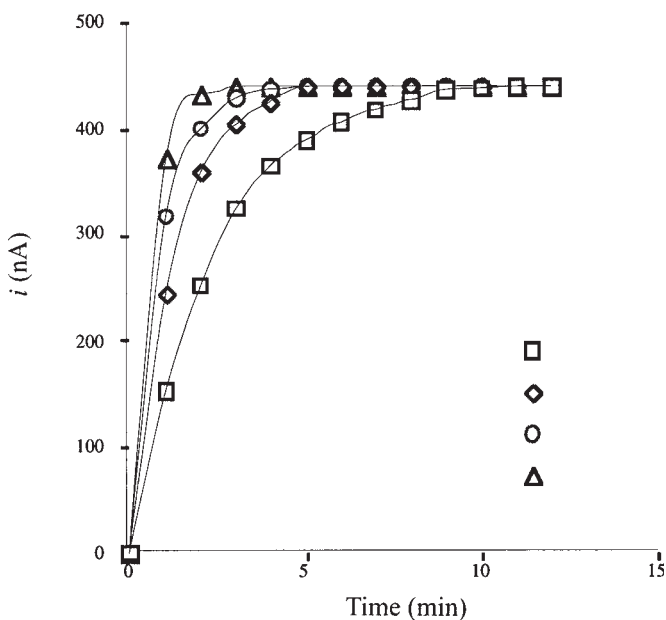


Fig. 3. Effect of the amounts of invertase on the GOD/HRP/FcH electrode response. Sucrose concentration: 100 mg/L; amount of mutarotase: 30 U/mL; amount of invertase: (□) 23, (◇) 45, (○) 68, and (△) 113 U/mL.

sucrose must occur as quickly as possible, the effect of the amounts of both invertase and mutarotase were investigated and optimized. By keeping the amount of mutarotase constant and equal to 30 U/mL and varying the amount of invertase from 23 to 113 U/mL, we noticed that the hydrolysis time for a 100 mg/L sucrose solution varied from 10 to 2 min (Fig. 3).

Spontaneous and catalyzed mutarotation reactions were compared. Figure 4 shows that the total sucrose hydrolysis and the equilibrium between α -glucose and β -glucose were reached about 2.5 min after the addition of a large amount (30 U/mL) of mutarotase to a 100 mg/L sucrose solution containing 118 U/mL of invertase. Simultaneous optimizations of

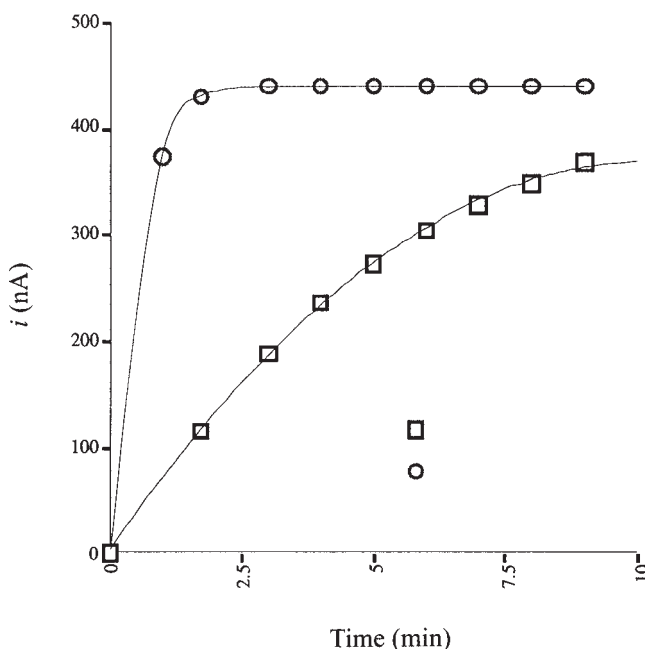


Fig. 4. Effect of the mutarotase on the GOD/HRP/FcH electrode response to sucrose solution: in the absence (□) and presence (○) of mutarotase (30 U/mL). Sucrose concentration, 100 mg/L; amount of invertase, 118 U/mL.

the amounts of invertase and mutarotase were carried out. The best result was obtained when, respectively, 188 and 13 U/mL of enzymes were used in the assay. For these concentrations, the hydrolysis was effective in less than 2 min, even for the highest sucrose concentration (160 mg/L) of the linear range of the electrode.

