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COMPOSITE HYDROGELS CONTAINING POLYPYRROLE AS SUPPORT MEMBRANES FOR AMPEROMETRIC ENZYME BIOSENSORS

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Dedicated to the memory of Professor Sukant K. Tripathy.

ABSTRACT

Conducting polymers and redox hydrogels are shown to be attractive materials for biocompatible electrodes in amperometric biosensors. We have combined electrically conducting polypyrrole (PPy) with crosslinked poly(2-hydroxyethylmethacrylate) (p-HEMA) to produce a novel composite hydrogel membrane. The high water content of these materials provides a biocompatible environment for the long-term immobilization of enzymes and a more favorable medium for the rapid movement of charge neutralizing ions. Electrode-supported composite films were prepared by UV polymerization of the hydrogel component (containing dissolved enzyme) followed immediately by electrochemical polymerization (+0.7V vs. Ag/AgCl) of the pyrrole component within the interstitial spaces of the pre-formed hydrogel network. Typical monomer compositions consisted of HEMA:TEGDA:pyrrole in an 85:10:05 vol%. (TEGDA = tetraethyleneglycol diacrylate). An optimized glucose biosensor displayed a wide linear response range of 5.0×10^{-5} to 2.0×10^{-2} M, a detection limit ($3S_{y/x}$/sensitivity) of 25 μM and a response time of 35-40

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seconds. The analytical recovery of glucose in serum samples ranged from 98 to 102% with mean coefficients of variation of 4.4% (within-day analyses) and 5.1% (day-to-day analyses). The optimized cholesterol and galactose biosensors also displayed wide linear response ranges ($5.0 \times 10^{-4} - 1.5 \times 10^{-2}$ M and $1.0 \times 10^{-4} - 1.0 \times 10^{-2}$ M, respectively) towards their respective substrates. All three biosensors retained > 70% of initial activity after 9 months when stored desiccated in the absence of buffer. An attractive feature with all the biosensors was their ability to effectively screen the endogenous interferents ascorbic acid, uric acid, L-cysteine and acetaminophen. This characteristic, coupled with the high biocompatibility of the polymeric hydrogel composites make these materials potential candidates for *in-vivo* biosensors.

Key Words: Hydrogel; Methacrylates; Conducting polymer; Polypyrrole; Amperometric biosensor; Glucose oxidase; Glucose; Cholesterol oxidase; Cholesterol; Galactose oxidase; Galactose; Ascorbic acid; Uric acid; Cysteine; Acetaminophen

INTRODUCTION

To date, the most popular, and arguably the most reliable, types of biosensors reported on in the literature and utilized commercially are those employing redox enzymes coupled with amperometric detection [1-3]. Both conducting polymers and redox hydrogels are attractive as materials in the construction of such biosensors in that they offer increased biocompatibility for the enzyme and/or facilitate rapid and enhanced electron transfer to the sensor surface. A range of conducting polymers including polypyrrole, polyaniline, poly(indole) and polyphenol have been employed in the construction of enzyme sensors [4-7]. However, of these materials, the most widely used have been films of polypyrrole and its derivatives [8]. This may be attributed to the ease with which these films can be grown from aqueous solutions and the high degree of selectivity due to the inherent size exclusion property of polypyrrole films.

Several researchers have also reported on the use of enzymes trapped within redox hydrogel films, the so-called "electrical or molecular wiring" of enzymes [9-11]. These redox polymers are characterized by their chemical inertness and rapid self-exchange rates. Researchers [12] have reported on the use of a hydrogel consisting of ferrocene-modified acrylamide to wire glucose oxidase electrically to glassy carbon and carbon paste electrodes. Others [13] have utilized hydrogels of poly(vinylpyridine) containing an osmium complex to wire glucose oxidase to vitreous carbon containing rotating disc electrodes.

The polymeric membrane of the current study consists of a hydrogel component of crosslinked poly(hydroxyethylmethacrylate) intimately combined with an electroactive component, polypyrrole, as shown in Figure 1. Such a composite hydrogel membrane, with its high water content, should provide a favorable biocompatible microenvironment for immobilized biomolecules. The polypyrrole

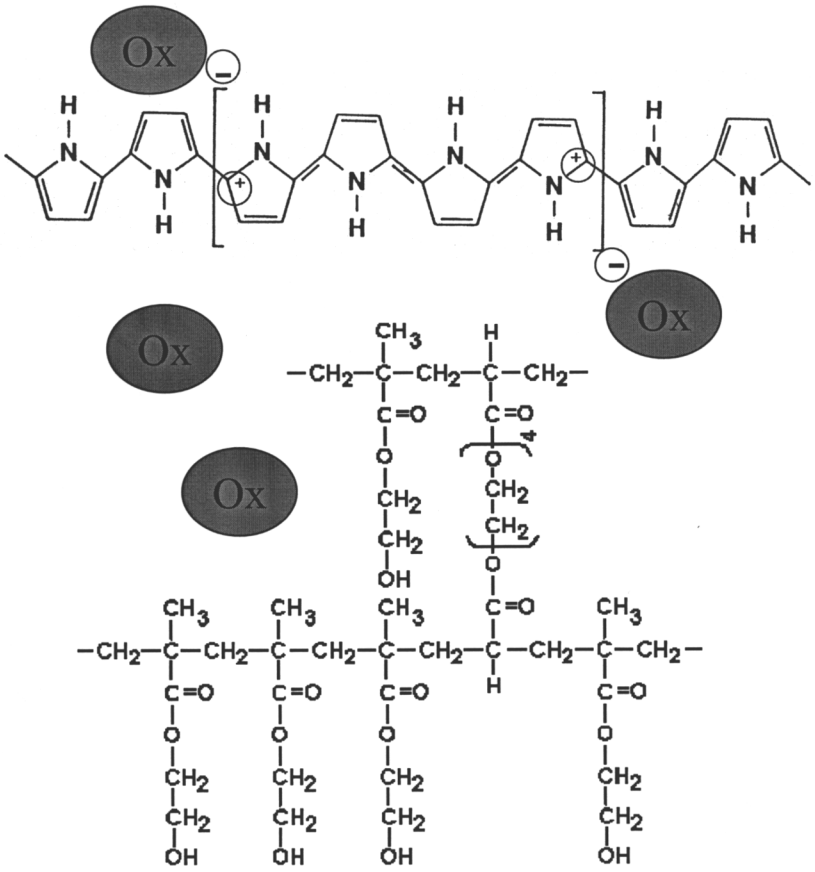

Figure 1. Schematic representation of the three components of the novel hydrogel composite matrix—the crosslinked hydrophilic hydrogel, the positively charged electroactive polypyrrole component and the negatively charged (net) oxidase enzyme.

