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# QUANTITATIVE ANALYSIS OF PERFORMANCE OF A HYDROGEN PEROXIDE-BASED GLUCOSE BIOSENSOR

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**Abstract.** A partial glucose biosensor was constructed using a selective internal layer, an enzyme layer and a gold electrode. Both membrane layers are based on cross-linked polyvinyl alcohol (PVA). The performance of the partial glucose biosensor was analyzed particularly with respect to the interfering effects of acetaminophen, a non-ionic molecule. The sensitivity of the immobilized enzyme to glucose was high but it was not high enough to counter acetaminophen interference. The addition of the moderately selective PVA internal layer did not seem to have a significant effect on interference. A mathematical model was then used to analyze the performance of a completed biosensor. A simulated external layer was added to the two layers. The mathematical model predicted that the addition of an external layer with lowered permeability to solutes could improve the performance of the sensor.

*Keywords:* Glucose biosensor; mathematical modeling; acetaminophen; electrochemical interference; cross-linked polyvinyl alcohol

**Abstrak.** Satu biosensor glukosa separa telah dibina menggunakan satu lapisan dalaman yang selektif, satu lapisan enzim dan satu elektrod emas. Kedua-dua lapisan membran tersebut adalah berasaskan polivinil alcohol (PVA) tersambung silang. Prestasi biosensor glukosa tersebut telah dinilai terutamanya prestasi terhadap gangguan asetaminofen, yang merupakan molekul tak ionik. Sensitiviti enzim tersekat gerak tersebut terhadap glukosa adalah tinggi tetapi ianya tidak mencukupi untuk melawan gangguan asetaminofen. Kehadiran lapisan dalaman PVA yang hanya sederhana selektif tidak menyebabkan kesan yang ketara pada gangguan. Satu model matematik kemudiannya digunakan untuk menganalisis prestasi biosensor yang lengkap. Satu lapisan luar ditambah secara simulasi kepada dua lapisan terdahulu. Model matematik tersebut meramalkan bahawa penambahan satu lapisan luar yang mempunyai kebolehtelapan yang rendah terhadap bahan larut mampu memperbaiki prestasi sensor.

*Kata kunci:* Biosensor glukosa; model matematik; asetaminofen; gangguan elektrokimia; polivinil alkohol tersambung silang

## 1.0 INTRODUCTION

Amperometric glucose biosensors are usually based on the detection of hydrogen peroxide due to its simple configuration which would facilitate miniaturization. However, this type of glucose sensor is usually affected by electrochemical interference from oxidizable chemical species such as ascorbic acid, uric acid and acetaminophen [1-9].

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Permeability and selectivity of the inner selective layer of a biosensor play a very important role in interference elimination, thus utmost care must be exercised when determining the values. Abdul-Aziz *et al.* [10] has shown that the material for the working electrode must be carefully selected to ensure that the method used in determining membrane permeability and selectivity would be effective. Although platinum has always been the common choice as the electrode material, gold was found to be more suitable for this application.

Numerous membranes have been investigated for their potential ability to repel ionic electroactive interferents [2–9]. To repel non-ionic compounds such as acetaminophen, cross-linked polyvinyl alcohol (PVA), a non-toxic water soluble synthetic material, is a potential selective inner membrane. By controlling the cross-link density of PVA, a variety of transport properties can be obtained [11]. Polyvinyl alcohol has been found to show moderate selectivity to peroxide over acetaminophen. If coupled to an external membrane with suitable characteristics and an enzyme layer with high activity, a glucose sensor with acceptable performance could be realized.

The development of an active glucose oxidase (GOD) membrane is also crucial in the determination of sensor performance. Poor enzyme immobilization results in significant loss of enzyme activity. A sensor incorporating an inefficient glucose oxidase membrane would suffer from low current response to glucose, which will exacerbate the problem of electrochemical interference. Various polymers have been investigated as potential enzyme immobilization matrix [2–9]. As a hydrogel, PVA is a very attractive matrix for enzyme immobilization. Azila *et al.* [12] immobilized glucose oxidase in cross-linked PVA with a variety of cross-linking densities and a range of enzyme activity was obtained. A moderate cross-linking density was found to be a favorable environment for the immobilized enzyme. The magnitude of the signal generated by the immobilized enzyme was rather high and the enzyme was also stable over time.