Using the optimized experimental conditions for sucrose hydrolysis, we calibrated the HRP/GOD/FcH electrode for both glucose and sucrose measurements. Figure 5 shows typical calibration curves. The ratio of the sensitivities (given by the slopes of the calibration curves for sucrose and glucose) was found to be equal to the molecular weight ratio (1.9), indicating that total conversion of sucrose was obtained. Hence, only one calibration curve (glucose) is then necessary to quantify both glucose and sucrose. As seen in Table 2, the errors for sucrose assays for four different standard solutions are acceptable. This shows that accurate measurement of sucrose can be obtained when only the glucose calibration curve is used. For each assay, the final sucrose concentration is recovered by multiplying the result obtained with the glucose calibration curve by the ratio of the molecular weights (1.9). For the simultaneous determination of glucose and sucrose in the mixtures that contain both sugars, a single calibration curve is used and glucose is measured before and after adding hydrolysis enzymes. The difference between total and initial glucose allows the calculation of sucrose concentrations.

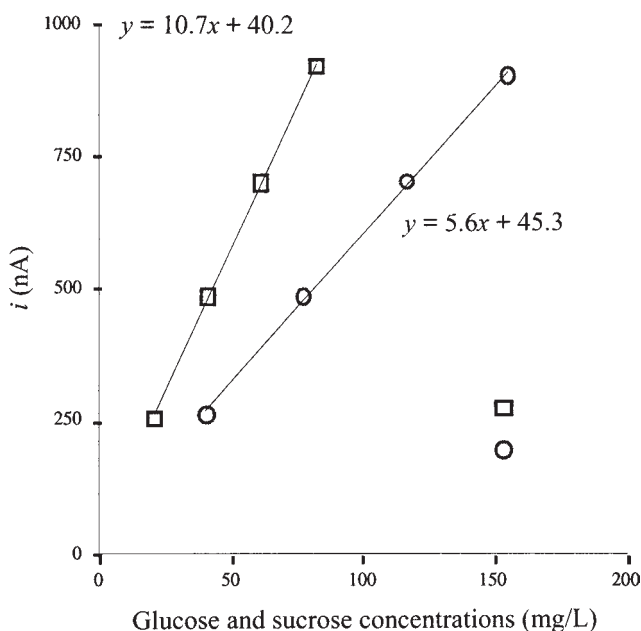


Fig. 5. Calibration curves obtained with standard glucose (□) and sucrose (○) solutions. Sucrose solutions contained 188 U/mL of invertase and 13 U/mL of mutarotase.

Table 2
Validation of Sucrose Measurements
When Glucose Calibration Curve Is Used

Standard sucrose concentration (mg/L)	Measured concentration (mg/L) ^a	Error (%)
40.3	39.3	-2.5
77.2	78.6	1.8
116.0	117.0	0.9
155.0	152.0	-1.9

^aMean of two measurements.

The accurate measurements of both sugars in complex matrices such as fruit juices require, in addition to the total hydrolysis of sucrose, the absence of interfering species. Ascorbic acid is the most known and prominent interfering specie in the fruit juices (2,12,21,22). Because of the high sensitivity of the HRP/GOD/FcH electrode and the high dilution factor of the juice samples, we found no, or negligible, effect of ascorbic acid on the electrode response. The dilution factor (800) generally used for the measurement of sugars in fruit juices using the FIA technique drastically affects the level of various interfering species and allowed us to escape the matrix effects.

