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Nanocomposite Polypyrrole-Glucose Oxidase/Poly-ortho-Phenylenediamine Bilayer Biosensor for Detection of Glucose

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Overview

- Introduction
- Ascorbic Acid Interference on Glucose Detection
- Aims of Study – Use of NanoLayers for Biosensing
- Layer by Layer Growth of PPy-GOx/P-oPDA Films
- Stability and Sensitivity of PPy-GOx/P-oPDA Electrode Response
- Stability of GOx in PPy-GOx/P-oPDA Bilayer Arrangement

Commonly Used Conducting Polymers

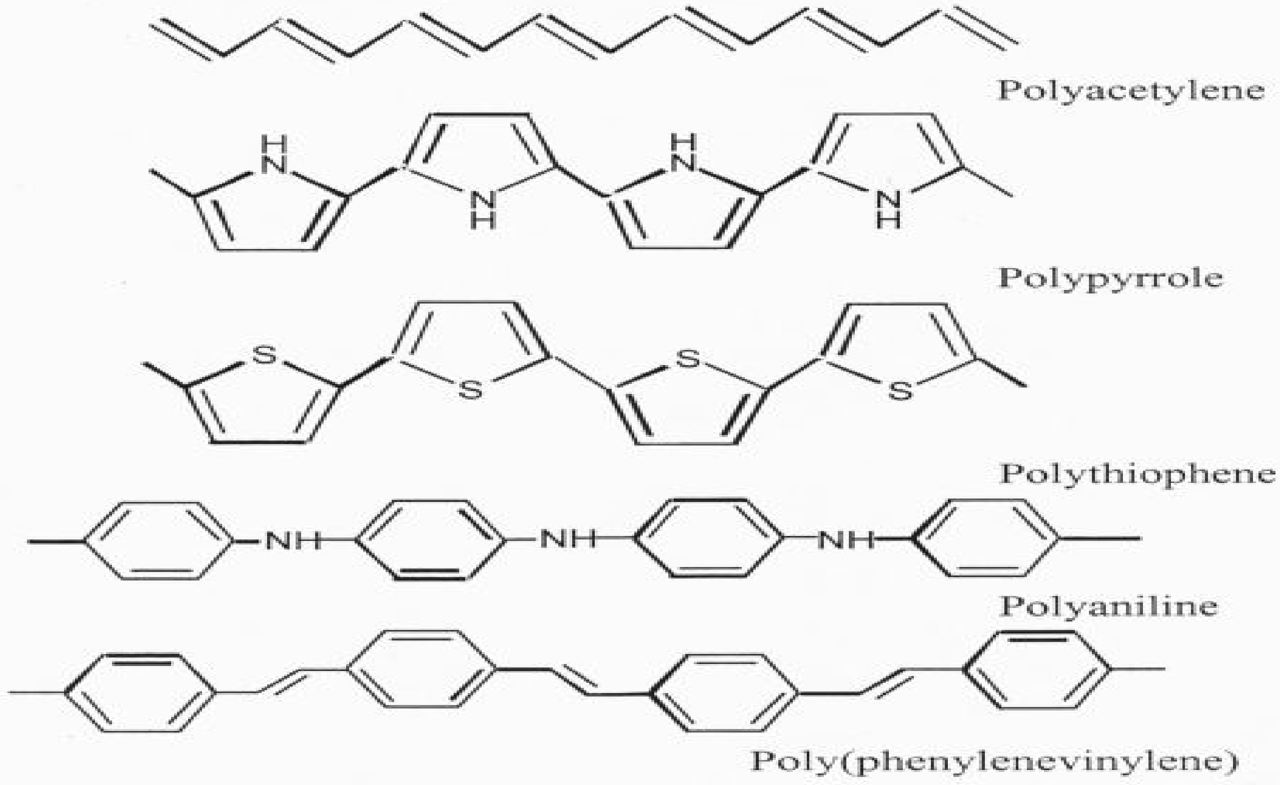