component should impact the electrochemical properties of the medium by influencing redox mediation, direct electronic tunneling or by its reaction with hydrogen peroxide. We studied the substrate responses of enzyme biosensors for glucose, cholesterol and galactose. We evaluated the impact of the polypyrrole content of the composite membrane on its sensitivity to hydrogen peroxide. Hydrogen peroxide is the detected species in amperometric biosensors for glucose, cholesterol and galactose in which the enzymes are entrapped within the composite hydrogel/polypyrrole membrane. We also measured the amperometric response of the composite membrane (of varying PPy content) to the common endogenous interferents ascorbic acid, uric acid, L-cysteine and acetaminophen. The biosensors were found to be stable over extended periods of storage under desiccation at 4°C. The magnitude of the amperometric response was found to decrease as the polypyrrole content was increased suggesting no electrocatalytic influence of the polypyrrole component. However, the effectiveness of the com-

posite membrane for shielding of these interferences was greatly enhanced as the polypyrrole content was increased. The foregoing suggests an optimum polymer composition for best performance of the biosensors.

EXPERIMENTAL

Materials

Glucose oxidase (E.C. 1.1.3.4 from Aspergillus niger), cholesterol oxidase (E.C 1.1.3.6. from Streptomycin species) and galactose oxidase (E.C. 1.1.3.9 from Dactylium dendroides) were obtained from Sigma Chemical Co., St. Louis, MO. The monomer, hydroxyethylmethacrylate (HEMA), was obtained from Polysciences Inc., Warrington, PA, while the crosslinker tetraethyleneglycol diacrylate (TEGDA), inhibitor remover columns, pyrrole monomer (Py), the photoinitiator dimethoxyphenyl acetophenone (DMPA), platinum foil (0.1 mm thick) and platinum wire (0.1 mm diam.) were all obtained from Aldrich Co. (Milwaukee, WI). Serum samples were obtained from the Mount Hope Maternity Hospital, Mount Hope, Trinidad, West Indies. The HEMA and pyrrole monomers were both vacuum distilled before use (1.3 mm Hg, 80°C and 3.5 mm Hg, 60°C, respectively). All other reagents used were of the Analytical Reagent grade (B.D.H., Poole, U.K.) and were used without further purification.

Methods

Construction of the Enzyme Electrodes

The working electrodes were constructed from platinum foil ($10 \times 10 \times 0.1$ mm) according to a widely used construction protocol (14). The platinum electrodes were first flamed and then washed ultrasonically with hexane, acetone, isopropanol and distilled water. This was followed by treatment at 60°C for 30 seconds with a solution comprising a 1:1:5 volume ratio of aqueous ammonia (0.1M), hydrogen peroxide (20% volume) and distilled water. The electrode surfaces were then polished with a diamond slurry paste (1 μm) followed by an alumina slurry (0.05 μm) and any residual abrasive particles were removed ultrasonically in DI water. Appropriate quantities (3 μL) of the enzyme-monomer mixtures were then applied to 0.25 cm^2 windows on the surface of the clean, polished platinum electrodes formed using adhesive backed polyimide tape. The mixture was immediately irradiated with U.V. light (366 nm, 2.3 watts/cm^2, Spectroline Model 330844) for 45 minutes under an inert argon atmosphere to effect polymerization of the hydrogel component. The electrode was then immersed into 3 mL of a deaerated phosphate buffered KCl solution (0.1M NaH_2PO_4 containing 0.1M KCl, pH 7.0) that was saturated with pyrrole monomer (ca. 0.4 M). The polypyr-

role component of the composite membrane was deposited within the interstitial spaces of the pre-formed hydrogel network by potentiostatic electropolymerization (+0.85 V *vs.* Ag/AgCl) for 100 seconds. The electrodes were then rinsed with phosphate buffer (0.1M, pH 7.0) to remove any residual monomer or weakly adsorbed enzyme. Finally, the composite polyHEMA/polypyrrole/enzyme film was further extensively oxidized at +0.7 V *vs.* Ag/AgCl (the working potential required to oxidize enzymatically generated H_2O_2) until the background current fell below 1.0 μA. When not in use, the electrodes were desiccated and stored at 4°C in the absence of buffer.

Quantitation of Substrate Response

The enzyme electrodes were each made, in turn, the working electrodes in an electrochemical cell configuration utilizing a coiled platinum counter electrode and a Ag/AgCl reference electrode. Phosphate buffer (0.1M, pH 7.0) was added to the cell and all electrodes were connected to a potentiostat (Bioanalytical Systems – BAS 100B Electrochemical Analyzer, West Lafayette, Indiana). A constant potential of +0.7 V was applied to the three-electrode cell with stirring (450 r.p.m.) until the background current decayed to a steady low current density value of 4 μA/cm^2. Aliquots (1 mL) of the respective analytes, glucose, cholesterol and galactose (1.0–100 mM) were each injected into the phosphate buffer (3 mL) containing the corresponding enzyme biosensor and the steady state currents produced as a result of oxidation of the enzymatically generated H_2O_2 were recorded.