The ultimate goal of the development of a peroxide-based amperometric glucose sensor is the construction of a sensor with better resistance to electrochemical interference. Usually, membrane performance is only inferred from the overall outcome of a biosensor, however, a mathematical model has been developed to provide insight into the parameters that can be exploited to improve sensor performance [1]. Interestingly, the model predicted that permeability and selectivity of both the external and internal layers could be manipulated to create a sensor with excellent properties.

In this work, the performance of a glucose sensor described by Abd. Aziz [1] was analyzed quantitatively. A partial glucose sensor was fabricated by sequentially adding an internal membrane and an enzyme membrane to a gold electrode. The sensor was then exposed to glucose and acetaminophen. Concurrently, a mathematical model of the partial glucose sensor was also generated using the transport properties obtained during the course of this work and the numerical results were compared to the experimental values. A hypothetical external membrane was then added to the assembly and the performance of a whole glucose sensor was predicted using the model.

## 2.0 MATERIALS AND METHOD

### 2.1 Materials

Glucose oxidase (EC 1.1.3.4, type X-S, 245 900 units/g solid and D-glucose (from corn sugar) were obtained from Sigma Chemical Co (Missouri, USA). Polyvinyl alcohol, 99+% hydrolyzed with an average molecular weight of 89,000-98,000 and 4-acetamidophenol (acetaminophen) with 98% purity were obtained from Aldrich Chemical Co (Missouri, USA). Sodium phosphate monobasic and sodium phosphate dibasic were from Fisher Scientific (Penssylvania, USA). All chemicals were used as received.

### 2.2 Instrumentation

For the amperometric experiments, an EG&G Princeton Applied Research Company Rotor Model 616 (Massachusetts, USA) was used with an EG&G Princeton Applied Research Scanning Potentiostat model 362 (Massachusetts, USA). For data collection, the system was equipped with 2 Keithley 175 Autoranging Multimeters (Ohio, USA) along with a Linseis Ly1400 X-Y Recorder (Linseis, USA). A conventional three-electrode electrochemical cell was employed. The counter electrode consisted of a platinum mesh from Alpha (99.99%) (Texas, USA) and a saturated calomel electrode (SCE) from Cole Parmer (Illinois, USA) was used as the reference electrode. The working electrode was gold with an active area of 19.63 $mm^2$.

### 2.3 Electrochemical Measurements

Glucose stock solution (0.25 M) was prepared by dissolving the appropriate amount of glucose in 0.1 M phosphate buffer, pH 6.7. The solution was then allowed to mutarotate overnight at room temperature before being used in the amperometic experiments. Unused glucose solutions were kept at 4°C for a maximum of 2–3 days. 10 mM acetaminophen in 0.1 M phosphate buffer was prepared fresh on the day of the experiment.

Electrochemical measurements were conducted to measure the effect of acetaminophen on the performance of a partial glucose sensor consisting of a PVA-GOD layer and an internal layer. The PVA-GOD layers used in the experiments were those in which the enzyme was immobilized in the PVA matrix with cross-linking ratios of 0.06 and 0.1 [12]. The internal layer was prepared according to the method by Abdul-Aziz *et al.* [11] where a base PVA layer with a cross-linking ratio of 0.03 was further reacted with 2.5% glutaraldehyde for 17 minutes. The PVA-GOD layers were prepared as unsupported layers whereas the internal layer was prepared directly on the electrode.

A PVA-GOD membrane was placed on top of a gold electrode coated with an internal PVA layer and gauze and rubber rings were used to secure the components

in place. The electrochemical measurements were performed by immersing the electrode in stirred 0.1 M phosphate buffer, pH 6.7 and applying a constant potential of 950 mV (vs. SCE). Following background current stabilization, an appropriate amount of the stock glucose solution was injected into the cell to give a pre-selected concentration. After the glucose-induced current had reached steady state, an appropriate amount of acetaminophen was introduced into the cell to make a 0.2 mM acetaminophen solution. Acetaminophen-induced current was also measured using just the enzyme layer or the bare gold electrode for comparison purposes. All measurements were done at 25 °C.

### 2.4 Permeability Determination for PVA-GOD Layer

PVA-GOD layers with a cross-linking ratio of 0.06 were chosen for these experiments. The enzyme layer was prepared as a loose layer, which was secured in place using gauze and rubber rings. Voltammograms for the oxidation of 1 mM hydrogen peroxide and 1 mM acetaminophen were recorded by scanning from 200 mV to 1150 mV (vs. SCE) at a scan rate of 1 mV/s. The rotation rates were randomly varied between 50 to 200 rpm for each set of experiment. All measurements were done at 25 °C.