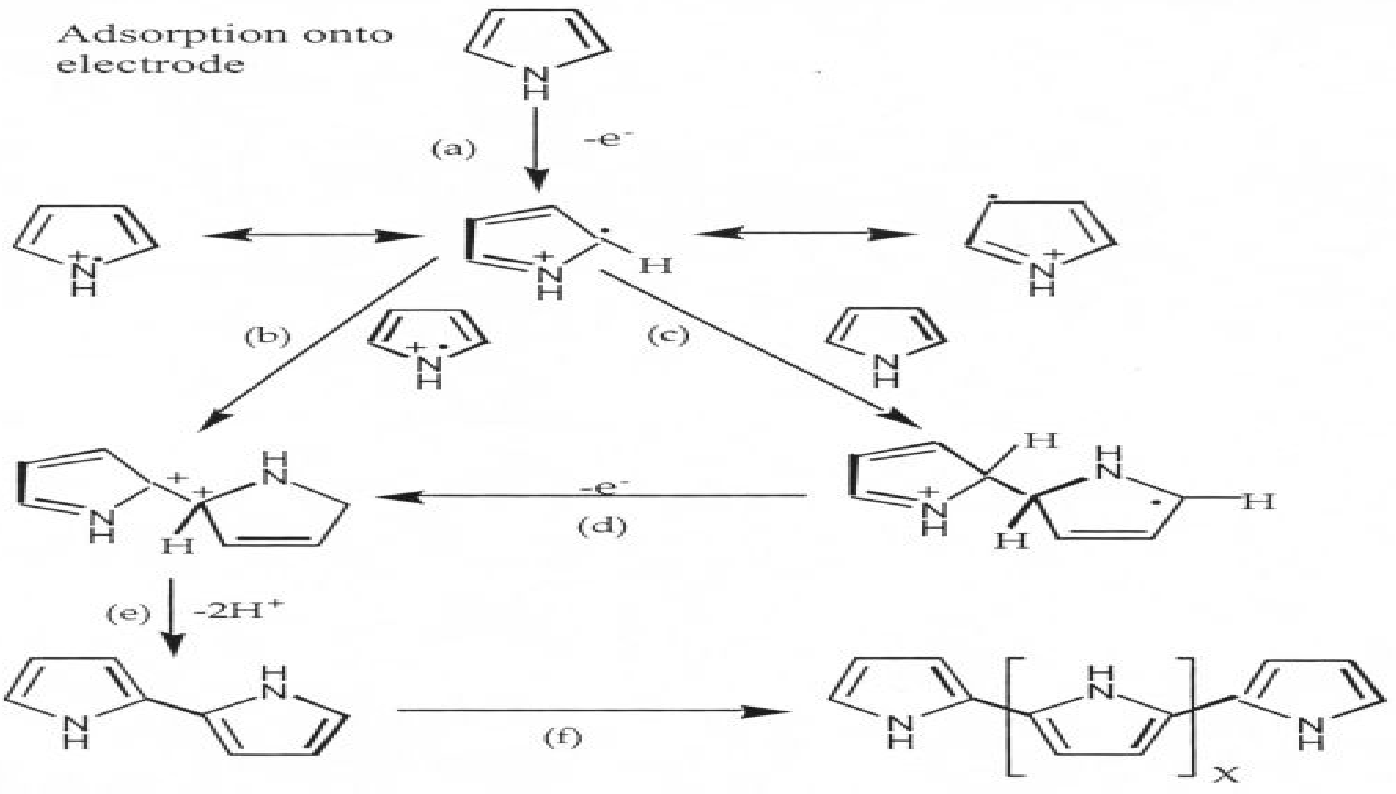
Figure *Chemical structure of some polymers which become electrically conducting after doping. An important feature is the extended domain of conjugated double bonds.*

Useful Properties of Conducting Polymers

Conductivities of Conducting Polymers

Polymer	Conductivity (S cm^{-1})	
	Dedoped Form	Doped Form
cis-Polyacetylene	10^{-7}	10^{-3} - 10^{4}
trans-Polyacetylene	10^{-4}	10^{-3} - 10^{4}
Polypyrrole	10^{-10}	10^{2} - 10^{3}
Polythiophene	10^{-10}	10^{2} - 10^{3}
Polyparaphenylene	10^{-12}	5×10^{2}
Polyparaphenylene Sulphide	10^{-12}	5×10^{2}

