Biosensors & Bioelectronics **10** (1995) 359–370

Galactose biosensors using composite polymers to prevent interferences

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(Received 1 March 1994; revised 8 August 1994; accepted 14 October 1994)

Abstract: A biosensor using a composite polymer to prevent interferences was used in a flow injection analysis system for the detection of galactose in human plasma. The biosensor consisted of galactose oxidase immobilized on a platinized carbon electrode that had been modified with a composite polymer. The composite polymer showed improved selectivity to hydrogen peroxide compared with either of its individual polymeric components, Nafion and a copolymer of diaminobenzene and resorcinol. The composite polymer minimized the effect of possible interference from urate, ascorbate, and acetaminophen. This analytical system had a minimum detection limit of 50 μM, linearity to 6 mM, a storage stability of greater than 30 days, and a high sample throughput (approx. 120 samples/h).

Keywords: biosensor, composite polymer, Nafion, 1,3-phenylenediamine, electropolymerization, galactose, 1,3-diaminobenzene, galactose oxidase, interferences

INTRODUCTION

The determination of physiologically important analytes in complex matrices, such as blood, plasma or urine, presents a challenging and significant problem to the biosensor. A common solution is to use polymer films to prevent or minimize interferences that occur in these complex matrices. A variety of polymers are being used for this application: for example, nonconducting polymers (Malitesta *et al.*, 1990; Sasso *et al.*, 1990; Geise *et al.*, 1991; Sohn *et al.*, 1991; Reynolds & Yacynych, 1993); anionic polymers such as Nafion and Eastman AQ (Palleschi *et al.*, 1986; Harrison *et al.*, 1988; Sanchez *et al.*, 1991; Fan & Harrison, 1992; Gilmartin *et al.*, 1992; Andrieux *et al.*, 1993; Navera *et al.*, 1993), and conducting polymers (Foulds & Lowe, 1986; Shinohara *et al.*, 1987).

Anionic polymers retard the progress of negatively charged interferents, such as ascorbate and urate, towards the electrode surface. However, they have difficulty blocking neutral species, such as acetaminophen, and are not completely effective against urate and ascorbate, especially at certain pHs (Palleschi *et al.*, 1986; Sanchez *et*

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al., 1991; Fan & Harrison, 1992; Navera *et al.*, 1993).

Geise *et al.*, (1991) and Sohn *et al.*, (1991) have shown that a copolymer of 1,3-diaminobenzene (1,3-DAB) and resorcinol (Res) effectively blocks the three most common interferents when used on different types of platinized carbon electrodes. The limited permselectivity of a non-conducting film of poly(1,3-DAB/Res) decreases the response to interferents compared with that to H_2O_2. These non-conducting polymers work well against acetaminophen and urate, but there is some interference from ascorbate at lower current levels.

Although these various types of electropolymerized films are excellent at blocking a large percentage of the signal caused by interferents, they are not sufficient when detecting analytes near the minimum detection limit (MDL) in systems that operate at low currents (*e.g.* ultramicroelectrodes, low analyte concentration detection and low activity biocomponents). It was our goal to make a biosensor that could detect low concentrations of galactose in human plasma. This objective required the development of an improved polymer coating to eliminate the effects of interferents at low current ranges.

We propose the use of a composite polymer made from a mixture of two types of polymer (Fortier *et al.*, 1992; Chen *et al.*, 1993; Moussy *et al.*, 1993): poly(1,3-DAB/Res), a non-conducting polymer, combined with Nafion, an anionic polymer, to create a composite that would be more effective than either of the polymers alone. Nafion is a popular polymer that has been shown to minimize the electrode response to negatively charged interferents (Palleschi *et al.*, 1986; Sanchez *et al.*, 1991; Fan & Harrison, 1992; Navera *et al.*, 1993), but as a single component film has difficulty in completely eliminating interferents (Palleschi *et al.*, 1986). It was hoped that a composite polymer of poly(1,3-DAB/Res) and Nafion would combine the best properties of both polymers to exclude interferents from the electrode surface.

Galactose is a clinically important sugar that is usually not detectable in physiological substances; it is present at a maximum concentration of 0·28 mM in normal subjects and 1·11 mM in neonates less than 5 days old. In disease states galactose concentrations greater than 7 mM have been observed during loading tests (Tietz, 1990; Birlouez-Aragon *et al.*, 1993a,b). The detection and control of galactose levels is extremely important because death can occur in undiagnosed galactosemic neonates. In elderly and diabetic patients, disturbed galactose metabolism is associated with cataract formation (Birlouez-Aragon *et al.*, 1993b).

Several other researchers have developed biosensors for galactose (Taylor *et al.*, 1977; Bertrand *et al.*, 1981; Fortier *et al.*, 1992) but composite polymers were not used. In addition, these biosensors were used in batch mode and would not be well-suited for kinetic studies or for monitoring large numbers of samples. The objective of this paper is to develop composite polymer films for use in biosensors that are required to operate at low current levels, such as those for the detection of galactose.