Optimization of the Biosensor Membranes

The amperometric response of each composite membrane of different polypyrrole composition (0, 5, 10, 20 vol% pyrrole) towards H_2O_2 oxidation was evaluated by making each electrode in turn the working electrode of the electrolytic cell containing phosphate buffer. Varying concentrations of H_2O_2 were subsequently injected and the magnitude of the amperometric current produced due to H_2O_2 oxidation was measured at +700 mV (vs. Ag/AgCl). In another set of experiments, twice the physiological concentrations of four endogenous interferents, acsorbic acid, uric acid, L-cysteine and acetaminophen were separately introduced into the electrolytic cell. The current produced by oxidation of each interfering species at each electrode surface coated with composite material of a different polypyrrole content was measured. The three enzyme biosensors constructed (glucose, cholesterol and galactose) were optimized with respect to monomer composition, enzyme loading and film thickness, and their performance was optimized with regard to pH and temperature. In each of these optimization studies, the response of each biosensor to solutions of their respective analyte was monitored as described in the preceding section.

RESULTS AND DISCUSSION

Preparation of PolyHEMA/Polypyrrole/Composites

During the electropolymerization stage, the anodic current *vs.* time plot obtained was one of increasing magnitude leading to a maximum followed by a gradual fall to low current values. This suggests that during this process the polypyrrole component continued to form from unreacted pyrrole monomer trapped within the preformed hydrogel membrane. The gradual fall in anodic current signals an approach to completion of electropolymerization and an attainment of background current values. Further evidence to support this was obtained from spectroelectrochemical studies performed on composite membranes cast onto ITO plates and subjected to different pyrrole oxidation times. Figure 2 clearly shows the progressive augmentation of a single absorbance band at 325 nm with increased oxidation time, suggesting further oxidative polymerization of unreacted pyrrole monomer and polypyrrole polymer formation.

Choice of Monomer Formulation

The dynamic response characteristics of the composite hydrogel membrane are expected to be governed by the network crosslink density. To investigate this, we evaluated a series of hydrogels of different HEMA/Py monomer ratio. Testing of different monomer formulations in the preparation of platinum-supported, glucose oxidase-containing polymer films shows that the best performance factors (Table 1) with respect to; linear calibration ranges, sensitivity, response time and precision; were obtained using an 85:10:05 vol% (HEMA:TEGDA:Py) monomer formulation. The blood glucose levels in normal patients fall well within the linear calibration range (10^{-2}-10^{1} mM) of the biosensor fabricated with this formulation. Additionally, 95% of the response time can be attained within 70 seconds. This monomer formulation was thus used throughout in the preparation of films for

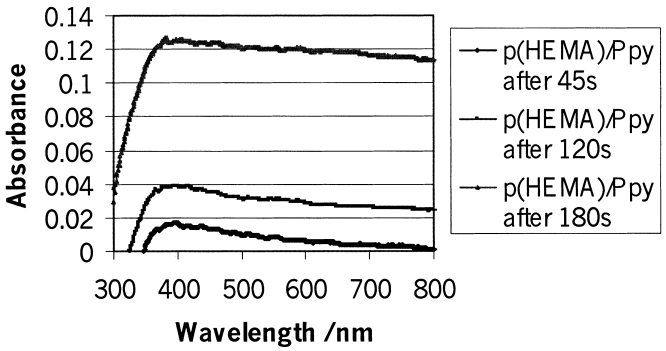

Figure 2. UV-Visible spectra of composite film of composition HEMA:TEGDA:Py (85:10:05 vol%) performed on indium-tin oxide plates over the wavelengths 300 nm-800 nm.

Table 1. Comparison of Performance Factors Among Pt | p(HEMA)/PPy/GOx Biosensors Fabricated via Electrochemical Polymerization of the Pyrrole Component Using Three Different Monomer Formulations

Performance Factor	Pt | p(HEMA)/PPy/GOx Electrodes Monomer Composition (H:T:Py (vol%))*		
	70:10:20	80:10:10	85:10:05
Linear glucose range/mM	5.0×10^{-1} - 2.0×10^{1}	5.0×10^{-1} - 2.0×10^{1}	5.0×10^{-2} - 2.5×10^{1}
Sensitivity/μA mM^{-1}	2.49×10^{-1}	6.9×10^{-2}	1.56×10^{-1}
Detection Limit / mM	0.15	0.10	0.02
Response Time / s (95% equilibrium)	160	200	70
Precision (%):			
1 mM	12.2	18.5	6.4
10 mM	8.2	7.0	2.8
Regression Equation[a]	y = 249× + 0.110	y = 69× + 0.173	y = 156× + 0.277
r^2	0.998	0.997	0.997

*H:T:Py ≡ HEMA:TEGDA:pyrrole.

[a]Regression equations of the form y (μA) × m (μA M-1) × [M] + c (μA). Conditions were: 1) enzyme loading = 760 units cm^{-2}; 2) film thickness = 200 μm; 3) pH = 7.0 (NaH_2PO_4, 0.1M); 4) Temp = 30ºC.º

further optimization. Comparable volumes (9 vol%) of another electroactive monomer, aniline, were previously reported in preparing monomer formulations for possible electroactive hydrogels [15].

Enzyme Loading

The response of each biosensor can be expected to vary depending upon the amount of retained bioactivity of the particular oxidase enzyme entrapped within the membrane. Composite hydrogels prepared from the 85:10:05 vol% monomer ratio were studied with varying amounts of each loaded enzyme; glucose oxidase (304–3040 units cm^{-2}), cholesterol oxidase (10–118 units cm^{-2}) and galactose oxidase (61–694 units cm^{-2}). Optimal biosensor performance characteristics were generated using a GOx loading of 1064 units cm^{-2}, a ChOx loading of 87 units cm^{-2} and a GalOx loading of 694 units cm^{-2} (Table 2). Both these optimum glucose and cholesterol biosensors displayed linear response ranges that span up to four times the normal physiological concentrations of each respective analyte. The galactose biosensor containing 694 units cm^{-2} exhibited the fastest response time (70 seconds) among all other enzyme loadings used. Hence, all further optimizations were carried out using the above three biosensor formulations.

