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SYSTEMATIC SELECTION OF MATERIAL FOR AN AMPEROMETRIC GLUCOSE BIOSENSOR

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Abstract. The internal selective membrane for an amperometric glucose biosensor was selected quantitatively based on its transport properties. A rotating disk electrode was used to obtain the permeability of acetaminophen, a common interferent for peroxide-based glucose biosensor, and hydrogen peroxide through cross-linked poly(vinyl alcohol) (PVA). All diffusion experiments were performed using a gold rotating electrode as platinum electrode exhibited slow electron transfer on its surface during the oxidation of acetaminophen, thus rendering it ineffective for this work. Selectivity of cross-linked PVA to peroxide was based on the permeability of peroxide compared to that acetaminophen. As hydrogen peroxide is small, its permeability was only affected when the mesh size of cross-linked PVA was significantly reduced. The permeability of the bigger acetaminophen, on the other hand, was linearly proportional to the mesh size of the cross-linked PVA. Cross-linked PVA membrane was found to display marginal selectivity towards peroxide.

Keywords: Glucose biosensor; interference; acetaminophen; permeability; cross-linked poly(vinyl alcohol)

Abstrak. Membran dalaman yang selektif bagi biosensor glukosa amperometrik telah dipilih secara kuantitatif berdasarkan kepada sifat pengangkutannya. Disk elektrod yang berputar digunakan untuk mendapatkan kebolehtelapan asetaminofen, satu penganggu yang biasa bagi biosensor glukosa berdasarkan peroksid, dan hidrogen peroksid melalui poli(vinil alkohol)(PVA) tersambung silang. Kesemua amali resapan dilakukan menggunakan elektrod berputar emas kerana elektrod platinum mempamerkan perpindahan elektron yang perlahan pada permukaannya ketika oksidasi asetaminofen yang menyebabkan ia tidak sesuai digunakan. Kememilihan PVA tersambung silang kepada peroksid adalah berasaskan kebolehtelapan peroksid berbanding keboletelapan asetaminofen. Peroksid mempunyai saiz yang kecil. Oleh itu kebolehtelapannya hanya terkesan bila saiz jejaring PVA tersambung silang dikurangkan dengan ketara. Keboletelapan asetaminofen yang bersaiz lebih besar pula adalah berkadar terus dengan saiz jejaring PVA tersambung silang. PVA tersambung silang didapati mempamerkan kememilihan yang sederhana terhadap peroksid.

Kata kunci: Biosensor glukosa; gangguan; asetaminofen; kebolehtelapan; poli(vinil alkohol) tersambung silang

1.0 INTRODUCTION

Over the years, a variety of physical principles for glucose sensing have been investigated. One concept that has found wide application in the development of

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glucose biosensors is the peroxide-based amperometric electrode. Despite having to compete with more sophisticated emerging technologies, the peroxide-based enzymatic sensor remains a popular choice due to its simple configuration, which would facilitate miniaturization. However, for this type of sensor, interference from electro-active species, such as ascorbate, urate and the widely used analgesic acetaminophen, is a major problem. At present, membrane performance is only inferred from the overall outcome of a sensor.

A wide variety of membranes have been investigated for their potential ability to provide selectivity to retard the interferents. A lipid layer [1], polypyrrole [2], polyion complex [3], cellulose acetate [4, 5] and nafion [6] have been shown to eliminate ionic interferents.

On the other hand, the interfering effect of uncharged molecules such as acetaminophen remains a problem. With the exception of the polyion complex layer, the other membranes provide little protection against acetaminophen. Acetaminophen, in fact, is a serious offender and it has been reported that it can cause up to 200% bias *in vivo* [7]. A few groups have investigated the effectiveness of composite membranes in reducing the bias caused by acetaminophen. The interference caused by acetaminophen has been reported to be alleviated using composite membranes such as cellulose acetate and nafion [8]; and γ-aminopropyltriethoxysilane, cellulose acetate and nafion [9].

In spite of this, no attempt has been made to systematically select the perm-selective membranes based on the effective diffusion coefficients of the electro-active species. Addressing this issue was the motivation behind this work. Acetaminophen was chosen as the representative interferent and cross-linked polyvinyl alcohol (PVA) was studied as a potentially viable internal membrane material. The permeabilities of the electro-active species were determined using a rotating disk electrode (RDE) system.