EXPERIMENTAL DETAILS

Apparatus

An ECO Instruments (Newton, MA) Model 549 or 550 potentiostat/galvanostat was used for platinizations in the galvanostatic mode. All other experiments used an EG&G Princeton Applied Research (Princeton, NJ) Model 264A potentiostat or a Bioanalytical Systems (BAS) (West Lafayette, IN) LC-4B potentiostat. A Rainin (Woburn, MA) rabbit peristaltic pump and a Rheodyne (Cotati, CA) Model 7125 fitted with a 20 μl loop were used for flow-injection analysis experiments. Data were recorded on a Houston Instruments (division of Bausch and Lomb, Houston, TX) Omnigraphic 2000 X-Y recorder or a Fisher (Springfield, NJ) Series 5000 Recordall strip chart recorder.

A flow-through cell (Fig. 1) with a BAS stainless steel auxiliary electrode block and Ag/AgCl (3 M NaCl) reference electrode was used. Platinized, wax-impregnated carbon rods were used as the working electrode. These electrodes were screwed into an electrode block (made in-house) that was mated to the BAS auxiliary electrode. A Struer (Denmark) polishing wheel was used for all electrode polishing.

Materials

Phosphate buffer (0·1 M) was prepared in distilled-deionized water using ACS certified potassium phosphate salts (Fisher, Springfield, NJ).

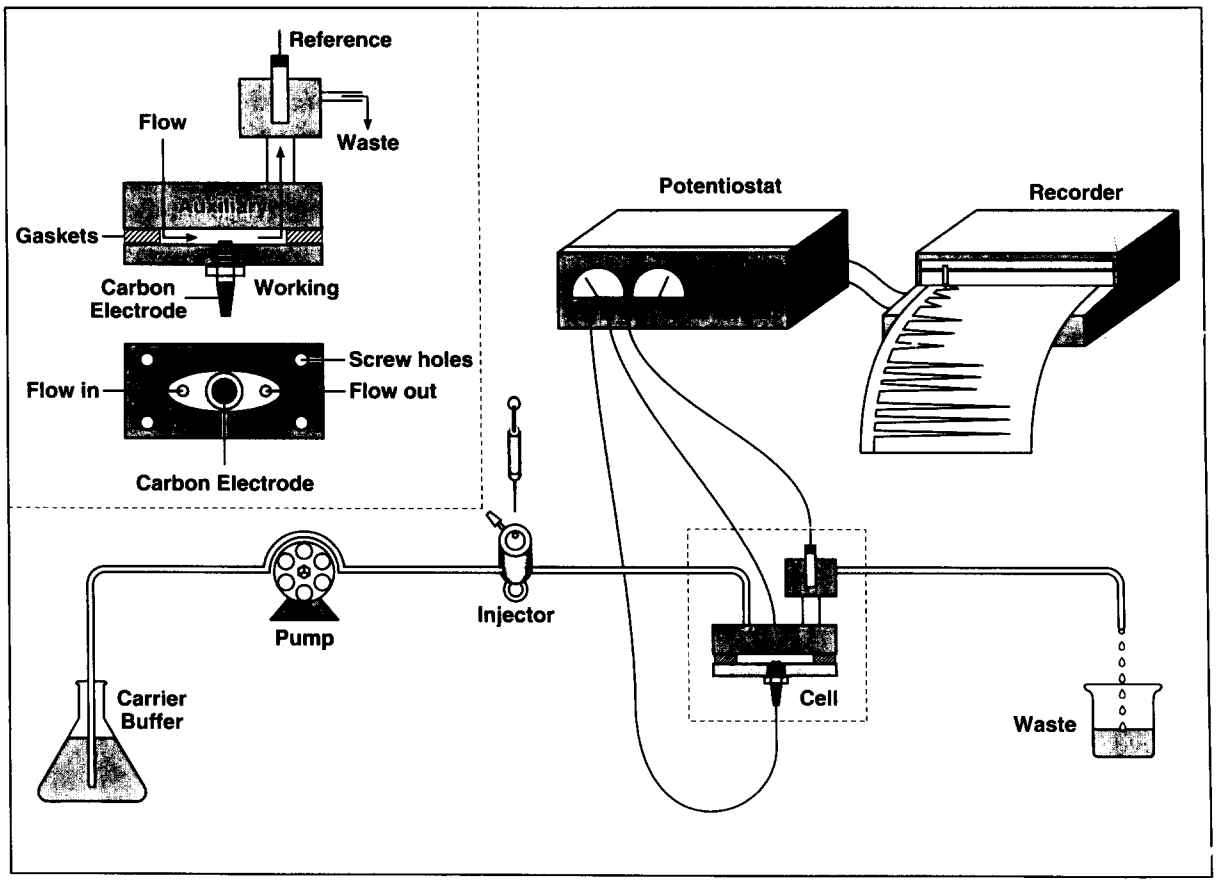

Fig. 1. Flow injection analysis system. Thin layer cell is shown in an expanded view. Carrier buffer: 0·1 M potassium phosphate buffer (pH 7·4) with 10 mM potassium ferricyanide. Injection volume: 20 μl. Flow rate: 1 ml/min. Cell volume: approximately 65 μl. Working potential: +0·70 V (vs Ag/AgCl, 3 M NaCl).

