the intestine of weanling rats have suggested that the bacterial flora may adapt to the presence of a bulking agent and more fully convert it to absorbable nutrients upon prolonged exposure (Grossklaus et al., 1984). However, fatty acids were quantitated only at single time points and therefore reflect the rates but not necessarily the extent of fatty acid formation. Moreover, those observations may not be relevant to older animals, since development of the adult pattern of intestinal transport mechanisms in the rat only begins in the third week (Batt and Schachter, 1969). Comparisons between single dose studies and studies in mature rats that had received daily doses for 90 days suggest that changes do not occur with an agent such as polydextrose (Figdor and Rennhard, 1981). Of particular relevance, studies in adult humans following repeated doses of polydextrose for 7 days yielded caloric utilization values similar to those in rats receiving single doses (Figdor and Bianchine, 1983). Studies in adult rats (or humans) presumably started with intestinal flora able to digest the bulking agents as well as was possible under these circumstances. The absence of adaptation in disposition studies is therefore not unexpected.

Registry No. Sorbitol, 50-70-4; isomalt, 64519-82-0.

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Enzyme Electrode for the Determination of Sucrose in Food Products

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A multienzyme electrode for the determination of sucrose in food and agricultural products was developed. Both the immobilization and experimental parameters were optimized. The electrode was evaluated for the determination of sucrose over analogous interferences. For sucrose, the linear dynamic range is 3.33×10^{-5} to 1.3×10^{-3} M for the initial-rate method and 3.3×10^{-5} to 1.5×10^{-3} M for the steady-state method. The electrode is very stable and gives fast response to sucrose.

Immobilized enzymes are receiving increasing attention (Guilbault, 1984). Although Clark and Lyon (1962) first introduced the concept of the "soluble" enzyme electrode, the first working electrode was reported by Updike and Hicks (1971). Today, enzyme electrodes are applied to the determination of a wide variety of sugars such as glucose (Clark and Lyon, 1962; Guilbault and Lubrano, 1973; Pfeiffer et al., 1979), lactose (Bertrand et al., 1981; Frank and Christen, 1984; Mason, 1983), galactose (Taylor et al., 1977), and maltose (Coulet and Bertrand, 1979). Techniques such as polarimetry, isotope dilution, chromatography, refractometry, and densitometry have been developed and employed for the determination of disaccharides, mainly sucrose (Schneider, 1982). These methods generally require laborious sample pretreatment

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and have poor sensitivity. Bertrand et al. (1981) described a sucrose electrode by immobilizing invertase, mutarotase, and glucose oxidase onto a collagen membrane. The electrode lacked sensitivity, required up to 30 min for steady-state response, and had no long-term stability. Scheller and Renneberg (1983) described a preliminary sucrose electrode based on the coupling of glucose oxidase, catalase, and invertase. The electrode was used for assay in some food products but lacked sufficient stability for long-term usefulness and is slow.

Other sucrose electrodes described in the literature suffer from either lack of sensitivity and very small linear range (Cordonnier et al., 1975; Satoh et al., 1976) or lack of stability and good response (Kulys, 1981; Satoh et al., 1976). We, here, present an improved assay method for sucrose based on the immobilization of three enzymes on an oxygen electrode, which can ultimately be used for the determination of total glucose and/or sucrose in food and agricultural products. The multienzymatic reactions of sucrose are presented in eq 1–3.

$$\text{sucrose} + H_2O \xrightarrow{\text{invertase}} \text{fructose} + \alpha\text{-D-glucose} \quad (1)$$

$$\alpha\text{-D-glucose} \xrightarrow{\text{mutarotase}} \beta\text{-D-glucose} \quad (2)$$

$$\beta\text{-D-glucose} + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} H_2O_2 + \text{gluconic acid} \quad (3)$$

The electrode is stable and sensitive, has good linear range, and responds very fast.

EXPERIMENTAL SECTION

Apparatus. An amperometric detector (Model PRG-GLUC, Tacussel Electronics) was used to apply −550 mV to the working electrode and measure the current. The signals were recorded on a Series 4500 microscribe strip chart recorder (Houston Instruments, Austin, TX). A platinum oxygen electrode (Catalog No. 4000-1) was used as the base O_2 sensor, and pig intestine (peptidase free) was used as a support for the enzyme immobilization (Universal Sensors, Inc., New Orleans, LA 70148). A Model 13-4615-10 dc amplifier, Series 4600, combined with a differentiator amplifier, Model 13-4615-71 (Gould Inc., Cleveland, OH), were used for initial rate measurement studies.