An efficient glucose sensor is expected to give a signal linearly proportional to blood glucose concentration. In order to successfully do this, the overall process has to be glucose diffusion controlled. For a peroxide based sensor, this means that the enzymatic reaction should not depend on oxygen and the rate of hydrogen peroxide oxidation at the electrode must be relatively fast. As most glucose sensors are constructed in such a way that the total enzyme loading is quite excessive in order to raise the sensitivity to glucose, the rate of electron transfer reaction is even more important. Thus, care should be exercised when selecting the electrode material.

A rotating disk electrode (RDE) system is an accurate means of measuring the diffusion coefficient of an electro-active active species of interest. A major advantage of this technique is that measurements are made at steady state, thus eliminating the need to consider the time of electrolysis.

The following equation is used to measure the diffusion coefficient of an electro-active species,

$$\frac{1}{i} = \frac{1}{i_k} + \frac{1}{0.62nFAD_{dl}^{2/3}\omega^{1/2}\nu^{-1/6}C_b} \quad (1)$$

where i is the current, n is the number of electrons involved in the electrode reaction, A is the electrode area, C_b is the bulk concentration, F is the Faraday constant, m is a mass transport parameter which depends on electrode geometries and solution hydrodynamics, i_k is the kinetic current, in the absence of any mass transfer effect, D_{dl} is the diffusion coefficient of the electro-active species in bulk fluid, ω is the rotation speed of the electrode and ν is the kinematic viscosity of the bulk solution.

A plot of $1/i$ versus $1/\omega^{1/2}$, known as a Koutecky-Levich plot, will give a straight line. The slope of the plot contains information about the diffusion coefficient of the electro-active species in the bulk solution. If the overall process is mass transfer controlled, the Koutecky-Levich plot will pass through the origin. A non-negligible intercept means that electron transfer at the electrode is slow, making it a surface reaction controlled event.

By covering the RDE with a membrane, the system can also be used to measure the diffusion coefficients of electro-active compounds through the membrane [10–12]. The equation that can be used to calculate the diffusion coefficients of the said species is as follows [13]:

$$\frac{1}{i_{\lim}} = \frac{d_m}{nFA\alpha D_m C_b} + \frac{1}{062nFAD_{dl}^{2/3}\nu^{-1/6}\omega^{1/2}C_b} \quad (2)$$

where α is the partition coefficient, d_m is the membrane thickness, d_{dl} is the diffusion layer thickness, C_b is the bulk concentration and D_m is the diffusion coefficient in the membrane.

By operating the RDE at different rotation speeds, the value of αD_m (also known as the effective diffusion coefficient of the substance through the membrane) can be calculated from the intercept of the plot of $1/i_{\lim}$ vs. $1/\omega^{1/2}$.

2.0 MATERIALS AND METHODS

2.1 Materials

4-acetamidophenol (acetaminophen) was obtained from Aldrich Chemical Co. Sodium phosphate monobasic and sodium phosphate dibasic were from Fisher Scientific. Hydrogen peroxide (30%, w/w, aqueous solution) was obtained from Sigma Chemical Co. Tetrammine platinum (II) hydrogen orthophosphate ($Pt(NH_3)_4HPO_4$) in powder form was obtained from Johnson Matthey. Orthophosporic acid was from Baker Analyzed. Disodium hydrogen orthophosphate and sodium hydroxide were obtained from J. T. Baker. All chemicals were used as received.

2.2 Instrumentation

For the RDE experiments, an EG&G Princeton Applied Research Company Rotor model 616 was used with an EG&G Princeton Applied Research Scanning Potentiostat Model 362. For data collection, the system was equipped with 2 Keithley 175 Autoranging Multimeters along with a Linseis Ly1400 X-Y Recorder. A conventional three-electrode electrochemical cell was employed. The counter electrode consisted of a platinum mesh from Alpha (99.99%) and a saturated calomel electrode (SCE) from Cole Parmer was used as the reference electrode. The rotating working electrode was comprised of a copper rod with a diameter of 0.5 cm and a length of 1.0 cm. The rod was imbedded in an epoxy casing and the whole assembly had a diameter of 1.5 cm. One end of the copper rod was fitted for electrical connection to the rotating shaft while the other end was electroplated with either gold or platinum to make it an active working electrode.

2.3 Electroplating Procedure

To prepare the electrodes for the electroplating procedure, the surfaces of the electrodes were polished repeatedly using aluminum oxide particles with diameters ranging from 15 μm to 0.3 μm on a moist polishing cloth. Subsequently, the electrodes were cleaned by immersing them in ethanol and sonicating for approximately 10 minutes. Then, the surfaces of the electrodes were etched with 10% sulfuric acid for approximately 10 minutes.