The pH was adjusted to 7·4 with concentrated potassium hydroxide. Phosphate buffer was prepared daily to minimize bacterial growth. Common anti-bacterial agents, *e.g.* EDTA, sodium azide, were not used because they are detrimental to galactose oxidase. The carrier buffer, used for all experiments, was phosphate buffer containing 10 mM potassium ferricyanide (ACS reagent, Fisher). Potassium ferricyanide was added to the buffer to keep the copper of galactose oxidase in the active +2 oxidation state. Hydrogen hexachloroplatinate (IV) hydrate (ACS reagent, Aldrich, Milwaukee, WI) was used for platinization. A 1 ml bed volume column with AG 501-X8 mixed bed ion exchange resin (Bio-Rad, Hercules, CA) was used for ion exchange experiments.

1,3-Diaminobenzene (99%), resorcinol (98%) (Aldrich), Nafion 5% solution (Aldrich), galactose oxidase (Boehringer-Mannheim, Indianapolis, IN), glutaraldehyde (Fluka, Ronkonkoma, NY), 4-acetamidophenol, ascorbic acid, and uric acid (Aldrich) were used as received. Stock solutions of galactose (250 mM, ACS reagent, Fisher) were allowed to mutarotate for 24 h at room temperature, and then divided into 5 ml aliquots and frozen at −20°C for storage. All standards were prepared from fresh dilutions of these stock aliquots. Spectroscopic carbon rods (diameter = 3·0 mm; Carbone, Bay City, MI), paraffin (Fisher), fittings (Alltech Assoc., Deerfield, IL), Epoxy 907 (Miller Stephenson, Danbury, CT) and alpha-alumina particle sizes 5·0 μm, 0·3 μm, and 0·05 μm (Buehler, Lake Bluff, IL) were used as received. Fresh frozen human plasma (NJ Blood Services, New Brunswick, NJ) was used as received. The anticoagulant used in the human plasma consisted of a 100 ml solution of: 2·63 g sodium citrate, 327 mg citric acid, 222 mg sodium biphosphate, 3·19 g dextrose, and 27·5 mg adenine. This solution was placed into a 500 ml blood bag prior to the

collection of whole blood and separation into plasma.

PROCEDURE

Working electrodes

Spectroscopic grade carbon rods were fixed with epoxy into Rainin end fittings; the epoxy was allowed to set as per manufacturers instructions. To decrease the porosity of the electrodes, they were placed in paraffin in a vacuum oven at 80°C for 12 h at approximately 120 mtorr. This decreases the electroactive surface area, lowering the background current and noise. The electrodes were allowed to cool, but were removed from the paraffin while it was still molten. The excess paraffin was removed from the electrodes after it had hardened. The electrodes were polished flush to the fitting with 600 grade Silicon Carbide paper; the electrodes were then polished with successively smaller diameter alumina on a velvet polishing cloth and sonicated in distilled/deionized water for 5 to 10 min to remove any loose carbon or alumina. Batches of three electrodes were electrochemically pretreated in parallel by applying ±1·53 V to the electrodes, starting with +1·53 V and ending with −1·53 V, for 5 min at each potential; this process was repeated once.

Electrochemical platinization was accomplished by placing the electrodes in a solution of 0·025 M hexachloroplatinate and using a constant current of −0·5 mA for 4 h. The solution was deaerated with nitrogen, before and during the plating. Approximately 20 ml of the plating solution was sufficient to platinize three electrodes. This process provided the electrode with a partial platinum surface, lowering the working potential required for the detection of H_2O_2. Furthermore, the platinized carbon electrode maintains some of the carbon surface which permits better enzyme immobilization than a bare platinum electrode. The electrodes were then electrochemically pretreated as before, except that the negative voltage was applied first and the positive last. Following this, the electrodes were ready for use.

Electrodes were modified with either poly(1,3-DAB/Res), Nafion and poly(1,3-DAB/Res), or Nafion alone. Electrodes were modified with Nafion by placing nine 5 μl aliquots of 1% Nafion (diluted 1:5 with methanol) onto inverted electrodes that had been previously cleaned with two 5 μl aliquots of methanol. Electrodes that were only modified with the electropolymerized films had methanol placed on them in the same manner as with the Nafion electrodes (*i.e.*, eleven 5 μl aliquots).