Electropolymerization of Pyrrole



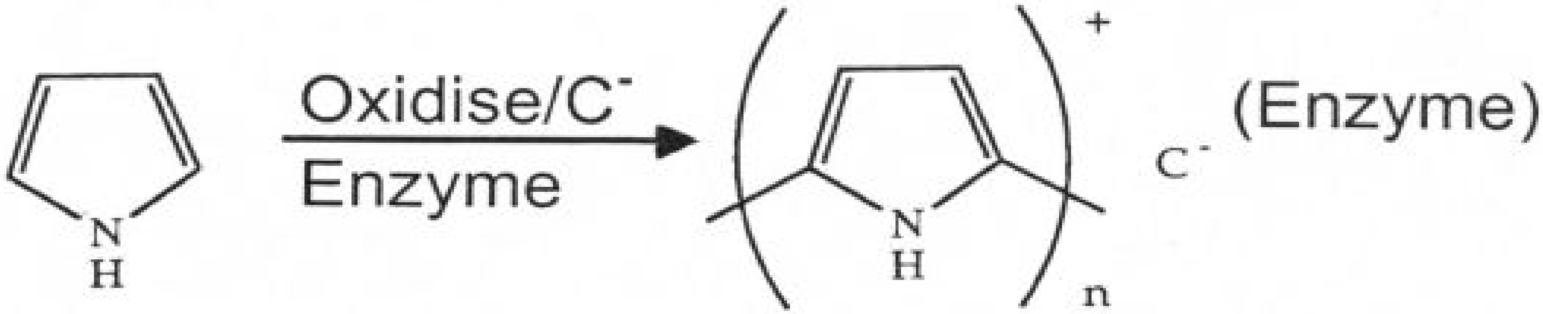
Mechanism for polypyrrole formation.

(a) Oxidation of monomer. (b) Radical-radical coupling. (c) Radical-monomer. (d) Oxidation of dimer radical. (e) Aromatisation. (f) Propagation to form polymer.

Benefits of Electropolymerization of Pyrrole for Enzyme Immobilization

- Enzyme Immobilization by Entrapment in Conducting Polymers is One of The Simplest, Quick and Most Attractive Methods for Fabrication of Biosensors
- Use of Polypyrrole Has Gained Much Interest in this Area because of the Ability to Form in Aqueous Solutions
- Polypyrrole is the Most Ideal Conducting Polymer – Easily Polymerised at Low Anodic Potentials from Aqueous Solutions
- Provides Excellent Sensing Medium for Detection of Various Catalytic Products

Electroimmobilization of Enzyme in Polypyrrole Film by Entrapment



- Used in this Study to Entrap Glucose Oxidase in 55 nm Thick PPy Film
- Measured Glucose by Detecting H_2O_2 Generated by Potentiometry or Amperometry

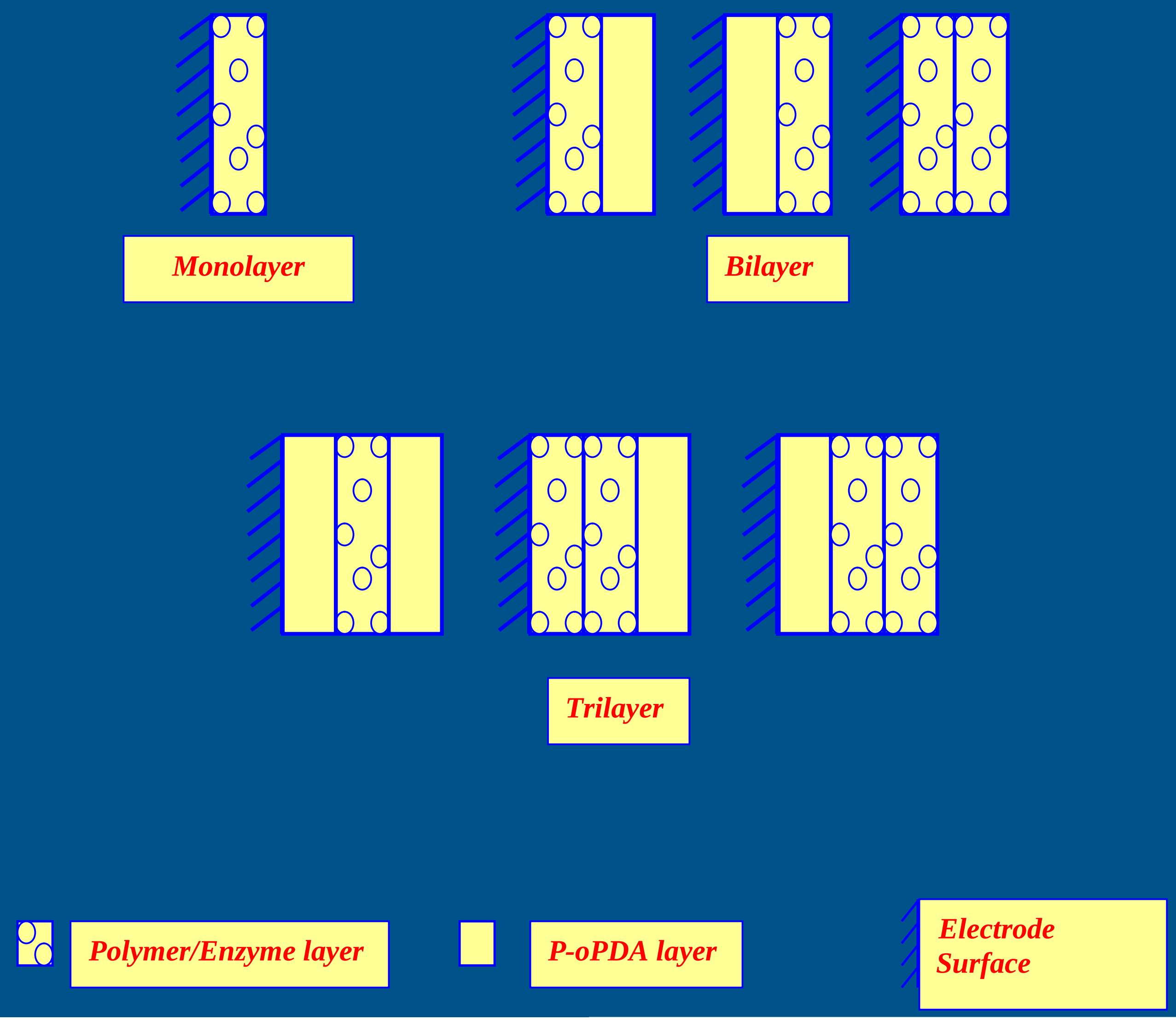
Interference of Ascorbic Acid on Glucose Measurement with PPy-GOx Electrode