The deposition of gold was done using a gold cyanide solution. The electrodes were electroplated at a current density of 1.1 mA/cm^2. The temperature for the reaction was 60 °C [14].

The bath for platinum deposition was prepared according to a procedure patented by Warren *et al*. for Johnson Matthey PLC [15]. Tetrammine platinum (II) dihydroxide was reacted with a stoichiometric amount of orthophosphoric acid to form 26 mmol/liter tertrammine platinum (II) hydrogen orthophosphate. A stoichiometric amount of disodium hydrogen orthophosphate was added to the solution to make up a 28 mmol/liter disodium hydrogen orthophosphate buffer solution. The pH was then adjusted with sodium hydroxide solution to pH 10-11 with pH 10.5 being the optimum pH.

The plating bath was deoxygenated with a fast stream of nitrogen gas before being used for the deposition experiments. The platinum electroplating experiments were carried out at 95 °C using a current density of 6.4 mA/cm^2 [16].

2.4 Rotating Disk Electrode Voltammetry Experiments

For the voltammetry experiments, 0.1 M phosphate buffer pH 6.7 was used as the supporting electrolyte [17]. The kinematic viscosity of this buffer at 25 °C is 0.9×10^{-6}

m^2/s [18]. Before any experiments were conducted, the electrode surface was conditioned by applying a constant potential of 600 mV vs. SCE while rotating at 100 rpm. Voltammograms of the background current were also recorded at different rotation speeds. All measurements were made at 25 °C and at a scan rate of 20 mV/s.

For acetaminophen experiments (1–10 mmol/liter), the solution was saturated with nitrogen before any measurement was made. Voltammograms were recorded by scanning from 200 mV to 1100 mV (vs. SCE) at various rotation rates (100–400 rpm). Rotation rates were varied in random order.

For hydrogen peroxide experiments (1–10 mmol/liter), the buffer solution was saturated with nitrogen before hydrogen peroxide was added. Then, voltammograms were scanned from 200 mV to 1100 mV (vs. SCE) at various rotation rates (100–400 rpm) in random order.

The permeability or the effective diffusion coefficient of hydrogen peroxide and acetaminophen in cross-linked PVA was determined using the Rotating Disk Electrode (RDE) system. All voltammograms were recorded by scanning from 200 mV to 1100 mV (vs. SCE) at a scan rate of 1 mV/s. The rotation rates were randomly varied between 100 to 400 rpm for each set of experiments. All measurements were made at 25 °C.

The thickness of the PVA gel layer coated on the gold electrode was determined using a precision micrometer (Fowler). The gold electrode, with and without the gel layer, was sandwiched between two glass slides and the thickness was measured repeatedly. The difference in the average of the two sets of measurements was taken as the gel thickness.

2.5 Membrane Preparation

The internal membrane was prepared by chemically cross-linking PVA in two steps. Initially, a PVA solution, with a cross-linking ratio of 0.03, was prepared using a previously reported technique [19]. A 10 wt% aqueous PVA solution was mixed with a cross-linking solution made up of 10% acetic acid (buffer), 50% methanol (quencher), 10% sulfuric acid (catalyst), in a volume ratio of 3:2:1, and an appropriate amount of glutaraldehyde. Cross-linking ratio is defined here as the ratio of the moles of glutaraldehyde per mole of PVA repeat unit. An aliquot of the resulting mixture was then spin-coated onto the gold working electrode at a rotating speed of 600 rpm for one minute and subsequently placed in an oven at 50 °C for 90 minutes to facilitate the cross-linking reaction. The gel was then allowed to dry at room temperature for 1 hour. To form a stable membrane that adheres strongly to the electrode, the membrane was further cross-linked by contacting it with a glutaraldehyde solution of varying concentration for 17 minutes, followed by drying at 50 °C for another 90 minutes. The membrane part of the electrode was then submerged in 0.1 M phosphate buffer (pH 6.7) and allowed swell to equilibrium prior to use.

2.6 Water Content Determination

As the epoxy casing of the electrode can also absorb water, the actual water content of the membrane coated on the electrode was not determined. An estimation of the water content of the cross-linked PVA coated onto the gold electrode was obtained using membranes cast onto glass plates and then removed. These membranes were prepared in the same manner as the membranes on the electrode.

Following synthesis and drying, the membranes were placed in 0.1 M phosphate buffer and left for a sufficiently long time to ensure equilibrium hydration. After the weights of the hydrated membranes were recorded, the membranes were then dried at 50 °C. Apparent water content of the membrane coated on the electrode, H^{app}, was determined according to the following equation:

$$H^{app} = \frac{(W_w - W_d)}{W_w} \times 100 \quad (3)$$

where W_w is the fully hydrated weight and W_d is the dehydrated weight of the membrane, respectively.