Electropolymerization

1,3-Diaminobenzene and resorcinol monomers, each at a concentration of 1·5 mM, were dissolved in deoxygenated phosphate buffer. The solution was used immediately after preparation, protected from light, and blanketed with nitrogen during the electropolymerization process. Three electrodes were modified in parallel. They were placed in the solution of monomers, and the potential was continuously cycled from +0·03 V to +0·83 V at 2 mV/s for 18 h. The electrodes were then tested for their response to interferents to ensure that the film had formed properly.

Interference testing

The modified electrodes were tested with interferents before and after polymer modification to determine their effectiveness in blocking interferents. The percentage of interferent blocked was determined by comparing the response to interferents of the modified electrode with its response before modification. Three electrodes were tested for each modification.

Enzyme immobilization

Galactose oxidase (GAO) was immobilized onto the polymer modified platinized carbon rods in the following manner. The electrodes were placed in a stirred solution of 1·25% glutaraldehyde for 1·0 h. The stirring was stopped, and the electrodes were allowed to soak in the glutaraldehyde solution for an additional 0·5 h; they were then rinsed with water and inverted. Successive 10 μl aliquots of enzyme solution (made by dissolving a bottle of GAO, 450 U, in 300 μl of distilled/deionized water) were then placed on each of three electrodes until all of the enzyme solution was used. The electrodes were allowed to dry at room temperature. Following this, a 10 μl aliquot of the 1·25% glutaraldehyde was placed on each electrode. The electrodes were again allowed to dry for 1·5 h. The electrodes were placed in the phosphate buffer overnight at 4°C, and then

rinsed with phosphate buffer to remove any loosely bound enzyme before use.

Recovery

Recovery of galactose was determined from human plasma samples. The fresh frozen human plasma was thawed at 37°C and then mixed. The plasma was centrifuged at 3000 g for 30 min. The supernatant was separated into 12 ml aliquots and frozen at −20°C until needed.

Galactose, in phosphate buffer, was added to human plasma in concentrations of 0·050, 0·10, 0·50, 1·00, and 2·00 mM. The stock galactose solution was diluted to varying concentrations so the volume of the galactose added to each of the plasma samples was constant.

Galactose standards, in phosphate buffer, were prepared in the same manner as the plasma samples. Recovery was calculated by comparing the response for galactose in plasma to the response for galactose in phosphate buffer at each concentration. The normal carrier buffer, phosphate buffer with 10 mM potassium ferricyanide added, was used for the recovery study.

RESULTS

Order of modification

The combination of Nafion with poly(1,3-DAB/Res) was carried out by sequential modification of the electrode with Nafion followed by poly(1,3-DAB/Res). This order was used because the electropolymerization of the DAB/Res monomers allows the monitoring of the electrode surface, ensuring the integrity of the film and complete coverage of the electrode surface with polymer. As shown in the cyclic voltammogram (Fig. 2), the electrode is completely covered when the current decreases to background levels. The reverse order of application (Moussy *et al.*, 1993), *i.e.* poly(1,3-DAB/Res) followed by Nafion, cannot be monitored to ensure the polymer integrity or complete coverage of the electrode. In addition, it has been shown that this approach suffers from a substantial interference from acetaminophen and the stability of the composite polymer is questionable (Moussy *et al.*, 1993).

Electropolymerization of 1,3 DAB and Resorcinol

The electropolymerization of 1,3-DAB/Res on the platinized carbon rod was essentially the same with (Fig. 2A) and without (Fig. 2B) Nafion present. For the Nafion modified electrode, the anodic peak was broader and the anodic current was less than that of a bare electrode, as would be expected. This lower current indicates that the electrode was modified by the Nafion and there was less electrode surface area available for the electropolymerization.

Operational characteristics

A typical calibration curve for galactose is shown in Fig. 3. The biosensors constructed had a good linear range from 0·050 to 6·0 mM. The minimum detectable limit (MDL) was defined as three times the noise. It was 50 μM on average, well below the maximum normal plasma galactose levels. The electrodes were tested in the batch mode to determine if the MDL could be made even lower. In the batch mode, the supporting electrolyte buffer was used to pretreat the human plasma samples, making them more compatible with the biosensor. Unfortunately, due to dilution of the sample when using a batch mode system, and the presence of $K_3Fe(CN)_6$ to keep the enzyme active, the MDL was not lowered.

Table 1 summarizes other operational parameters of the flow injection analysis galactose biosensor.

Interferences

Figure 4 shows the typical response of an electrode to interferents before and after modification with the composite polymer. Although an interference is seen for ascorbate, it should be noted that the level tested was five times that usually encountered in human plasma and the response was approximately 100 times less than the response at the bare electrode.