Table 2. Performance Factors for the Optimized Pt | p(HEMA)/PPy/Ox Biosensors

Parameter	GOx	ChOx	GalOx
Linear range/mM	0.05–20	0.15–15	0.05–10
Sensitivity/μA mM^{-1}	0.475	0.195×10^{-1}	0.937
Detection Limit/mM	0.025	0.120	0.025
Response Time/s (95% equilibrium)	40	30	75
Mean Precision (%):	4.4	3.0	3.8
Regression Equation[a]	y = 475× + 0.105	y = 19.05× − 0.029	y = 937× + 0.171
r^2	0.997	0.999	0.999

[a]Regression equations of the form y (μA) = m (μA M^{-1}) × [M] + c (μA). Conditions were: 1) enzyme loading = 760 units cm^{-2}; 2) film thickness = 120 μm; 3) pH = 7.0 (0.1M NaH2PO4); 4) temp = 30ºC.

Film Thickness

Table 3 summarizes the performance factors for four glucose biosensors of varying film thickness (120-800 μm). All electrodes showed broad linear response ranges for physiological glucose determination (> 10mM). However, the response time (time to achieve 95% of maximum signal) was found to increase as the film thickness increases, varying from approximately 40 seconds (120 μm thick) to in excess of 300 seconds (800 μm thick). A similar trend was previously reported for the fabrication of a p(HEMA)-based glucose biosensor [16]. A final composite

Table 3. Comparison of Performance Factors Among Glucose Electrodes of Different Film Thickness

	Polymer Film Thickness /μm*			
Performance Factors	120	200	400	800
Linear glucose range/mM	0.1–100	0.05–50	0.1–10	0.02–25
Sensitivity/μA mM^{-1}	0.441	0.548	0.629	0.583
Detection Limit / mM	0.06	0.02	0.08	0.01
Response Time / s (95% equilibrium)	40	80	100	310
Precision (%):				
1 mM	6.3	5.4	7.3	8.5
5 mM	2.6	3.5	1.9	1.7
Regression Equation[a]	y = 441× + 0.161	y= 548× + 0.277	y = 629× + 0.103	y =583× + 0.142
R^2	0.997	0.997	0.998	0.997

*Conditions were: 1) monomer formulation = 85:10:05 vol% (HEMA:TEGDA:Py); 2) enzyme loading = 1064 units cm^{-2} GOx; 3) pH = 7.0 (0.1M NaH_2PO_4); 4) temp = 30ºC.
[a]Regression equations of the form y (μA) = m (μA M^{-1}) x [M] + c (μA).

membrane thickness of 120 μm was chosen as the optimum value for further studies on all three clinical biosensors.

pH Studies

The influence of pH of the assay solution on the amperometric response to glucose, cholesterol and galactose of the three clinical biosensors was investigated. Bioactive membranes that were optimized with respect to monomer formulation, enzyme loading and film thickness were investigated over the pH range 4.5-8.0. The resulting activity profiles are shown in Figure 3. The glucose biosensor exhibited a broad pH profile with response optimum around 6.0. This is similar to that reported for solution-borne GOx [17]. The cholesterol biosensor showed an optimum response between pH 6.0–6.5, similar to that reported for immobilized ChOx entrapped in a variety of matrixes [18, 19]. The influence of pH on the galactose biosensor's response to a fixed galactose concentration revealed a maximum response at pH 7.0, with a marked decrease in response below pH 6.5. Of significance is that all three clinical biosensors retained greater than 80% of optimal activity at physiological pH (7.3), thus favoring an anticipated use in clinical chemistry without the need to adjust the pH of samples prior to analysis.

Temperature Studies

The response of all biosensors to solutions of their respective analytes at the optimized pH over the temperature range 10–60°C was also investigated. The resulting activity profiles for the three systems are shown in Figure 4. These three

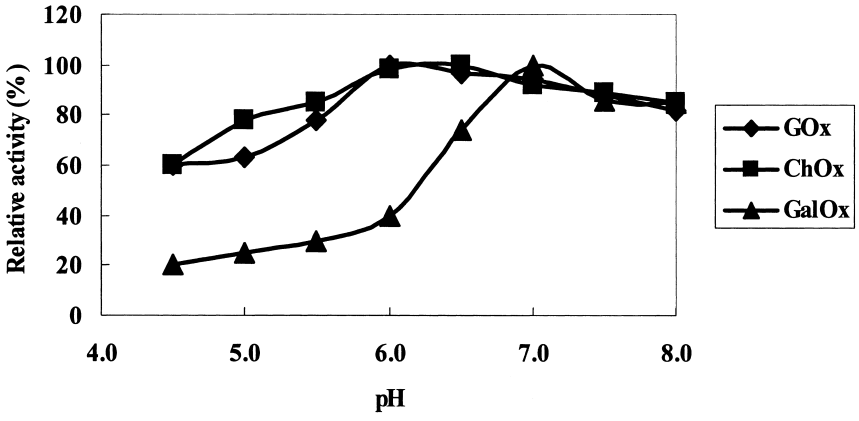

Figure 3. The pH profiles of the three Pt|p(HEMA)/PPy/Ox biosensors. Conditions were : 1) monomer composition = 85:10:05 vol% (HEMA:TEGDA:Py); 2) film thickness = 120 μm; 3) Temp = 30°C; 4) Buffer = 0.1M.