3.0 RESULTS AND DISCUSSION

3.1 Comparison of Gold and Platinum

As expected, the oxidation of H_2O_2 took place readily on platinum compared to gold. This is in agreement with the work of Zhang and Wilson [20], which showed that the oxidation of 0.1 M H_2O_2 at pH 7.4 was effective on platinum in the range of 400 to 800 mV (vs. Ag/AgCl). In this work, the oxidation current for H_2O_2 on gold reached its limiting value of ca. 950 mV (vs. SCE). This value matches the value of the oxidation potential of H_2O_2 on gold as reported by Gerlache *et al.* which was 870 mV (vs. Ag/AgCl) [21].

Plots of $1/i_{lim}$ versus $1/\omega^{1/2}$ gave straight lines for all measurements using both gold and platinum electrodes. However, an interesting observation eliminates platinum from being a suitable electrode material for this work. A non-negligible intercept is observed for the oxidation of acetaminophen on platinum (Figure 1), indicating that the oxidation of acetaminophen on platinum is not mass transfer controlled. As the objective of this work is to compare the effective diffusion coefficients of peroxide and acetaminophen through a membrane, a slow electron transfer rate at the electrode surface will pose a problem as the intercept of the plot of $1/i_{lim}$ vs. $1/\omega^{1/2}$ for RDE experiments using platinum coated with a membrane will now contain information about both the kinetic current from the slow electron transfer rate and the permeability of the membrane to acetaminophen. For that reason, it is befitting to use gold instead of platinum for the diffusion experiments. The experiments thereafter were carried out on gold electrodes only.

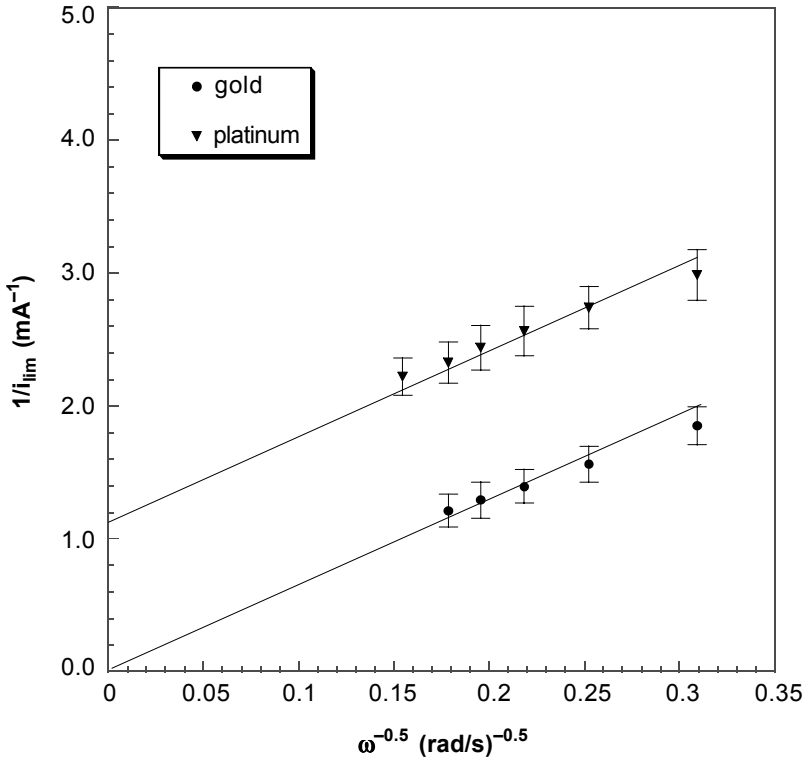

Figure 1 Plot of the reciprocal of limiting current against the reciprocal of the square root of the rotation rate for the oxidation of acetaminophen on gold and platinum

3.2 Determination of Diffusion Coefficients of Electrochemical Species in Buffer

To determine the diffusion coefficients of the electro-active species in buffer, knowledge of the number of electrons involved in the oxidation process is required. The electrode reaction involving H_2O_2 is a two-electron process [20, 22]. For acetaminophen oxidation, the number of electrons involved was found to be 2.1 ± 0.1 using a coulometric method [23]. The product of the oxidation process is N-acetyl-*p* quinoneimine (NAPQI). However, there are by-products and simple two-electron, two-proton reaction chemistry is unlikely.