The composite polymer modified electrodes exhibit a better selectivity to H_2O_2 than those modified with only one polymer. Selectivity for hydrogen peroxide is defined as the response to 0·1 M H_2O_2 divided by the total response to all three interferents. As can be seen in Table 2, the signal of all three of the electrodes in the presence of interferents was decreased substan-

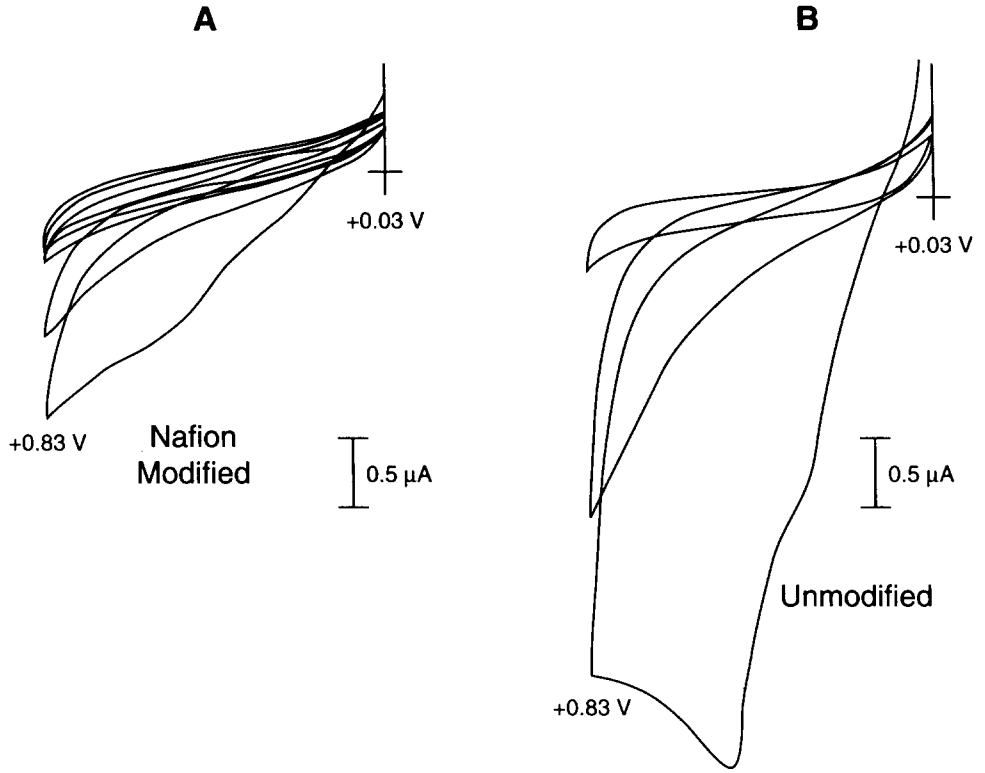

Fig. 2. Electropolymerization of 1,3-DAB and Res onto (A) a Nafion modified and (B) a bare electrode. Conditions given in text.

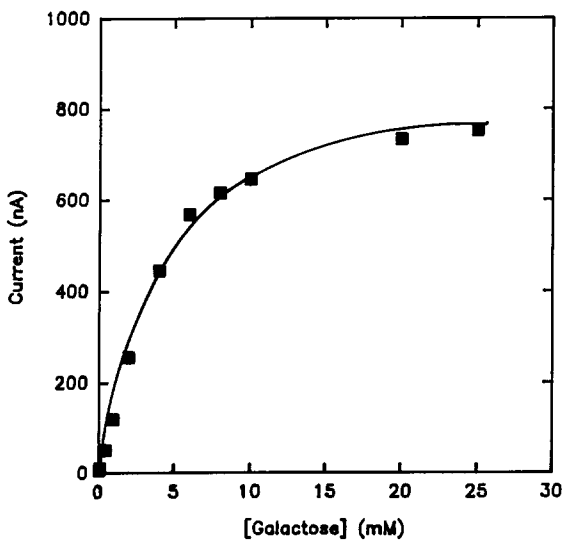

Fig. 3. Calibration curve of galactose in carrier buffer.

TABLE 1 Operational parameters of galactose biosensors (n = 3 electrodes).

Property	*Galactose biosensor*
Minimum detection limit (3 × noise)	50 μM
Linear range	0·05 to 6·0 mM
Coefficient of variation (0·5 mM: All)	<5%: <10%
Response time (95%)	18s
Sample throughput	120 samples/h
Recovery	104·7 ± 14·7%
Storage stability	> 30 days

tially after modification, but the composite polymer's selectivity for H_2O_2 was almost two times better than the other polymer modified electrodes.