Materials. Glucose oxidase (E.C. 1.1.3.4), invertase (E.C. 3.2.1.26), mutarotase (E.C. 5.1.3.3), α-chymotrypsin (E.C. 3.4.21.1), glutaraldehyde, bovine serum albumin, sucrose, glucose, lactose, melibiose, fructose, and raffinose were of the highest purity available from Sigma Chemical Co. (St. Louis, MO). Reagent-grade mono- and dibasic sodium phosphates were from J. T. Baker Chemical Co. (Phillipsburg, NJ). All other chemicals were reagent grade and were used without further purification.

PROCEDURE

Electrode Preparation. The method reported previously with minor modification was used for the immobilization of enzymes (Nabi Rahni, 1985). Phosphate buffer (0.2 M, pH 6.88) is the buffer used for all solution preparations and assays. A pig intestine membrane was mounted on the tip of an oxygen electrode with an O ring. It was treated with 250 μL of α-chymotrypsin solution (0.5 mg/0.5 mL of buffer) for 10 min and then washed with buffer. A 50-μL enzyme solution containing 0.5 mg of glucose oxidase (73 U), 1.0 mg of invertase (825 U), and 12650 U of mutarotase suspended in 3.2 M $(NH_4)_2SO_4$ solution was placed on the pig intestine. Bovine serum albumin (15 μL, 10% solution) was then added to it and the resultant mixture stirred for 30 s. Finally, 5 μL of glutaraldehyde 10% solution was added, stirred vigorously for 15 s, and let dry at room temperature for 3 h. It was then washed with buffer and stored in buffer at 5–6 °C when not in use. The glucose enzyme electrode was prepared, according to the method reported in the literature, using highly purified glucose oxidase from *Aspergillus niger* (Sigma).

Electrode Measurements. For every assay, the enzyme electrode was first equilibrated in 1.5 mL of phosphate buffer (0.2 M, pH 6.88) with constant stirring at room temperature (25 °C) until a steady base line was obtained. Known volumes of stock sucrose solution (0.01 M, prepared fresh daily in buffer) were then injected into the buffer solution. The signal was monitored by both the initial-rate and the steady-state methods in a standard addition and discrete assay fashion. The initial rate measurement generally takes about 30 s, and recovery time is on the order of 2–3 min. On the other hand, if the steady-state method is used, the measurement time is 2–3 min and the recovery time is 3–5 min. With a glucose electrode, stock solutions of sucrose were checked periodically for possible presence of glucose. In a food sample, the sucrose electrode measures the concentrations of both sucrose and glucose, while a glucose electrode measures the glucose present in the sample. The difference between the two is the sucrose concentration according to the calibration curves constructed for glucose and sucrose.

RESULTS AND DISCUSSION

Optimization of Parameters. At first, attempts were made to optimize the immobilization and reaction parameters. The electrode preparation was the result of numerous experiments, in which the effect of membrane pretreatment (e.g. chymotrypsin), the optimum contents of the various enzymes in the membrane (73 U of glucose oxidase, 825 U of invertase, 12650 U of mutarotase) and immobilizing matrix (5 μL of 10% glutaraldehyde, 15 μL of 10% BSA, 50 μL of enzymes) were elucidated. The main objective in the pretreatment of the pig intestine with chymotrypsin was to modify the polypeptide chains in the membrane, thus making more amino groups available for this immobilization method. Finally, the reaction parameters were tested, and 0.2 M phosphate, pH 6.88, and temperature 25 °C were found to be optimum.

Construction of Calibration Curves and Response Time. Figure 1 represents the standard calibration curves for the determination of aqueous sucrose solutions with the sucrose electrode. Figure 1A is a calibration curve obtained when discrete assays were performed in a steady-state mode (response time 2–3 min, depending on concentration), giving rise to a linear dynamic range of 3.3×10^{-5} to 1.5×10^{-3} M. Figure 1B is a calibration curve obtained by initial-rate method (less than 1-min measurement time) for discrete assays with a linearity from 3.3×10^{-5} to 1.3×10^{-3} M. Figure 2A is a calibration curve for glucose with a glucose electrode, whereas Figure 2B is the response of the glucose enzyme electrode to sucrose standard solution pretreated with invertase (825 U/1.0 mL) at room temperature for 20 min. Good agreement is thus observed between the glucose standard and the glucose response of the glucose electrode probe to this sugar liberated from sucrose. The small difference is perhaps due to incomplete mutarotation. The steady state was obtained in 2–5 min, the rate of reaction, in 1 min. Thus, the electrode has a faster response than previously described sucrose probes.