A plot of the reciprocal of the limiting current against the reciprocal of the square root of the rotation rate for the oxidation of acetaminophen and H_2O_2 on gold is shown in Figure 2. From the slope of the plot, the diffusion coefficients of H_2O_2 and acetaminophen in a 0.1 M phosphate buffer (pH 6.7) at 25 °C were calculated. The values obtained for H_2O_2 and acetaminophen were 1.57×10^{-5} and 7.5×10^{-6} cm^2/s, respectively.

The diffusion coefficient for H_2O_2 in this work is certainly comparable with the other reported values [13, 22, 24, 25], indicating that the technique used is indeed an

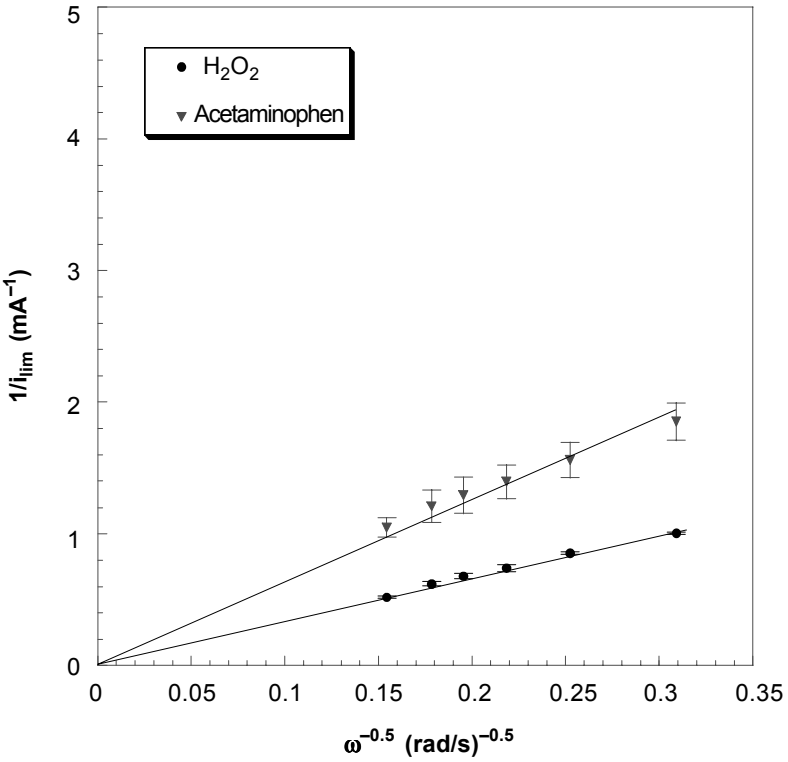

Figure 2 Plot of the reciprocal of limiting current against the reciprocal of the square root of the rotation rate for the oxidation of H_2O_2 and acetaminophen on gold

accurate means of measuring diffusion coefficients for electro-active species of interest. The diffusion coefficient of acetaminophen is comparable to the value calculated using the Wilke-Chang method [26].

3.3 Characterization of Cross-linked PVA Gels

One way of characterizing cross-linked PVA gels is through the hydration level of the gels. A gel with high water content indicates that the density of the cross-links of the gel is low (mesh size is considerably big) and vice versa. Figure 3 shows the dependence of apparent water content of the gel on the concentration of glutaraldehyde used during the second cross-linking reaction. As expected, the apparent water content decreased with an increase in the concentration of the additional cross-linker solution. The decrease in apparent water content was sharp when the concentration of additional cross-linker solution was increased from 1.25% to 2.5%, however, the decrease in apparent water content was more gradual when the concentration of cross-linker was further increased to 5%. The results implied that the mesh size of the PVA hydrogel was already small enough after exposure to 2.5% glutaraldehyde solution. Thus, increasing cross-linker solution to 5% would not result in marked decrease in mesh size of the hydrogel.