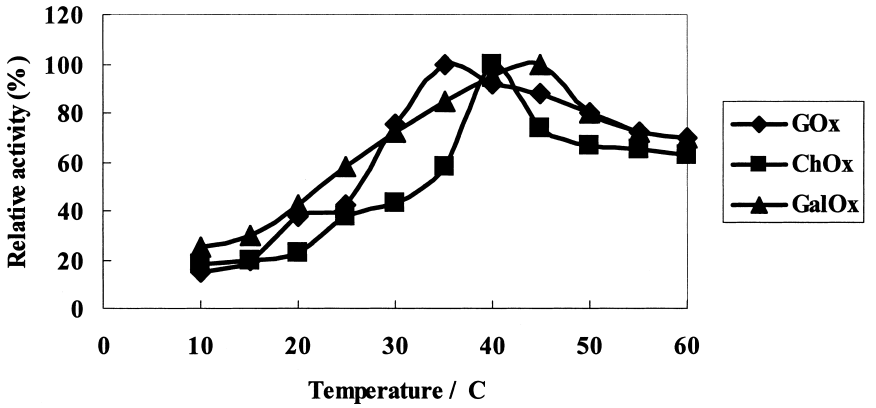

Figure 4. The temperature profiles of the three Pt|p(HEMA)/PPy/Ox biosensors. Conditions were: 1) monomer composition = 85:10:05 vol% (HEMA:TEGDA:Py); 2) film thickness = 0.12 mm; 3) pH = 7.0; 4) Buffer = 0.1M NaH_2PO_4.

systems display temperature dependent activity maxima within the range 35–40°C. The physiological body temperature of 37.5°C is accommodated within this range, thus indicating the potential use of the present biosensors for *in vivo* diagnostics. The composite hydrogel matrix, acting as a kind of scaffold, prevents denaturation that may otherwise arise from extensive conformational changes. This might explain the increased activity at higher temperatures.

Analytical Performance Characteristics of the Optimized Biosensors

The optimized biosensors all exhibited large linear response ranges to their respective analytes (Table 2). The glucose and galactose biosensors showed a dynamic measuring range extending over three orders of magnitude (0.05–20 mM and 0.05–10 mM, respectively) and with a sensitivity of 4.75×10^{-1} and 9.37×10^{-1} $\mu A\ mM^{-1}$ respectively. The cholesterol biosensor gave a current response that increased linearly over the concentration range of 0.15–15 mM cholesterol, with a sensitivity of 19.5 nA mM^{-1}. All biosensors displayed linear response ranges that were noticeably larger than those of previously reported amperometric enzyme electrodes for glucose [20], cholesterol [21] and galactose [22]. We attribute this extension of linearity and retained enzyme activity to the biocompatible microenvironment afforded to the oxidase enzymes by the novel composite hydrogel membrane. These materials provide a milieu that has a high water content similar to that of the native solution-borne enzyme. The time taken to reach 95% of the steady state response was 40 seconds for the glucose biosensor, 30 seconds and 75 seconds for the cholesterol and galactose biosensors respectively. The limit of detection, as determined from the expression $3S_{y/x}$/sensitivity (where $S_{y/x}$ is the estimated standard deviation for the points used to construct the calibration graph

and the sensitivity its slope) for each biosensor was 25.0 μM, 120 μM and 25.0 μM for glucose, cholesterol and galactose, respectively.

The amperometric responses of the three biosensors to two concentrations of glucose, cholesterol, and galactose were investigated at three different stirring speeds (150, 450, and 800 r.p.m.). The results (Table 4) indicate that there were no significant differences in the glucose and galactose electrodes' response towards both low and high substrate levels at the different stirring speeds. Thus, under these experimental conditions, both these biosensors are operating under kinetically controlled conditions. For the cholesterol biosensor however, it can be seen that variation of the stirring speed upon addition of cholesterol substrate significantly influences the amperometric response towards both low and high cholesterol concentrations. From these observations, it can be concluded that the cholesterol biosensor response is dependent upon the mass transfer of the cholesterol substrate from the bulk solution to the surface of the immobilized enzyme and of diffusion of H_2O_2 generated within the polymer matrix to the surface of the electrode. These results suggest that the rate determining step for the cholesterol biosensor is the formation of enzyme-substrate complex. On the other hand, the glucose and galactose biosensors' response to substrate is limited preferentially by the kinetics of the enzymatic reaction.

Repeated analyses using serum samples obtained from hospitalized patients demonstrate the precision of the clinical biosensors. The mean precisions were

Table 4. Effect of Stirring on Steady-State Current for the Biosensors

Stirring Speed / r.p.m.	Background Condition : Substrate Condition†	Steady State Current					
		Glucose (μA)		Cholesterol (nA)		Galactose (μA)	
		5 mM	7 mM	2 mM	8 mM	1 mM	10 mM
150	q:q	1.32	3.33	7.26	98.24	1.06	10.25
	q:s	1.37	3.60	20.97	236.23	1.22	10.39
	s:s	1.48	3.79	13.51	220.80	0.90	10.28
	s:q	1.24	3.16	2.30	103.74	0.88	10.46
450	q:q	1.32	3.61	7.31	103.80	1.08	10.35
	q:s	1.34	3.93	31.79	313.00	1.41	11.03
	s:s	1.12	3.89	25.58	215.30	1.28	10.91
	s:q	1.39	3.39	7.71	99.60	1.09	10.85
800	q:q	1.33	3.79	4.21	114.90	1.41	10.62
	q:s	1.30	3.98	37.81	386.30	1.48	10.76
	s:s	1.11	3.92	35.14	321.70	1.46	11.01
	s:q	1.36	3.85	3.29	98.06	1.39	10.04

Conditions were: 1) Biosensor film characteristics: Monomer composition = 85:10:05 vol% (HEMA:TEGDA:Py) film thickness = 120 μm; 2) temp = 30ºC; 3) buffer = 0.1M NaH_2PO_4, pH 7.0.

†Background condition refers to the condition of the buffer [stirred (s) or unstirred (q)] in which analyses are to be made prior to addition of substrate. Substrate condition refers to the condition of the buffer [stirred (s) or unstirred (q)] upon addition of the substrate.