## 3.0 RESULTS AND DISCUSSION

### 3.1 Effect of Acetaminophen on a Two-layer Sensor

Acetaminophen has always been considered as one of the most difficult electrochemical interferents for a peroxide-based glucose sensor due to its non-ionic nature. The fact that its molecular weight is only approximately 4 times that of peroxide complicates the matter further. In this work, a partial glucose sensor consisting of an enzyme layer and an internal layer, both based on PVA, was challenged with glucose and acetaminophen. The concentration of acetaminophen used (0.2 mM) corresponds to the higher end of the range that might be experienced following the maximum therapeutic dose of 1000 mg. Figure 1 shows the effect of acetaminophen on the typical response of a partial sensor, which consists of a PVA-GOD layer where the cross-linking ratio of the matrix was either 0.06 or 0.1 with the same internal PVA layer for each case. The introduction of a PVA internal layer, with a selectivity of 4 to peroxide, did not seem to have a significant effect on the ability of the sensor to withstand the onslaught of acetaminophen interference. Simplifying the definition of percent error as the percent of glucose-induced current that results in response to acetaminophen, electrochemical oxidation of 0.2 mM acetaminophen resulted in approximately 147% error and 691% error relative to the response from 5 mM glucose for the case of the sensor where the cross-linking ratio of the enzyme matrix was 0.06 and 0.1, respectively. When glucose concentration was increased to 20 mM, a value that can be experienced by a diabetic during a hyperglycemic episode, the error was reduced to roughly 65%

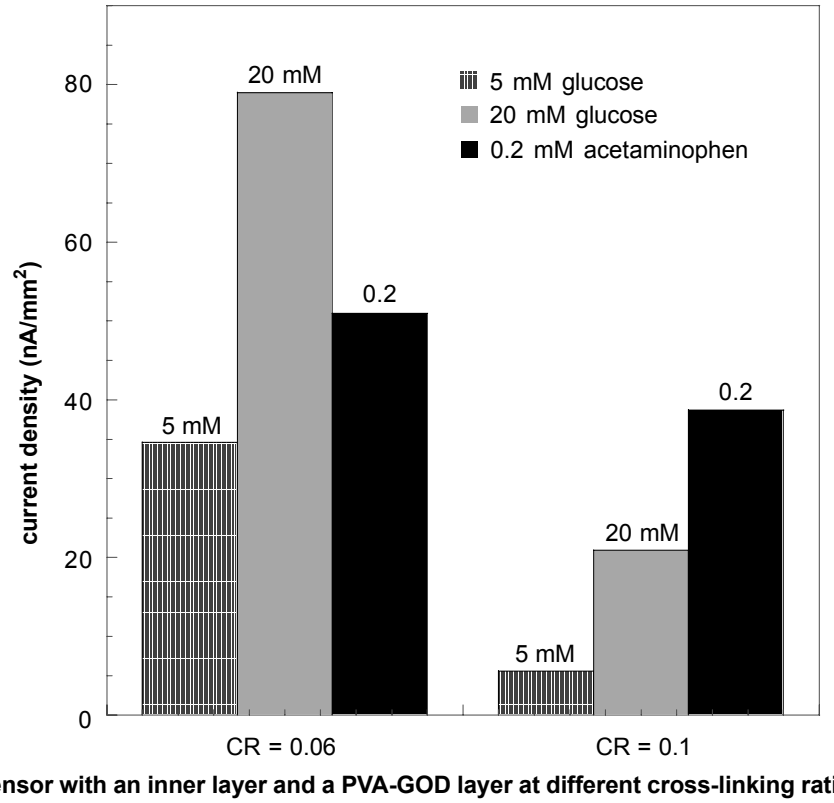


**Figure 1** The effect of acetaminophen on the performance of a two-layer sensor comprising an enzyme layer and an inner layer

and 185%, respectively. The reduction in error is due to the larger amount of peroxide generated by the enzyme layer during the oxidation of 20 mM glucose. Moatti-Sirat and co-workers [2] reported a 76% error (using the definition of error in this work) in the *in vitro* current readings for 5 mM glucose resulting from 0.2 mM acetaminophen. The needle-type glucose sensor that they used consisted of a polyurethane outer layer and a cellulose acetate-GOD layer. Comparing the value of the reported error to the values obtained in this work, it can be hypothesized that the presence of an external layer and the selectivity of cellulose acetate helped in reducing the error. An external layer can increase sensitivity of a sensor to glucose by limiting the amount of peroxide that diffuses out into the bulk solution [1].