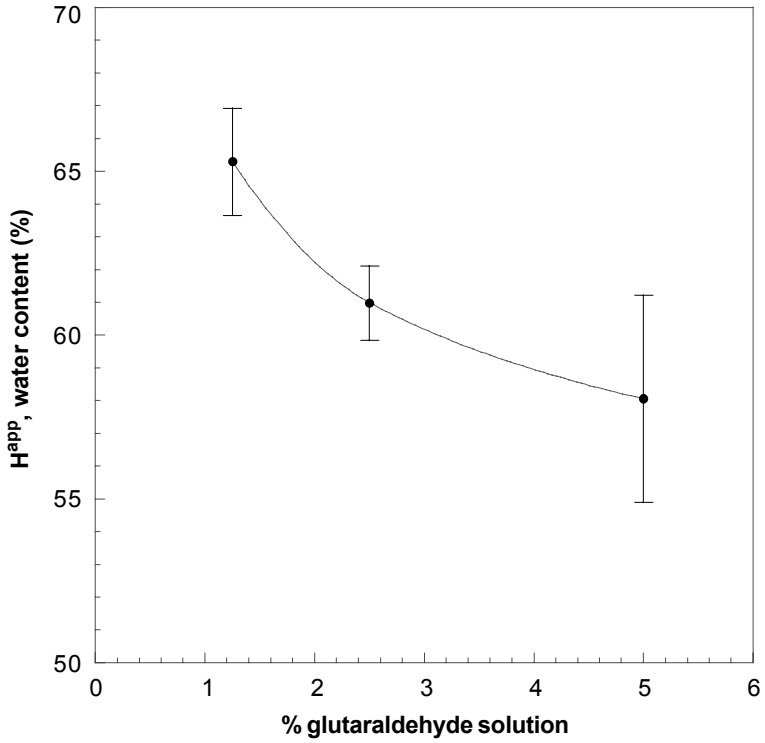

Figure 3 The dependence of apparent water content on the concentration of glutaraldehyde used during the second cross-linking reaction

3.4 Analysis of Transport Properties of Membranes

The transport characteristics of the cross-linked PVA gels are shown in Table 1. The permeability of hydrogen peroxide, a very small molecule, in the gel was only significantly affected when the mesh size was small enough to markedly obstruct the molecule. The permeability of the gel to peroxide was practically unchanged when the concentration of glutaraldehyde used to further increase the cross-link density of the gel was raised from 1.25% to 2.5% even though water content decreased (Figure 4(a)). On the other hand, the effective diffusion coefficient of acetaminophen, a slightly larger molecule, decreased immediately with an increase in cross-linker concentration from 1.25% to 2.5% (Figure 4(b)). A further increase of glutaraldehyde concentration to 5.0%, reduced the effective diffusion coefficient of acetaminophen to a lesser extent. The trend in the change of the permeability of the gel to acetaminophen as a result of the change in glutaraldehyde concentration followed the dependence of cross-linking density to water content closely (Figure 3 and Figure 4(b)). The reasoning in section 3.3 on the effect of cross-linker solution on mesh size applies here. When the gel was exposed to the increase in cross-linker solution from 1.25% to 2.5%, the mesh size of the gel was markedly reduced which resulted in the drastic reduction in the permeability

Table 1 Characteristics of homogeneous cross-linked PVA gels that were further reacted with a cross-linking solution of varying concentration

Cross-linker concentration in solution(%)	D_{eff} H_2O_2 ($\times 10^6 cm^2/s$)	D_{eff} Acetaminophen ($\times 10^6 cm^2/s$)	Apparent water content(%)
1.25	2.93	1.18	65.3
2.5	2.9	0.74	61
5	1.99	0.51	58.1