Table 5. Glucose Recovery Study Performed on a Serum Sample

Added glucose mg dL^{-1} (mM)	Glucose found mg dL^{-1} (mM)	Recovery %
—	19.64 (1.09)	—
5 (0.28)	24.14 (1.34)	98.0
15 (0.83)	34.05 (1.89)	98.3
30 (1.66)	50.45 (2.80)	101.6
70 (3.88)	91.17 (5.06)	101.7
125 (6.94)	145.41 (8.07)	100.5

found to range from 4.4%, 3.0%, and 3.8% for the glucose, cholesterol and galactose biosensors respectively for within-day analyses. Day-to-day analyses show the mean precision of the above biosensors to range from 5.0%, 3.9%, and 4.3%, respectively. Recovery yields performed using these same patient serum samples, now diluted and spiked with standards of each respective substrate, show recoveries in the range 98.0 to 101.7% for glucose (Table 5), 96.6 to 103% for cholesterol (Table 6) and between 96.8 to 105% for galactose (Table 7). Data obtained from the analysis of serum samples from hospitalized patients using the glucose and cholesterol biosensors are presented in our earlier manuscripts [23, 24]. An important prerequisite for the design and construction of biosensors is that they should be stable over an adequate length of time. The response of each biosensor to a fixed concentration of substrate was monitored over a period of 260 days. The biosensors were stored desiccated in the absence of buffer at 4°C when not in use. All three biosensors retained > 70 % of the initial response after 260 days of storage (Figure 5).

Response to H_2O_2 and Interferents

The amperometric response of each electrode-supported composite film of different polypyrrole content towards varying H_2O_2 concentrations is shown in Figure 6. Included in this figure is the response of a polypyrrole–free pHEMA gel membrane that serves as the control. There is progressive reduction in the magni-

Table 6. Recovery Studies Using the Optimized Cholesterol Biosensor

Added cholesterol mg dL^{-1} (mM)	Cholesterol found mg dL^{-1} (mM)	Recovery %
—	3.87 (0.10)	—
8.08 (0.21)	12.31 (0.32)	103.0
22.14 (0.57)	25.49 (0.66)	98.0
31.86 (0.82)	36.69 (0.95)	102.7
44.68 (1.16)	46.90 (1.21)	96.6

Table 7. Recovery Studies Using the Optimized Galactose Biosensor

Added galactose mg dL^{-1} (mM)	Galactose found mg dL^{-1} (mM)	Recovery %
—	3.24 (0.18)	—
2.16 (0.12)	5.23 (0.29)	96.8
12.62 (0.70)	15.50 (0.86)	97.7
18.04 (1.00)	22.34 (1.24)	105.0
32.80 (1.82)	35.68 (1.98)	99.0
68.84 (3.82)	72.80 (4.04)	101.0

tude of the oxidative current at all H_2O_2 concentrations as the percentage of incorporated polypyrrole is increased. These results suggest that there is no electrocatalytic influence provided by the polypyrrole component within the crosslinked hydrogel matrix. The development of useful amperometric biosensors is hampered by the presence of endogenous oxidizable molecules such as ascorbic acid, uric acid, L-cysteine, glutathione and acetaminophen in whole blood and blood serum samples. The efficiency of the polymer composite in suppressing interference caused by three very common electroactive interferents, ascorbic acid, uric acid and acetaminophen was investigated. The amperometric response of electrodes synthesized with varying contents of polypyrrole (0–20 vol%) to buffer solutions containing twice the physiological levels of each interferent is shown in Figure 7. As the % of polypyrrole incorporated into the hydrogel membrane was increased, there was a reduction in the magnitude of current produced due to interferent oxidation at the electrode surface. For a 5 vol% polypyrrole content, there was a

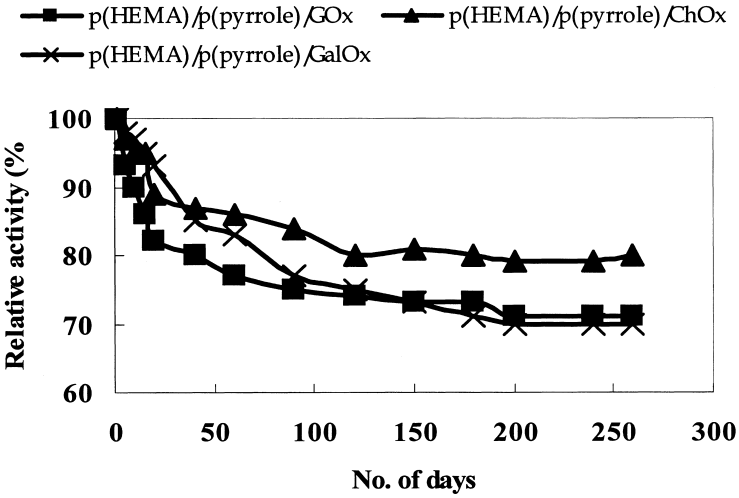

Figure 5. Storage stability profiles measured over a period of 260 days at 4°C for the three Pt | p(HEMA)/PPy/Ox biosensors.