One explanation for why the problem of electrochemical interference is so prevalent in enzymatic sensors is poor sensitivity of the sensor to glucose. Immobilizing the enzyme results in conformational changes that reduce the amount of active enzyme. The needle-type sensor used by Moatti-Sirat, *et al.* [2], had a sensitivity of 1–2 nA/mM glucose in the linear region of the calibration curve. The PVA-GOD layers with cross-linking ratios of 0.06 and 0.1 used in this work had sensitivities of 4.9 + 0.45 nA/

(mM-mm$^2$) and 1.41 ± 0.54 nA/(mM-mm$^2$) glucose in the linear region of the calibration curve, respectively. Figure 2, which compares the current obtained from the oxidation of acetaminophen on a bare gold electrode with the current obtained when the electrode is coated with the enzyme layer or two layers comprised of the enzyme and an internal layer, supports this hypothesis. The current from acetaminophen was reduced more than 90% by the addition of the additional layer(s). However, this reduction in error was obviously not enough to curb acetaminophen interference due to the poor sensitivity to glucose.

The PVA-GOD layer with a cross-linking ratio of 0.1, despite having a lower sensitivity to glucose, had better resistance towards acetaminophen interference than the one with a cross-linking ratio of 0.06 due to a lower permeability to solutes as evident by its low water content (60.1% compared to 66.3%) [11].

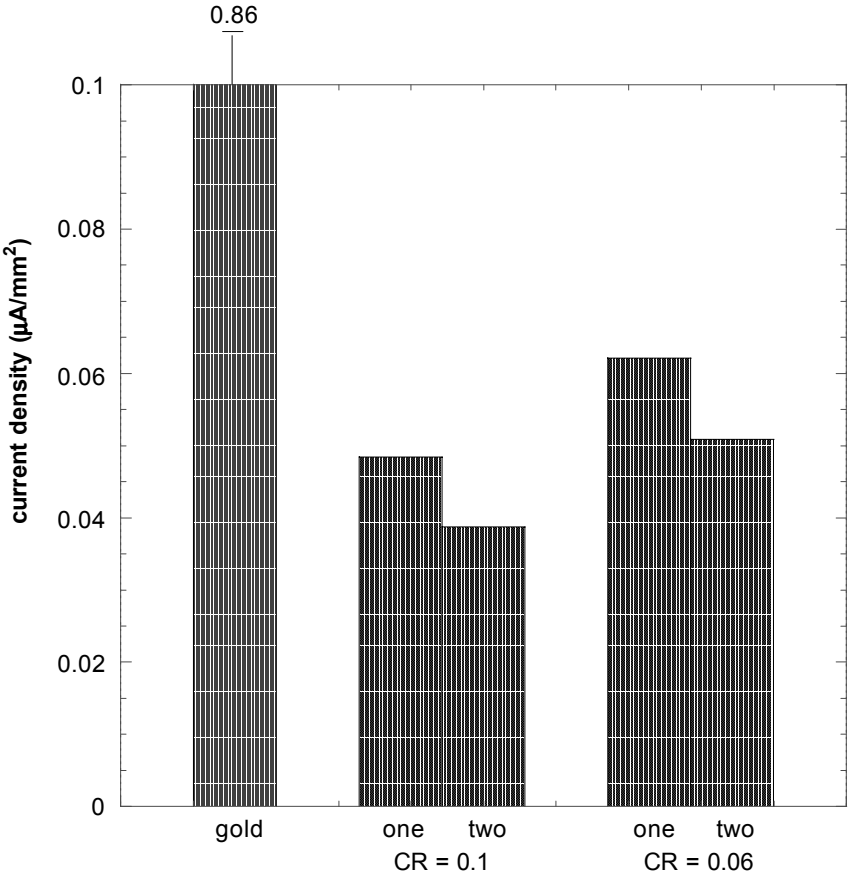


**Figure 2** Comparison of the oxidation of acetaminophen on a bare gold electrode with a gold electrode coated with just the enzyme layer (one) or both the enzyme and inner layers (two)