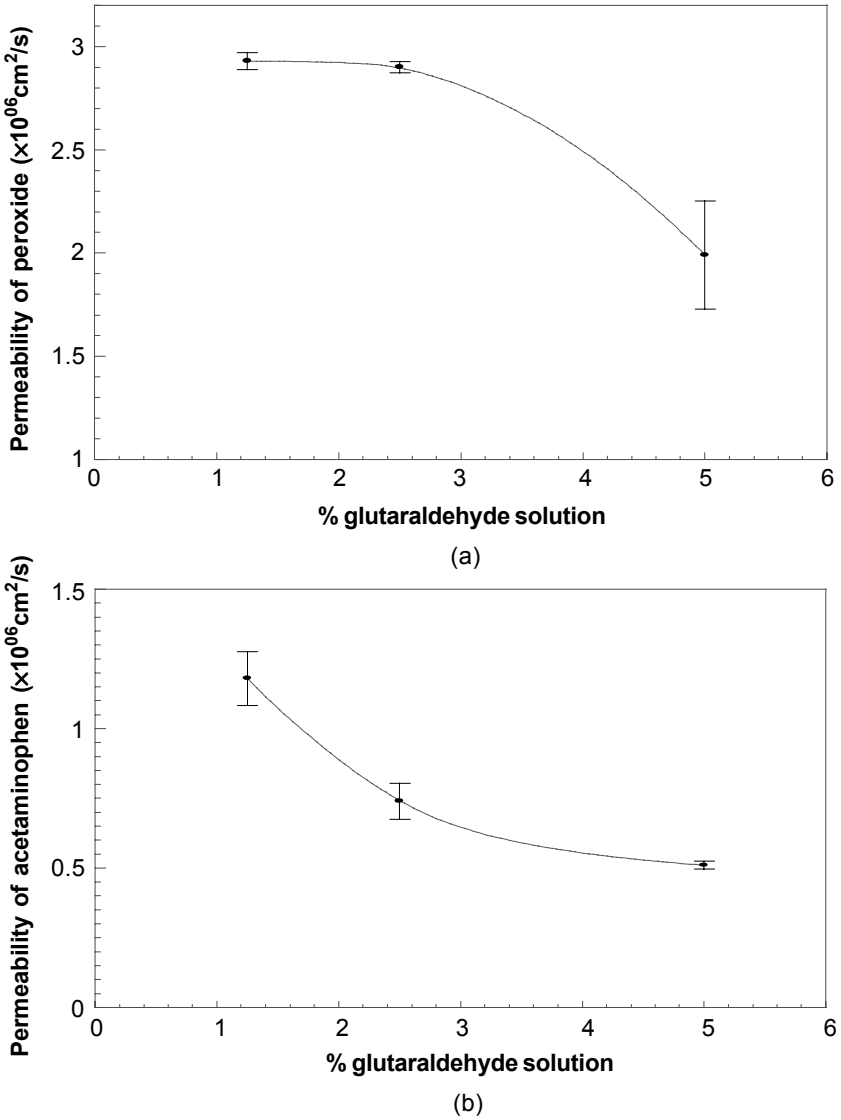

Figure 4 Effect of additional reaction with cross-linker on the permeability of (a) hydrogen peroxide and (b) acetaminophen

of the gel to acetaminophen. Reducing the cross-linker solution to 5% resulted in slight reduction in mesh size and thus minor reduction in permeability of acteminophen. This phenomenon is evident in Figure 5 which shows that permeability decreases according to the decrease in water content.

In this work, selectivity of the membrane to peroxide was defined as the ratio of the permeability of the membrane to peroxide to the permeability of the membrane to acetaminophen. Since the number of electrons involved in both the oxidation of peroxide and acetaminophen is two, selectivity to peroxide can also be estimated from the ratio of the intercept of the acetaminophen plot to that of peroxide, if the same PVA-gold electrode is used for both experiments and the concentration of the substances in both sets of experiments is the same.

In Figure 6, the selectivity of PVA to peroxide, σ, is plotted against water content. Doubling cross-linker concentration from 1.25% to 2.5% (water content decreased from ~65% to ~61%) increased selectivity markedly. Excellent selectivity was obtained due