The data in Table 2 were subjected to statistical tests to determine which modifications were significantly different. Levene's test showed no violation to our assumption of homogeneity of variances. This allowed us to do a post-hoc analysis of variance that showed the ability of 1,3-DAB/Res to block out ascorbate and urate was significantly lower than the Nafion and the composite modifications. Each of the modifi-

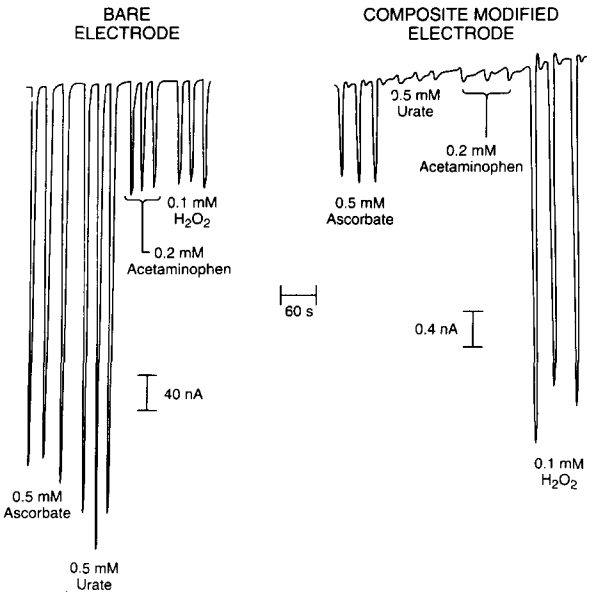

Fig. 4. Response to interferences before and after modification with composite polymer.

cations had a similar ability to block out acetaminophen. The composite had a significantly higher selectivity than the other modifications.

Plasma interference

The injection of plasma creates an interference (Fig. 5) that yielded the same current response as approximately 0·10 mM galactose in phosphate buffer. This response was similar, but in reverse, to that seen when phosphate buffer was injected. Removal of ferricyanide from the carrier buffer

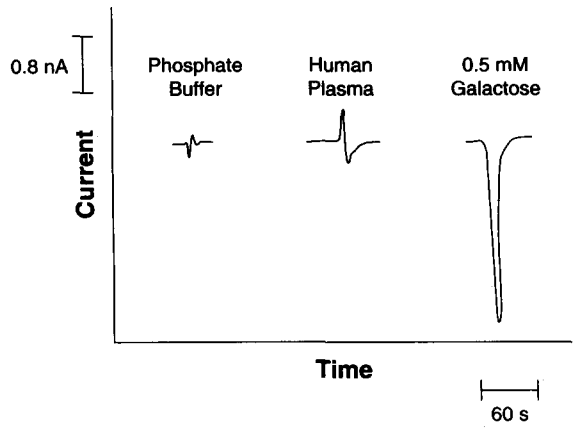

Fig. 5. Comparison of injections of 0·1 M potassium phosphate buffer, human plasma, and 0·5 mM galactose.

did not eliminate the injection interference for plasma, but did eliminate it for phosphate buffer.

This noise was not seen at bare electrodes, indicating that the noise was a result of a species in the plasma interacting with the polymer layer in some manner. All three modifications resulted in the same noise response, suggesting that the creation of a diffusional barrier on the electrode was the source of the problem. The following changes to the system did not eliminate the noise: different types of injectors; movement of the auxiliary electrode downstream; different potentiostats, and different reference electrodes. Therefore, the sample preparation was investigated to narrow down the problem.

Deproteinization with Centricon filtering (MWCO 30,000), addition of varying concentrations of sodium EDTA, ionic strength adjustment with a variety of salts, size exclusion separation, and changes to the composition of the carrier stream did not eliminate the noise. Dialysis (MWCO 3,500) and ion exchange with a mixed bed ion exchange column did eliminate the noise; this indicated that some lower molecular weight charged species was the cause of the noise. These pretreatments could not be used with samples containing galactose because the galactose would diffuse out of the sample in the former case and stick to the ion exchange resin in the latter, as evidenced by lower recoveries of galactose in phosphate buffer (data not shown). Therefore, the positive injection interference from plasma was subtracted from all of the galactose responses for the plasma samples.

Recovery

The average recovery was found to be 104·7 ± 14·7% (85–120%). The response to galactose in plasma was linear (r = 0·9992) and had a 22% greater slope than the response to the same levels of galactose in phosphate buffer (Fig. 6).

DISCUSSION

Galactose monitoring

The monitoring of galactose is important for the diagnosis of several disorders from galactosemia to liver dysfunctions. In neonates, the level of galactose in serum is usually less than 1·11 mM

TABLE 2 Comparison of individual polymers with the composite polymer. (n = 3 electrodes for each modification).

Electrode modification	*Percent of 0·5 mM ascorbate blocked*[b]	*Percent of 0·2 mM acetaminophen blocked*[b]	*Percent of 0·5 mM urate blocked*[b]	*Selectivity of 0·1 mM H_2O_2 over total interferences*[a]
Bare electrode	NA	NA	NA	0.123 ± 0.008
Nafion	99.71 ± 0.15[#]	99.60 ± 0.27	99.91 ± 0.06	2.37 ± 0.43
1,3-DAB/Res	85.40 ± 3.59	99.51 ± 0.20	99.33 ± 0.13	1.77 ± 0.58
Composite	99.82 ± 0.09	99.89 ± 0.07	99.97 ± 0.03	4.17 ± 0.51

[a]Response to 0·1 mM H_2O_2 divided by the total response to 0·5 mM ascorbate, 0·2 mM acetaminophen, and 0·5 mM urate.
[b]Percent blocked = 100 − (response at modified electrode/response at bare electrode) × 100
NA = not applicable
[#]Standard deviation (n−1)