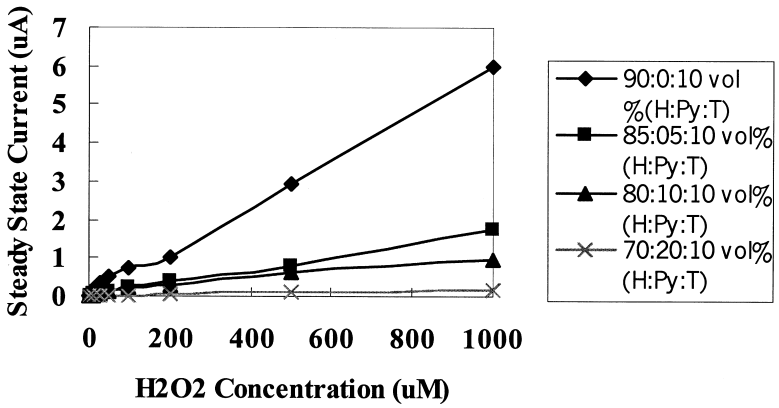

Figure 6. Effect of varying the composition of polypyrrole content (vol%) of the composite hydrogel membrane on the amperometric current generated by oxidation of H_2O_2.

reduction in oxidation current of 85%, 81%, and 61% for ascorbic acid, uric acid and acetaminophen respectively compared to the pure hydrogel membrane. There was further increase in interferent suppression with increasing content of polypyrrole in the composite membrane. This suggests that the polypyrrole component acts as a screen or barrier against the diffusion of these interferents into the polymer composite.

We postulate a dual permselectivity and electrostatic repulsion model for the role of the extensively oxidized polypyrrole in enhancing the overall selectivity of

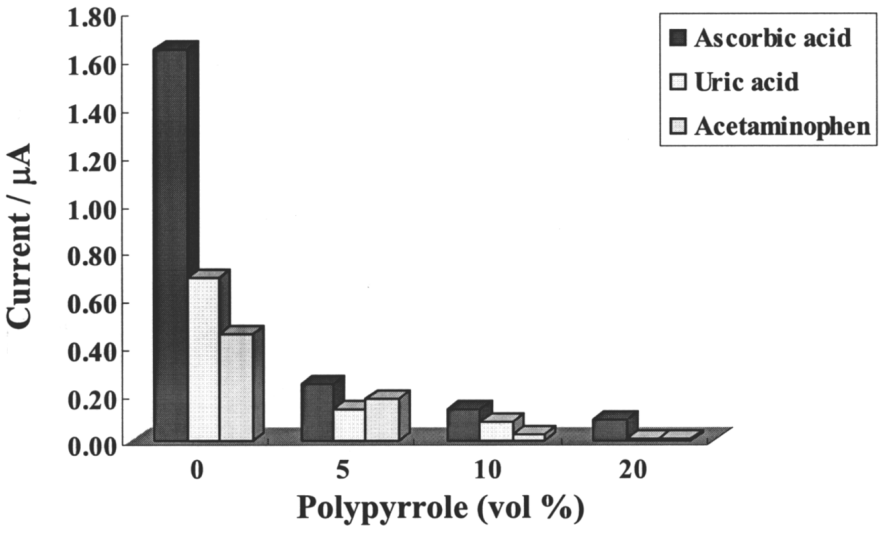

Figure 7. Effect of varying the composition of polypyrrole content (vol%) of the composite hydrogel membrane on the amperometric current generated by oxidation of interferent at the electrode surface.

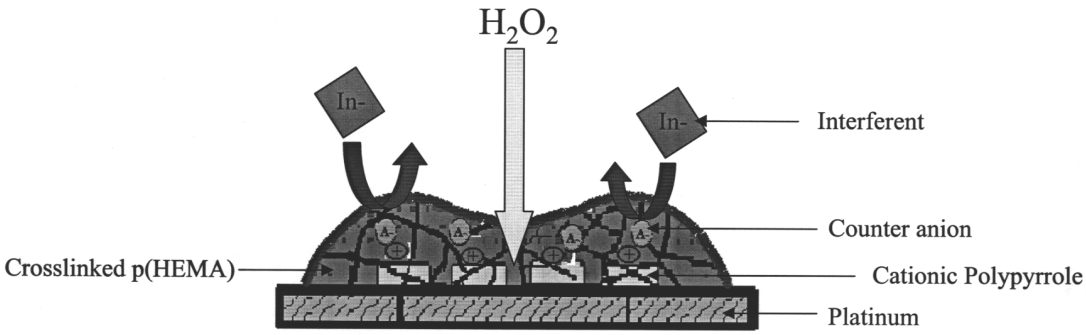

Figure 8. Schematic illustration of the screening characteristics of the p(HEMA)/PPy composite membrane.

the composite polymer membrane towards interferents. In the present study, polypyrrole was grown within the hydrogel film to produce an intimate combination of polypyrrole within the hydrogel layer. The cationic polypyrrole films confer remarkable permselectivity stemming from not only size-exclusion properties [19], but now also anion-exclusion properties due to the influx of anionic groups (dopant anions) from the buffer solution. This is schematically illustrated in Figure 8. As the majority of endogenous interferents occur in their anionic forms at physiological pH, this novel composite membrane establishes a unique avenue for diminishing electrode interference.

CONCLUSION

Three amperometric enzyme biosensors were synthesized employing a novel polymer composite configuration of p(HEMA) hydrogel containing polypyrrole by a simple, two-step polymerization process. The first step involved UV induced polymerization of the hydrogel component from a monomer cocktail containing the enzyme. This was followed by electrochemical polymerization of the pyrrole component within the preformed hydrogel network. The resulting polymer composite film displayed excellent exclusion properties towards common physiological interferents. The polymer composite also provided a biocompatible microenvironment for the entrapped enzymes that afforded > 70% retained bioactivity over a 260 day storage period. These electrodes display relatively rapid response, expanded linear response range and good reproducibility and recovery towards their respective substrate. The most attractive feature of these biosensors is that they are constructed almost entirely from a material (p[HEMA]) which has been used extensively for medical implants and contact lenses testifying to its *in vivo* compatibility and thus make these electrodes potential candidates for stable subcutaneous clinical biosensors.