### 3.2 Mathematical Simulation

Mathematical simulations were performed using the transport properties and kinetic constants obtained from previous works [3, 4]. The mathematical model is described elsewhere [1]. $K_m^{app}$ was selected to be 13 mM, and the concentration of the enzyme was adjusted accordingly. The internal layer chosen was the PVA base layer with a cross-linking ratio of 0.03 that was further reacted with 2.5% glutaraldehyde for 17 minutes [11]. For the enzyme layer, the voltammetric experiments conducted using a rotating disk electrode for the PVA-GOD layer with a cross-linking ratio in the matrix of 0.06 yielded a peroxide permeability of $3.8 \times 10^{-06}$ $cm^2/s$ and a selectivity of 2 [12]. Figure 3 compares the theoretical transient response of the PVA-GOD layer alone to 5 mM glucose to a typical experimental response. The model prediction suggests that the activity of the immobilized enzyme was significantly less than the 1% used in the simulation [1]. The major difference between the two is the response time. The model predicted a smaller response time (90 s) compared to the one obtained experimentally (200 s). This can be partly attributed to ineffective mixing of the sample. For the simulation, when glucose was added to the system, the change in concentration was

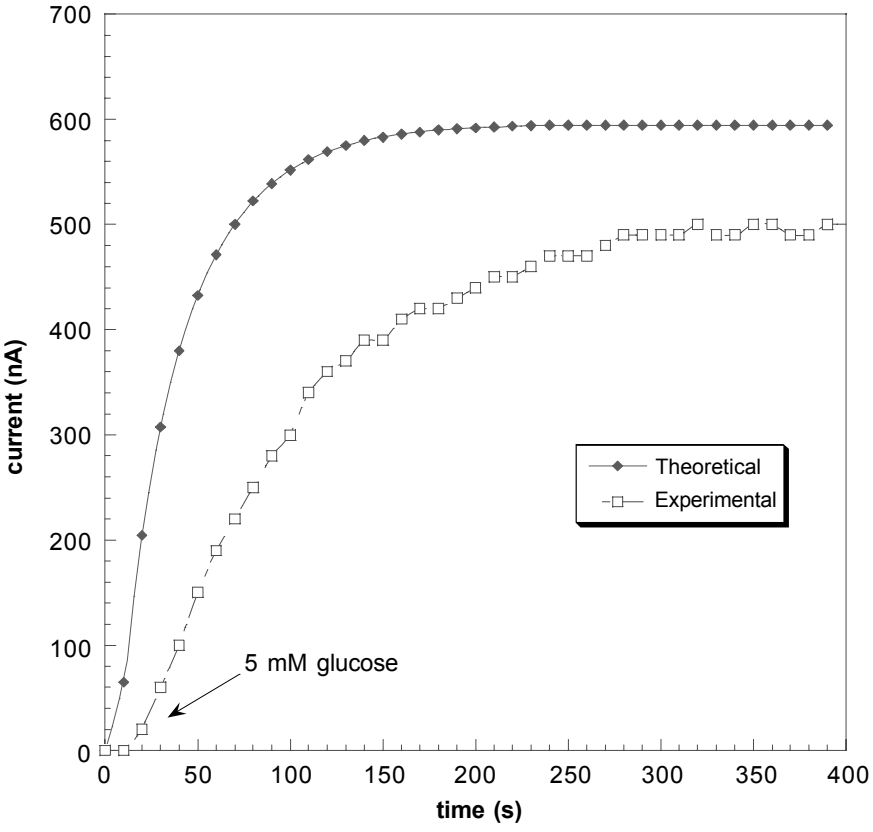


**Figure 3** Transient response a PVA-GOD layer to 5 mM glucose

assumed to be instantaneous. Experimentally, there would be a lag in the change in concentration, and with effective mixing, this lag could be minimized. Another possible reason for the disparity in response time is the absence of the effect of oxygen in the simulation. In the model, the effect of oxygen was neglected and the enzymatic reaction was assumed to follow the classical Michaelis-Menten kinetics for a one-substrate system. In fact, the enzymatic reaction follows the ping-pong mechanism for two substrates: glucose and oxygen [13]. Without an external layer selective for oxygen over glucose, the enzyme reaction will not be glucose limited.