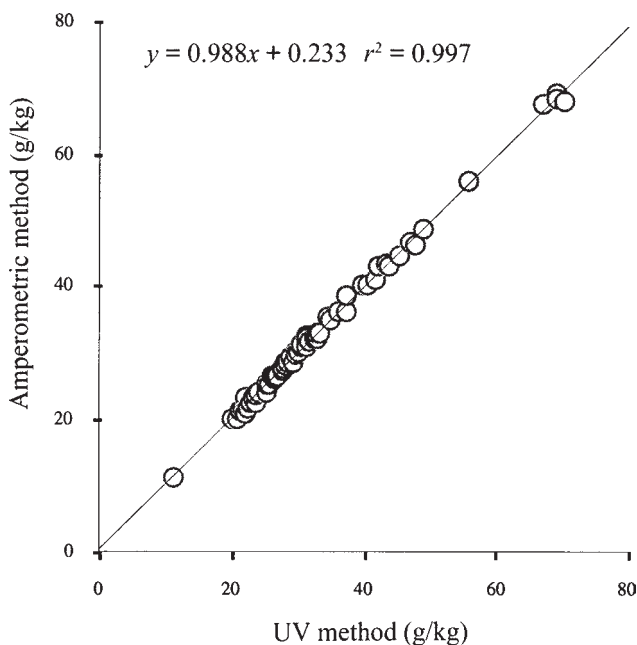


Fig. 6. Glucose determination in fruit juice samples. Comparison of the results was obtained by amperometric and UV methods.

To evaluate the reliability of the electrode response in real experimental conditions, we examined the repeatability of the method by analyzing the results of 10 successive assays applied to the same orange juice sample. The RSDs for glucose and sucrose concentrations were, respectively, 0.5 and 1.2, indicating that the proposed method gives very repeatable results.

The accuracy of the developed method was finally examined. The results for glucose and sucrose measurements in 80 different fruit juices were compared with those obtained by the standard UV method. The plots of the values obtained by the two different techniques for glucose and sucrose assays are shown in Figs. 6 and 7, respectively. The regression coefficients are 0.997 for glucose and 0.992 for sucrose determination, indicating a very good correlation between the two methods. A statistical test (23), used for the comparison of these two methods in terms of accuracy, showed no significant difference between the measurement series for both glucose and sucrose.

Conclusion

A modified carbon paste electrode was used in association with soluble invertase and mutarotase for glucose and sucrose determination in different mixed samples. Experimental conditions for glucose measurement and sucrose hydrolysis were optimized. A quantitative sucrose hydrolysis with <3% error was obtained. Only a calibration curve with glucose was neces-

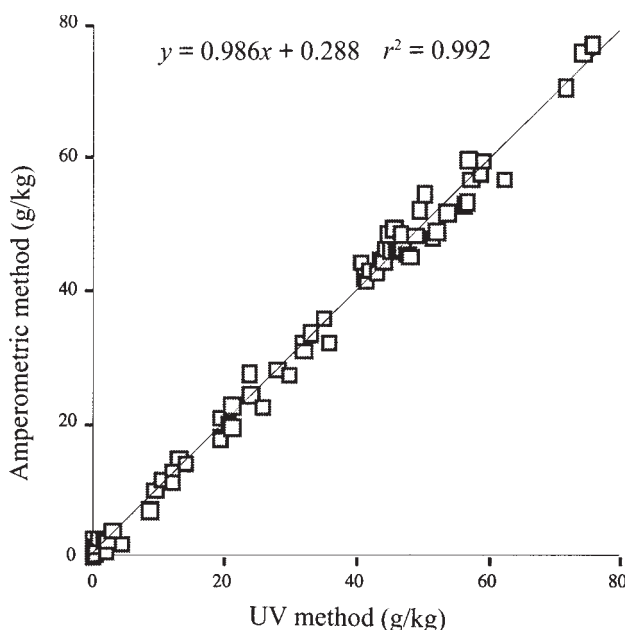


Fig. 7. Sucrose determination in fruit juice samples. Comparison of the results was obtained by amperometric and UV methods.

sary for the determination of both sugars. The method was shown to be accurate and repeatable and was sufficiently evaluated for real sample analysis and compared to the standard UV method. Very good correlation between the two techniques was obtained for both glucose and sucrose.

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