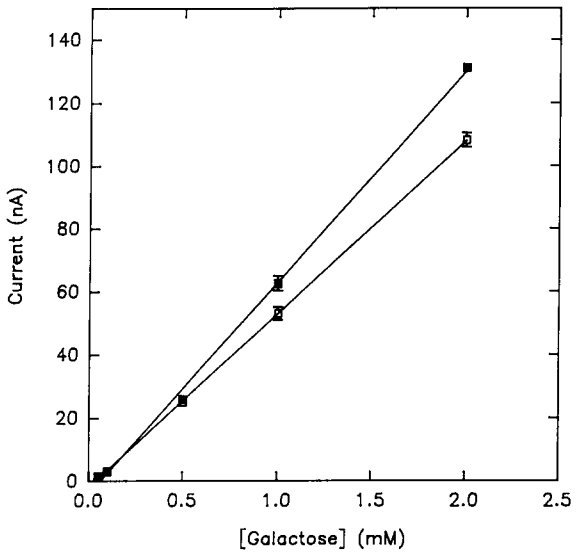

Fig. 6. Galactose response of samples prepared in phosphate buffer (□) and in human plasma (■).

(Tietz, 1990), but in galactosemic neonates the levels can rise to be much higher. If these elevated levels are not detected, death can occur in neonates.

In adults, fasting subjects, who are otherwise normal, usually have galactose levels that are less than 0·28 mM (Tietz, 1990). Galactose loading tests can raise the blood galactose levels of these individuals to greater than 5 mM in whole blood; normally these levels decrease quickly. In comparison, the level of glucose in the serum of individuals rarely drops below 3 mM or rises above 12 mM. Therefore, the detection of galactose in biological fluids covers a wide concentration range, with a large percentage of subjects having very low levels of galactose. A variety of techniques, *e.g.* spectrophotometric, microbiological, and fluorometric methods, have been used to detect galactose, each having distinct advantages and limitations. These have been reviewed (Segal, 1989).

The purpose of the present study was to develop a galactose biosensor that could encompass a larger linear dynamic range, as well as being able to operate at relatively low galactose concentrations for a majority of the samples tested. The operation of the galactose biosensor at these low levels of galactose creates the need for better polymer films to eliminate interferences that become a larger problem as the signal decreases near the minimum detection limit.

Several other researchers have constructed biosensors for the detection of galactose (Taylor *et al.*, 1977; Bertrand *et al.*, 1981; Fortier *et al.*, 1992). All of these biosensors are batch mode electrodes. These galactose biosensors have longer analysis times than the proposed flow injection analysis biosensor. The research by Bertrand *et al.*, (1981) and Fortier *et al.* (1992) did not evaluate the effects of interferents on the galactose response or detect galactose in physiological matrices. In addition, the biosensor proposed by Bertrand *et al.* (1981) did not have the linear range to encompass all of the physiological galactose concentrations.

The biosensor made by Taylor *et al.* (1977) was fully tested for selectivity, response to interferences, stability, and the detection of galactose in whole blood and plasma. It showed

excellent discrimination between interferents and galactose when potassium ferricyanide was present in the sample chamber (Taylor *et al.*, 1977). This excellent discrimination was seen because of the relatively thick membranes used, which were physically assembled onto the electrode surface. Such membranes are, however, limited to relatively large electrodes and simple electrode geometries. The thickness of these membranes leads to increased response times and the need for more frequent calibrations. The biosensor proposed here uses ultrathin membranes that are chemically applied to the electrode surface. These membranes decrease the response time and frequency of calibration, while still providing excellent discrimination from interferents. The increased flexibility of chemical construction methodologies allows for the application of the proposed composite to a large variety of complex surface geometries and to miniaturized electrodes (Stoecker & Yacynych, 1990).

Choice of polymers

Choosing the polymers with which to create the composite is important in determining the properties and effectiveness of the composite. A composite polymer was utilized because neither poly(DAB/Res) nor Nafion were as effective in eliminating the interferents relative to the signal levels of the analyte.

Geise *et al.* (1991) and Sohn *et al.* (1991) have shown that DAB and DAB/Res polymers are effective in decreasing interferents for glucose biosensors that operate at higher signal levels. These films were found to be less effective on the galactose biosensor because of operation at lower currents.

Palleschi *et al.* (1986) have stated that ". . . it [Nafion] did not eliminate, or selectively reduce, any of the interference, except possibly ascorbic acid. It is not recommended for practical use." The results presented here indicate that neither poly(DAB/Res) nor Nafion is completely effective by itself. However, the combination of these polymers creates a composite that is more effective than either single polymer.

Composite polymer

The combination of the two polymers most likely creates a unique composite polymer. Nafion, being non-conductive, does not allow the DAB/Res monomers to electropolymerize on top of itself. The DAB/Res monomers are electropolymerized at the electrode surface only in the pores of the Nafion layer. This creates a composite polymer that is a mix of Nafion and poly(DAB/Res).