Figure 4 shows the transient response of a typical partial glucose sensor to 5 mM glucose, 20 mM glucose and 0.2 mM acetaminophen. The experimental values were compared to the transient values predicted by the simulation. Here the model underestimated the value of both the current response and the response time for both glucose and acetaminophen. With respect to glucose, the explanations above for the PVA-GOD layer still apply. For acetaminophen, one possible explanation is that a rapid

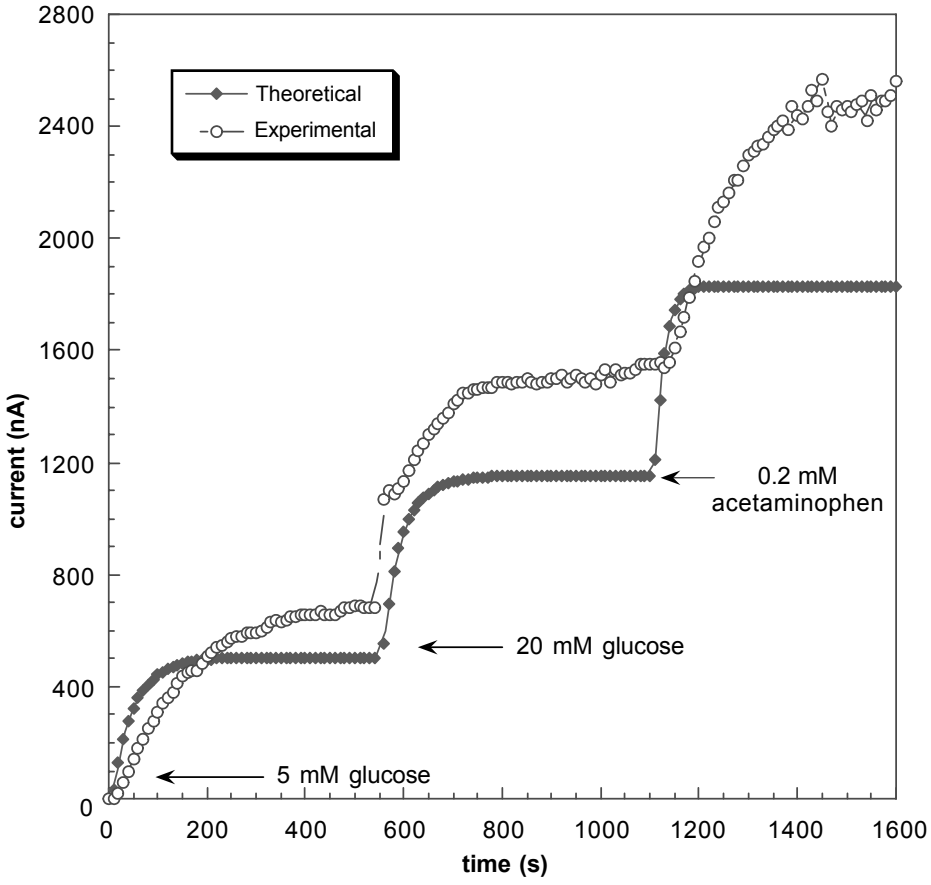


**Figure 4** Transient response of the partial sensor to glucose and acetaminophen

catalytic reaction occurs between the reduced flavin center of glucose oxidase and the electro-oxidized acetaminophen. This possibility had not been considered in the previous studies of acetaminophen interference on peroxide-based sensors. Moore *et al.* [14] reported this finding in 1994 and claimed to be the first group to study the previously unknown reaction between glucose oxidase and acetaminophen. This scenario was not considered in the mathematical model. In the model, acetaminophen was assumed to only diffuse through the system. The enzymatic reaction involving acetaminophen may account for the slow observed response time of the partial sensor to acetaminophen.

The model predicted the interference caused by acetaminophen to 5 mM glucose and 20 mM glucose to be approximately 134% and 59%, respectively, whereas the experimental values were 147% and 65%. These values are in good agreement with each other, indicating that even though the model did not accurately predict the response time of the sensor, the response current was predicted fairly accurately considering the simplicity of the mathematical model.

The results presented in this study indicate that the moderately selective polyvinyl alcohol internal layer, by itself, was not effective in reducing the error caused by acetaminophen. The model predicted that the properties of the outer layer could also be manipulated to improve the performance of the sensor [1]. Here, the effect of a hypothetical external layer on the performance of the partial sensor is presented. However, only the permeability of the outer layer was varied. The selectivity of the outer layer was not varied as high selectivity can have a counter-productive effect on the 3-layer sensor [1]. Figure 5 shows the effect of the permeability of the hypothetical external layer on the response time and the percent error of the sensor. The following parameters were used for the simulation:

For the enzyme layer: $D_{P2} = 3.8 \times 10^{-06}$ cm$^2$/s; $D_{A2} = D_{S2}$; $\sigma = 2$; thickness = 150$\mu$.
For the inner layer: $D_{P3} = 2.9 \times 10e^{-06}$ cm$^2$/s, $\sigma = 4$; thickness = 20$\mu$.
For the outer layer: $D_{P1}$ was varied such that $D_{P3}$ was 1 to 30 times greater than $D_{P1;}$ $\sigma = 4$; thickness = 20$\mu$.