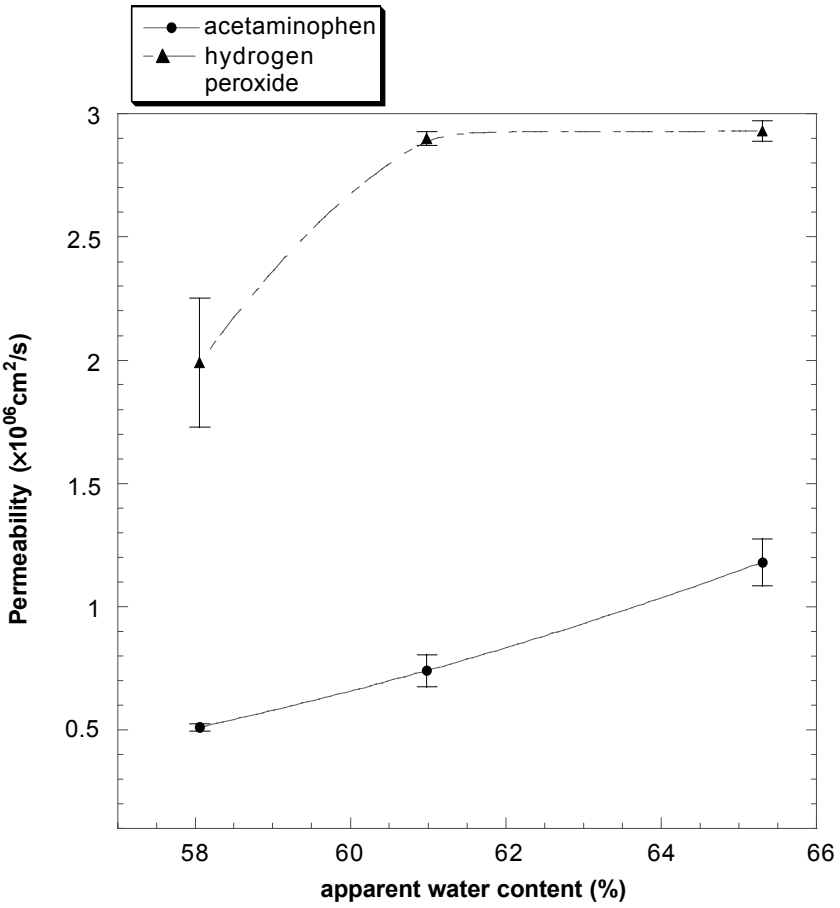

Figure 5 Dependence of permeability of peroxide and acetaminophen on apparent water content

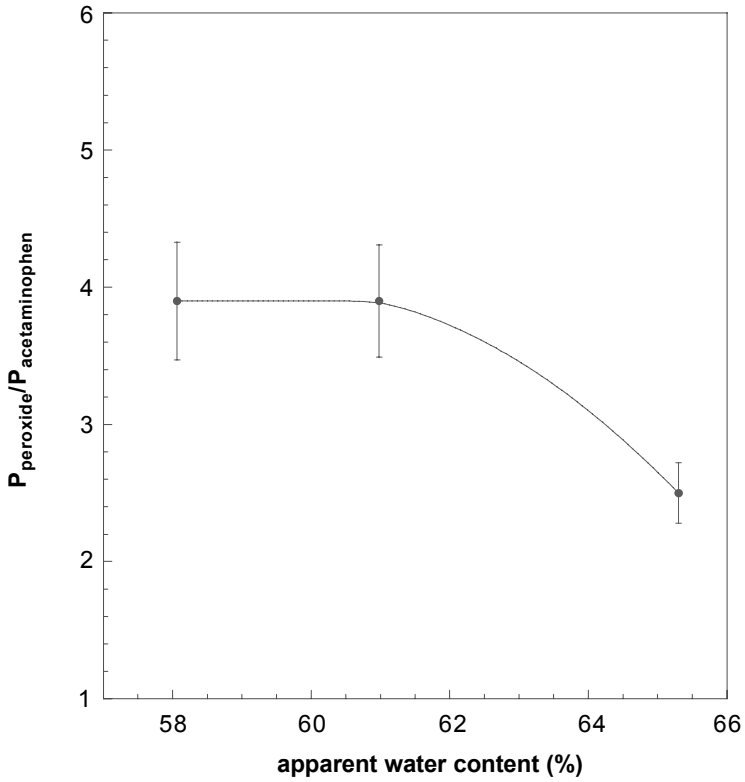

Figure 6 The dependence of selectivity on apparent water content

to the almost constant effective diffusion coefficient of peroxide through PVA and the decrease in the effective diffusion coefficient of acetaminophen. However, doubling the cross-linker concentration further (water content decreased from ~61% to ~58%) no longer results in any change in selectivity indicating that after a certain cut-off cross-linker concentration, the permeability of both peroxide and acetaminophen will be decreased to the same degree. Thus, increasing cross-linker concentration for the modification beyond after that concentration will no longer be beneficial as it would just serve to decrease the permeability of PVA to both solutes without affecting selectivity.

4.0 CONCLUSIONS

Platinum and gold were considered as the electrode material for a Rotating Disk Electrode (RDE) system that was to be used to measure the diffusion coefficients of H_2O_2 and acetaminophen in 0.1 M phosphate buffer, initially, and through a polymeric membrane, eventually. Despite the fact that oxidation of H_2O_2 occurs more readily on platinum, slow electron transfer at the platinum electrode surface during the oxidation of acetaminophen renders it ineffective for this work. Thus, all the diffusion experiments

were done on a gold electrode. In this work, cross-linked poly(vinyl alcohol) was investigated as a potentially viable internal membrane material. Selectivity of the membrane to peroxide was based on the permeability of peroxide relative to that of acetaminophen through the membrane. The permeability of hydrogen peroxide and acetaminophen (a representative interferent) was determined using a rotating disk electrode. Since peroxide is very small, the permeability of peroxide through cross-linked PVA was affected only when the mesh size was significantly reduced. On the other hand, the permeability of acetaminophen was linearly proportional to water content, and hence mesh size, in the range investigated. The cross-linked PVA membranes showed marginal selectivity towards peroxide.

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