The maximum thickness of the resulting composite is probably only as thick as the Nafion polymer because the non-conductive poly(DAB/Res) copolymer, which forms an ultrathin membrane at the electrode surface, is thinner than the Nafion.

Interferences

Table 2 compares the ability of the polymer films to exclude interferences. Other researchers using Nafion have found that the interference from acetaminophen and ascorbate is still present (Palleschi *et al.*, 1986; Harrison *et al.*, 1988; (Sanchez *et al.*, 1991; Navera *et al.*, 1993). The amount of interferents blocked from the electrode surface by all of the polymers was excellent. However, the polymers not only decreases the response to interferences, but also to the analyte, H_2O_2. Therefore the critical parameter in comparison of the electrodes is selectivity. The electrodes modified with single polymers were not as good in terms of selectivity as the composite modified electrode. This new composite decreases interference from acetaminophen, urate, and ascorbate over that of either individual polymer, as evidenced by the increased selectivity for H_2O_2 (Table 2).

The fact that the percentage of interference blocked by the films does not significantly change between the different polymer modifications means that the use of selectivity becomes an increasingly important parameter. The H_2O_2 response divided by the total interference response shows a much larger difference between modifications, clearly indicating the composite to be an improved protection against interferences. The response to H_2O_2 rather than the analyte, galactose, was used because it is a consistent standard that is not dependent on enzyme activity.

In Fig. 4, the absolute response to the interferents is shown before and after modification with the composite polymer. Although there is a response to ascorbate, it should be noted that the maximum physiological (brain) concentration of 0·5 mM was used rather than the maximum blood concentration of approximately 0·1 mM. In

addition, the current generated by the interferents was at least 100 times less for the modified electrode as compared to the bare electrode. The advantage of using the selectivity for the evaluation of polymer modifications is shown in Fig. 4 by the larger response to H_2O_2 at the modified electrode, on comparison with the interferences, than at the bare electrode.

Operational characteristics

As previously stated, the levels of galactose in normal human serum is quite low, requiring a low minimum detection limit. The galactose biosensor has a MDL of approximately 50 μM. While this is well below the maximum cut off for normal plasma galactose, an even lower MDL would be helpful in quantitating normal plasma galactose levels. Although the galactose biosensor was used in a flow injection analysis system, it is very versatile and could be removed from the housing to perform as a batch mode biosensor. There was no significant lowering of the MDL for the biosensor when used in this mode.

Other operational parameters for the flow injection analysis system are summarized in Table 1. All of these characteristics are well within the normal parameters for flow injection analysis biosensors. The response time and sample throughput are excellent, and linear dynamic range encompasses most of the plasma samples that would be encountered.

Plasma interference

Other researchers have noted similar types of injection interferences when detecting analytes in samples with complex matrices (Lundback & Olsson, 1985; Olsson *et al.*, 1985). It is our belief that this interference (Fig. 5) is created by an ionic strength difference between the sample and the carrier buffer, coupled with the diffusional barrier created by the composite polymer. This response is similar to that seen with human serum samples.

The interference was constant and could be quantified. It was also small and had little effect on the response to galactose. Therefore, the positive interference was subtracted from the galactose spiked plasma samples to determine the response to galactose.

Recovery and plasma galactose response

The average recovery of galactose in plasma was 104·7% with a range from 85 to 120%. The magnitude of this range is similar to that (51–100%) reported for the galactose biosensor evaluated by Buffone *et al.* (1980). The difference seen between the slope of response to galactose in plasma compared with that to galactose in phosphate buffer, may have been due to a sample matrix interacting with the enzyme or changing galactose mass transport to the enzyme. A galactose sample in phosphate buffer was injected after the plasma samples;this resulted in a 15% increase in response over the same sample run before the plasma samples. Therefore, it is probable that the sample matrix was affecting the enzyme. This difference in the slope of galactose response in plasma has been reported by other researchers (Taylor *et al.*, 1977).

CONCLUSION

The use of composite polymers to improve the protection of electrodes from electroactive interferents such as ascorbate, urate, and acetaminophen, has been demonstrated. The composite polymers consist of a non-conducting copolymer of diaminobenzene and resorcinol and an anionic polymer of Nafion. The inclusion of the composite polymers facilitated the development of a sensitive galactose biosensor for the analysis of samples with complex matrices. These biosensors have a linear range from 50 μM to 6·0 mM, and a storage stability of one month. These biosensors demonstrated excellent recovery of plasma galactose.

ACKNOWLEDGEMENT

The authors would like to acknowledge Dr Gad Avigad, Dr Stanley Stein, Dr Ronald Poretz, and Henry Lackland for their helpful suggestions. This work was supported by grants from the UNICO Foundation and the National Institute on Alcohol Abuse and Alcoholism Grant No. R01-AA07799.

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