Reducing the permeability of the outer layer can reduce the interference experienced by the partial sensor to a very low value. However, as the enzyme layers were quite thick, a significant increase in response time accompanies the reduction in percent error. As a comparison, the interference resulting from 0.2 mM acetaminophen was reduced to 24.4% for a 3-layer glucose sensor utilizing an internal layer comprising a composite of Nafion and cellulose acetate [3]. The resulting response time (time to reach 90% steady-state current) was only 216 ± 60 seconds.

Since the thickness of the enzyme layers analyzed during the course of this work were not optimized, the mathematical model was utilized to investigate the effect of the permeability of a hypothetical external layer for the case where the thickness of the

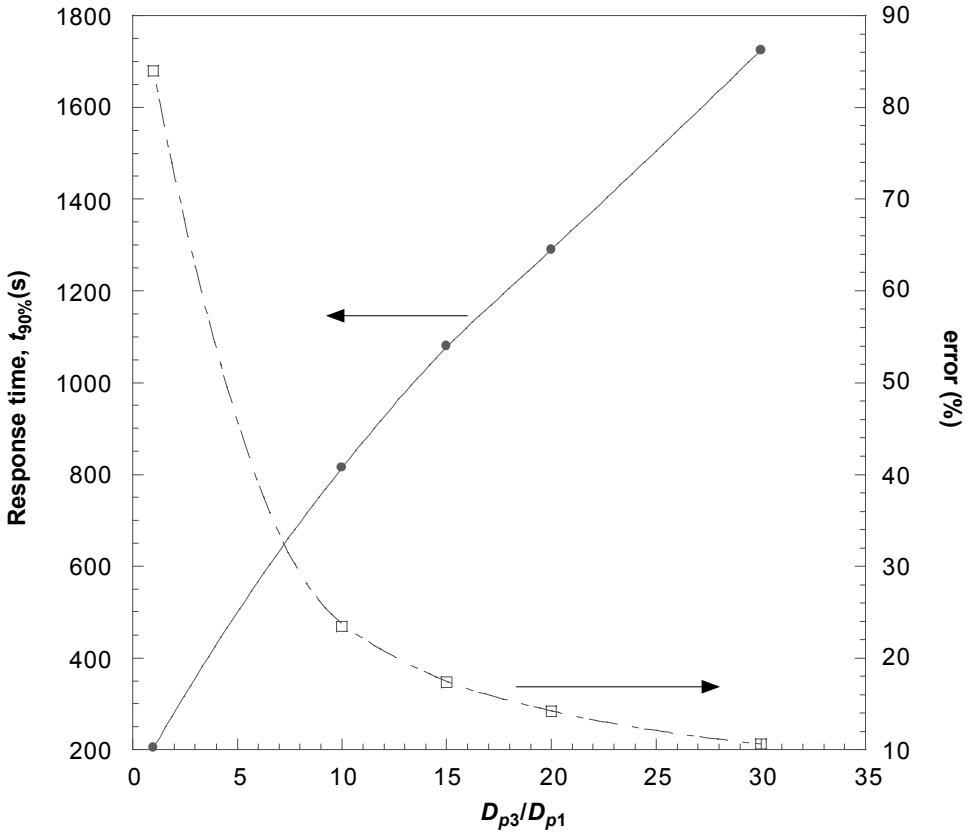


**Figure 5** The effect of permeability of the outer layer on the response time and % error of a glucose sensor. The thickness of the enzyme layer is 150 microns and the thickness of the outer and inner layers is 20 microns

enzyme layer was reduced to 50 microns. The thin enzyme layer was assumed to possess the same available activity as the thicker one. Figure 6 shows the effect of the permeability of the external layer on the performance of this modified sensor. The same extent of error reduction was observed as before (Figure 5). However, even though the response time decreases with the decrease in permeability, the magnitude of the response time was much smaller than the previous case. A glucose sensor with acceptable performance can be realized if a compromise between response time and percent interference is reached.