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REFERENCES

1. Cosnier, S. Biomolecule Immobilization on Electrode Surfaces by Entrapment oor Attachment to Electrochemically Polymerized Films. A Review. Biosensors and Bioelectronics **1999**, *14* (5), 443–456.
2. Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Electroactive Hydrogels for the Construction of Clinically Important Biosensors. Proceedings of the American Chemical Society, Division of Polymer Materials: Science and Engineering; 2000, 83, 514.
3. Tká, J.; Vostiar, I.; Turdík, E.; Gemeiner, P.; Mastihuba, V.; Annus, J. Fructose Biosensor Based on D-Fructose Dehydrogenase Immobilized on a Ferrocene-Embedded Cellulose Acetate Membrane. Anal. Chim. Acta **2001**, *439*(1), 39-46.
4. Guiseppi-Elie, A.; Wallace, G.G.; Matsue, T. Chemical and Biological Sensors Based on Electrically Conducting Polymers. *In Handbook of Conductive Polymers;* Skotheim, T., Elsenbaumer, R., Reynolds, J.R., Eds.; Marcel Dekker: New York, 1995.
5. Cooper, J.C.; Hall, E.A.H. Electrochemical Response of An Enzyme-Loaded Polyaniline Film. Biosensors and Bioelectronics **1992**, *7*, 473-485.
6. Shaolin, M.; Jinquing, K.; Jianbing, Z.J. Bioelectrochemical Responses of the Polyaniline Uricase Electrode. Electroanal. Chem. **1992**, *334*, 121-132.
7. Bartlett, P.N.; Cooper, J.M. A Review of the Immobilization of Enzymes in Electropolymerized Films. J. Electroanal. Chem. **1993**, *362*(1), 1-12.
8. Adeloju, S.; Moline, A. Fabrication of Ultra-Thin Polypyrrole–Glucose Oxidase Film From Supporting Electrolyte-Free Monomer Solution for Potentiometric Biosensing of Glucose. Biosensors and Bioelectronics **2001**, *16*(3), 133-139.
9. Rajagopalan, R.; Atsushi, A.; Heller, A. Effect of Quaternization of the Glucose Oxidase "Wiring" Redox Polymer on the Maximum Current Densities of Glucose Electrodes. J. Phys. Chem. **1996**, *100*, 3719-3727.
10. Hale, P.D.; Boguslavsky, L.I.; Inagaki, T.; Karan, H.I.; Lee, H.S.; Skothiem, T.A.; Okamoto, Y. Amperometric Glucose Biosensors Based on Redox Polymer-Mediated Electron Transfer. Anal. Chem. **1991**, *63*, 677-682.
11. Rohde, E.; Dempsey, E.; Smyth, M.R.; Vos, J.G.; Emons, H. Development of a Flow-Through Electrochemical Detector for Glucose Based on a Glucose Oxidase-Modified Microelectrode Incorporating Redox and Conducting Polymer Materials. Anal. Chim. Acta **1993**, *378*, 5-16.
12. Calvo, E.J.; Danilowicz, C.; Diaz, L. Enzyme Catalysis at Hydrogel-Modified Electrodes with Redox Polymer Mediator. J Chem. Soc., Faraday Trans. **1993**, *89*(2), 377-384.
13. Gregg, B.A.; Heller, A. Redox Polymer Films Containing Enzymes. 2. Glucose Oxidase Containing Enzyme Electrodes. J. Phys. Chem. **1991**, *95*, 5976-5980.

14. Pishko, M.W.; Katakis, I.; Lindquist, S.-L.; Ye, L.; Gregg, B.A.; Heller, A. Direct Electrical Communication between Graphite Electrodes and Surface Adsorbed Glucose Oxidase/Redox Polymer Complexes. Angew. Chem. Int. Ed. Engl. **1990**, *29*, 82-84.
15. Guiseppi-Elie, A.; Wilson, A.M.; Sujdak, A.S.; Brown, K.E. Electroconductive Hydrogels: Novel Materials for the Controlled Electrorelease of Bioactive Peptides. Polymer Preprints **1997**, *38*(2), 608-609.
16. Walt, D.R.; Li, L. Dual-Analyte Fibre-Optic Sensor for the Simultaneous Measurement of Glucose and Oxygen. Anal. Chem. **1995**, *67*, 3746-3752.
17. Gibson, Q.H.; Swoboda, B.E.P.; Massey, V. Kinetics and Mechanism of Action of Glucose Oxidase. J. Biol. Chem. **1964**, *239*, 3927-3934.
18. Charpentier, L.; El Murr, N. Amperometric Determination of Cholesterol in Serum with the Use of a Renewable Surface Peroxidase Electrode. Anal. Chim. Acta **1995**, *318*, 89-93.
19. Vidal, J.C.; Garcia, E.; Castillo, J.R. *In situ* Preparation of a Cholesterol Biosensor: Entrapment of Cholesterol Oxidase in an Overoxidized Polypyrrole Film Electrodeposited in a Flow System-Determination of Total Cholesterol in Serum. Anal. Chim. Acta **1999**, *385*, 213-222.
20. Liu, H.; Li, H.; Ying, T.; Sun, K.; Qin, Y.; Qi, D. Amperometric Biosensor Sensitive to Glucose and Lactose Based on Co-Immobilization of Ferrocene, Glucose Oxidase, β-galactosidase and Mutarotase in β-cyclodextrin Polymer. Anal. Chim. Acta **1998**, *358*, 137-144.
21. Besombes, J.-L.; Cosnier, S.; Labbe, P.; Reverdy, G. Improvement of the Analytical Characteristics of an Enzyme Electrode for Free and Total Cholesterol Via Laponite Clay Additives. Anal. Chim. Acta **1995**, *317*, 275-280.
22. Kurtz, K.S.; Crouch, S.R. Anal. Chim. Acta. **1991**, *254*, 201.
23. Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Amperometric Determination of Cholesterol in Serum using a Cholesterol Oxidase Biosensor with a Polypyrrole/Hydrogel Membrane. Anal. Chim. Acta; in press.
24. Brahim, S.; Narinesingh, D.; Guiseppi-Elie, A. Polypyrrole Containing Hydrogels for the Construction of Clinically Important Biosensors. Biosens. Bioelectron; in press.