Interference Studies. Standard solutions of fructose, lactose, melibiose, and raffinose were tested with the sucrose electrode to study their possible interference at

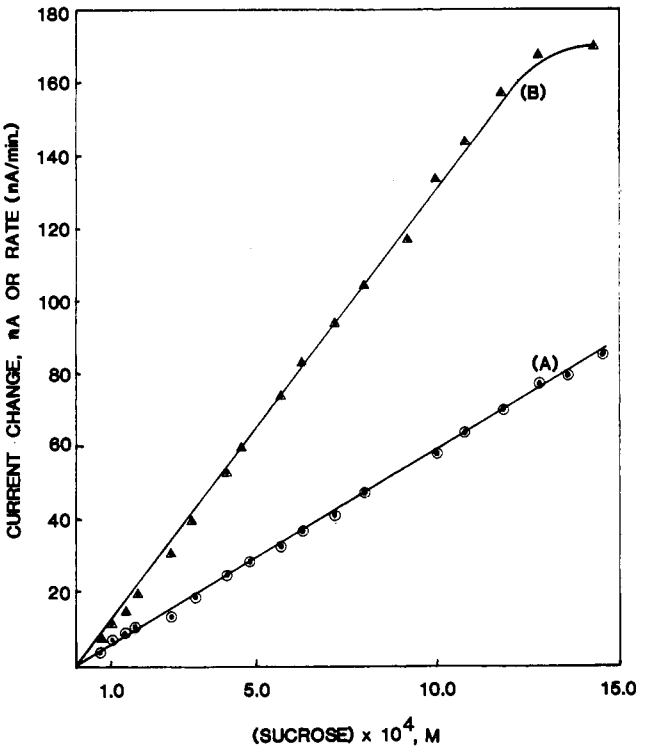

Figure 1. Standard calibration curves of sucrose: (A) steady-state method (response measured in 2–3 min); (B) initial-rate method (response measured within 1 min). T = 25 °C, 0.2 M phosphate buffer, pH 6.88.

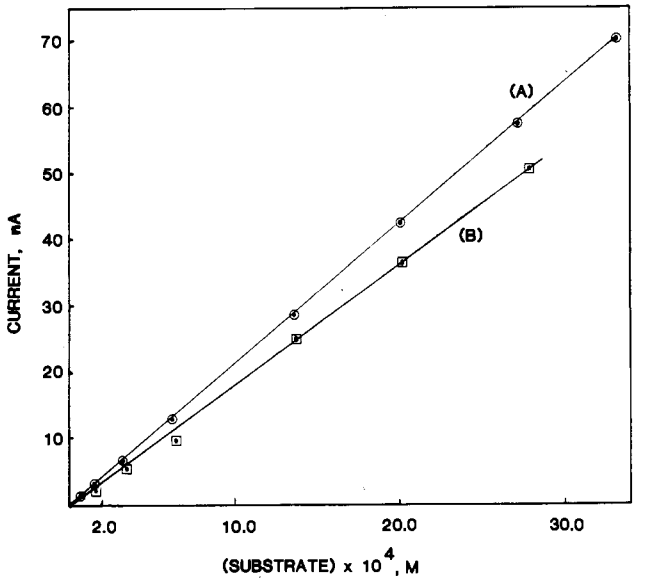

Figure 2. (A) Glucose calibration curve with glucose electrode. (B) Sucrose calibration curve with glucose electrode and soluble invertase. T = 25 °C, 0.2 M phosphate buffer, pH 6.88, initial-rate method.

concentrations up to 2.0×10^{-3} M. There was no response, and thus no interferences from any of these carbohydrates.

Stability Studies. Calibration curve controls were measured every other day with a sucrose electrode. All measurements were performed at ambient temperature, i.e. 24–26 °C. Figure 3 shows the relative activity of the enzyme electrode as a function of time when a sucrose solution of 6.25×10^{-4} M is analyzed. The electrode was used for over 500 assays over a 4-month period with almost no loss of activity. This is a distinct advantage over all previous electrodes described. An initial rise in electrode activity is observed, as is typical for most immobilized

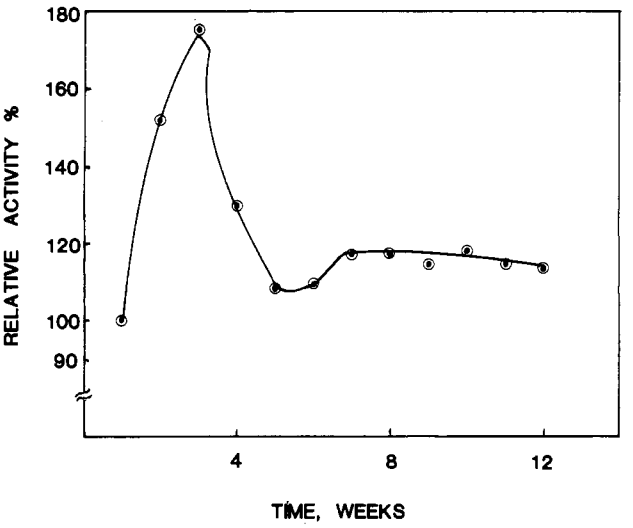

Figure 3. Long-term stability of sucrose electrode. Initial-rate measurement of electrode to sucrose 1.2×10^{-3} M, in 0.2 M phosphate buffer, pH 6.88, 25 °C, over a period of 12 weeks.