## 4.0 CONCLUSIONS

In this work, the performance of a partial sensor constructed from internal and enzyme layers was analyzed. Even though the sensitivity of the enzyme layer to glucose was quite high compared to the values reported in literature, it was still not high enough to counter acetaminophen interference. The addition of a moderately selective PVA

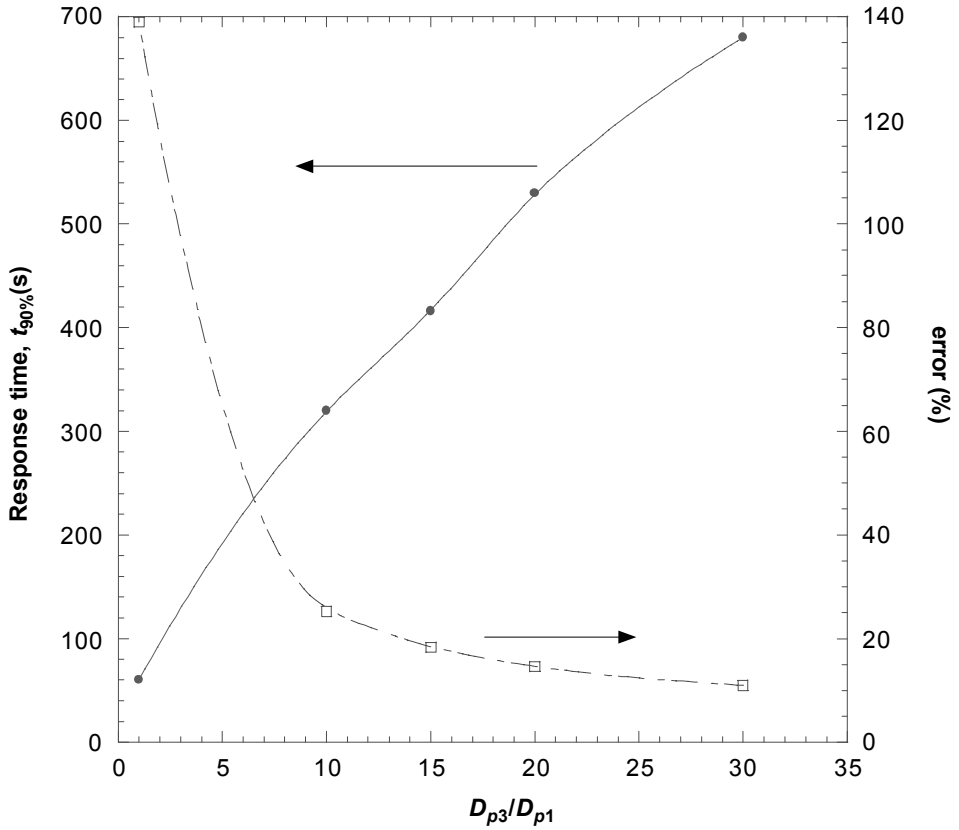


**Figure 6** The effect of permeability of the outer layer on the response time and % error of a glucose sensor. The thickness of enzyme layer is 50 microns and the thickness of the outer and inner layers is 20 microns

internal layer did not have a significant effect on interference. However, the mathematical model predicted that the addition of an external layer with lowered permeability to the solutes could improve the performance of the sensor. Highly cross-linked PVA with a very small amount of water content can be a potential candidate for this external layer. Poly(2-hydroxyethyl methacrylate) (pHEMA), a biocompatible hydrogel, can also be considered as a likely candidate. pHEMA membranes have been shown to have greatly reduced effective diffusivities for creatinine, a molecule similar in size to glucose. Values for creatinine in the range of $1.1 \times 10^{-07}$ to $9 \times 10^{-07}$ $cm^2/s$ have been reported[15]. These values could be further reduced by increasing the amount of ethylene glycol dimethacrylate (EGDMA), which acts as a cross-linker during the polymerization of the HEMA monomer.

The model and experimental data suggest that membrane configuration of the inner and outer layers can be engineered to address the issues of electrochemical interference. However, the sensitivity of the enzyme layer to glucose seems to be a greater concern here. Even though the sensitivity of the PVA-GOD to glucose was

relatively high, the model predicted that less than 1% of the immobilized enzyme was active. To effectively reduce electrochemical interference, there is a pressing need for an efficient immobilization technique that will yield an enzyme layer with a heightened sensitivity to glucose.

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