Table I. Aqueous Recovery Studies for Sucrose (Every Concentration Representative of Duplicate Assays)

added, M	found, M	rec, %
3.5×10^{-5}	3.7×10^{-5}	106
6.6×10^{-5}	6.7×10^{-5}	102
1.5×10^{-4}	1.5×10^{-4}	100
4.5×10^{-4}	4.5×10^{-4}	98
9.0×10^{-4}	8.9×10^{-4}	99
1.1×10^{-3}	1.0×10^{-3}	91
1.3×10^{-3}	1.2×10^{-3}	92

enzyme systems, followed by a decrease to a steady activity. This is believed due to the creation of diffusional channels for substrate transport in the enzyme layer.

Recovery and Reproducibility Studies. Aqueous solutions of sucrose ranging from 3.5×10^{-5} to 1.3×10^{-3} M were analyzed in duplicate. Table I indicates the recoveries to be between 91 and 106%. A reproducibility study was performed using 6.25×10^{-4} M sucrose. The mean steady-state currents and relative standard deviation (the coefficient of variation of 26 measurements) were 46.34 nA and ±4.41%, respectively. The cause of the rather high value of coefficient of variation is probably due to lack of temperature control during the assays (25 ± 2 °C), which has an effect on rate measurements.

Comparison Studies. In order to assess the reliability of this developed system, random aqueous solutions of sucrose of varying concentrations were assayed. The results obtained were compared to those acquired by a glucose electrode method. In the glucose electrode method, soluble invertase (825 U/1.0 mL) was added to the sucrose solution and the glucose generated was assayed after 20 min. Increasing activity occurs with increasing concentration of soluble invertase and a sucrose concentration of 1.176×10^{-3} M, reaching a maximum at about 800 U. Figure 2B shows a typical standard curve for sucrose when a glucose electrode coupled with soluble invertase is employed. Table II indicates the results obtained with the two methods for sucrose concentrations varying from 3.32×10^{-5} to 1.3×10^{-3} M. The linear regression equation was $y = 1.07x - 8.55$, where x and y are the glucose electrode and sucrose electrode methods, respectively. For 10 assays, the correlation coefficient was 0.99.

Table III demonstrates the analyses of sucrose and glucose contents in various food products according to both methods. The soft drink samples were degassed before assay. The linear regression equation was $y = 1.08x + 1.11$

Table II. Comparison of the Sucrose Electrode Method and Glucose Electrode/Soluble Invertase Method for the Assay of Aqueous Sucrose Solutions (μM)

sucrose electrode (y)	glucose electrode/invertase (x)
33.2	32.0
35.0	34.5
66.5	66.1
128	122
150	166
450	467
625	640
900	925
1100	1160
1300	1410

$y = 1.07x - 8.55$
$r = 0.99$
$n = 10$

Table III. Comparison of the Proposed Method and the Glucose/Invertase Method When Food Products Are Analyzed

	sucrose found, mM	
food sample	sucrose electrode	glucose electrode/ invertase
soft drink	36.6	44.2
diet soft drink	0.0	0.0
grape juice	88.8	116
orange juice	262	274
banana baby food	28.7	30.0
peaches baby food	31.4	29.6
apricot baby food	33.9	31.0
wine A	214.0	241.0
wine B	45.5	46.9

$y = 1.08x + 1.11$
$r = 0.98$

for the sucrose determination according to the two methods, and the correlation coefficient was $r = 0.98$. The sugar composition of soft drinks can vary, but a typical composition was as follows: sucrose, 0.7%; fructose, 6.3%; glucose, 4.3%.

CONCLUSIONS

An enzyme electrode for the determination of sucrose in food and agricultural products was developed. It is based on the coimmobilization of three enzymes, glucose oxidase, invertase, and mutarotase, on a pig intestine pretreated with α-chymotrypsin, mounted on a platinum oxygen sensor. Experimental parameters were optimized. The linear dynamic range is 3.33×10^{-5} to 1.3×10^{-3} M for the initial-rate method and 3.33×10^{-5} to 1.5×10^{-3} M for the steady-state method.

Recoveries were between 90.9 and 105.7%, and the coefficient of variation was ±4.41%. The method was then evaluated for real food analyses vs. a reference method. The correlation coefficient was 0.98.

Advantages over all previously described electrodes are speed of response (less than 1 min by a rate method), stability (4 months or 500 assays), good linear range (3×10^{-5} to 1×10^{-3} M), and reliability.

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