- Fabricate a Nanometer Thick Polypyrrole-GOx Single Layer Electrode
- Gave "Super" Nernstian Response (up to 100 mV per Decade) for Potentiometric Detection of Glucose
- Detect as Little as 10 µM Glucose
- Performance was Affected by Presence of Ascorbic Acid, Resulting in Enhancement of Glucose Response
- Need for a Strategy to Reduce or Eliminate Ascorbic Acid Interferences to Attain Optimum Performance
- Consider Use of Non-Conducting (Insulating) P-o-PDA Film as an Additional Layer over the PPy-GOx Layer
- P-o-PDA Film can be Readily Permeated by Protons, but Not by Large Molecules

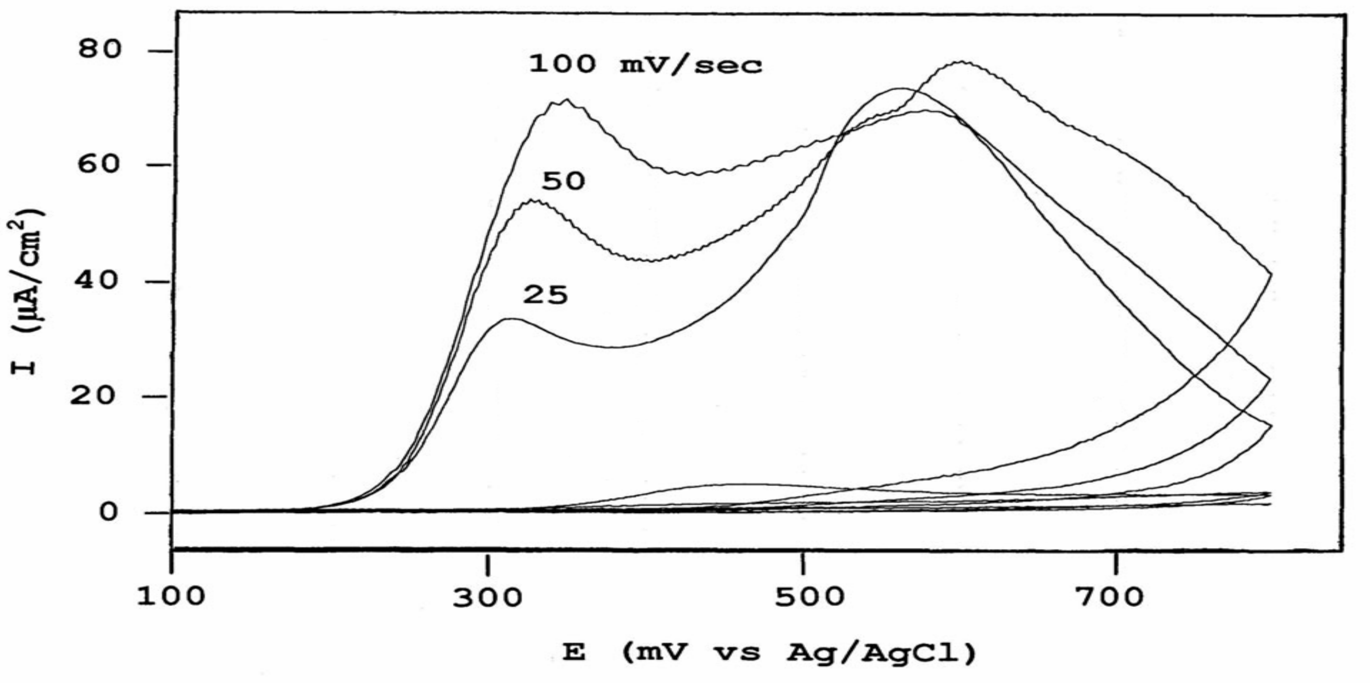
Aims of Study

- Develop Strategy for Growing Non-Conducting Poly-ortho-Phenylenediamine (P-o-PDA) Film on Conducting Polypyrrole-Glucose Oxidase Film
- Investigate the Effectiveness of the Use of Poly-ortho-Phenylenediamine (P-o-PDA) Film for Removing Ascorbic Acid Interference from Glucose Determination
- Study the Effects of Hydrodynamics on Potentiometric and Amperometric Biosensing of Glucose with the Bilayer Electrode

Strategy for Layer by Layer Electrochemical Fabrication of Composite Biosensors

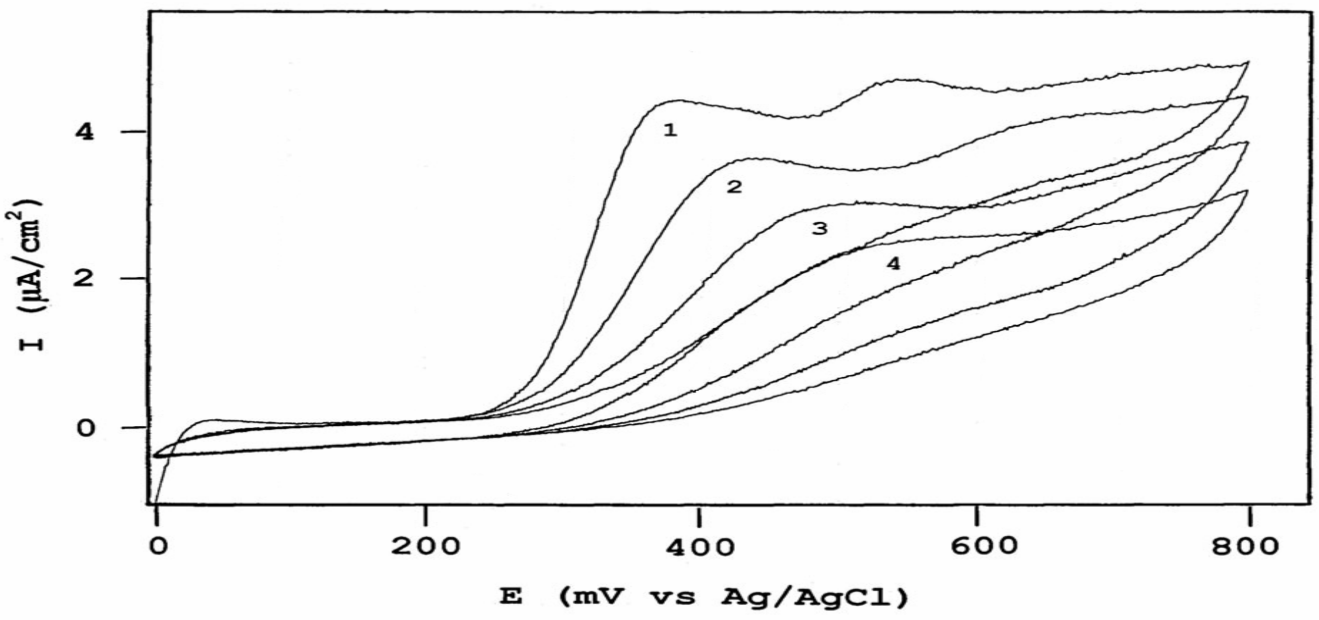


Electropolymerisation of o-PDA in KCl



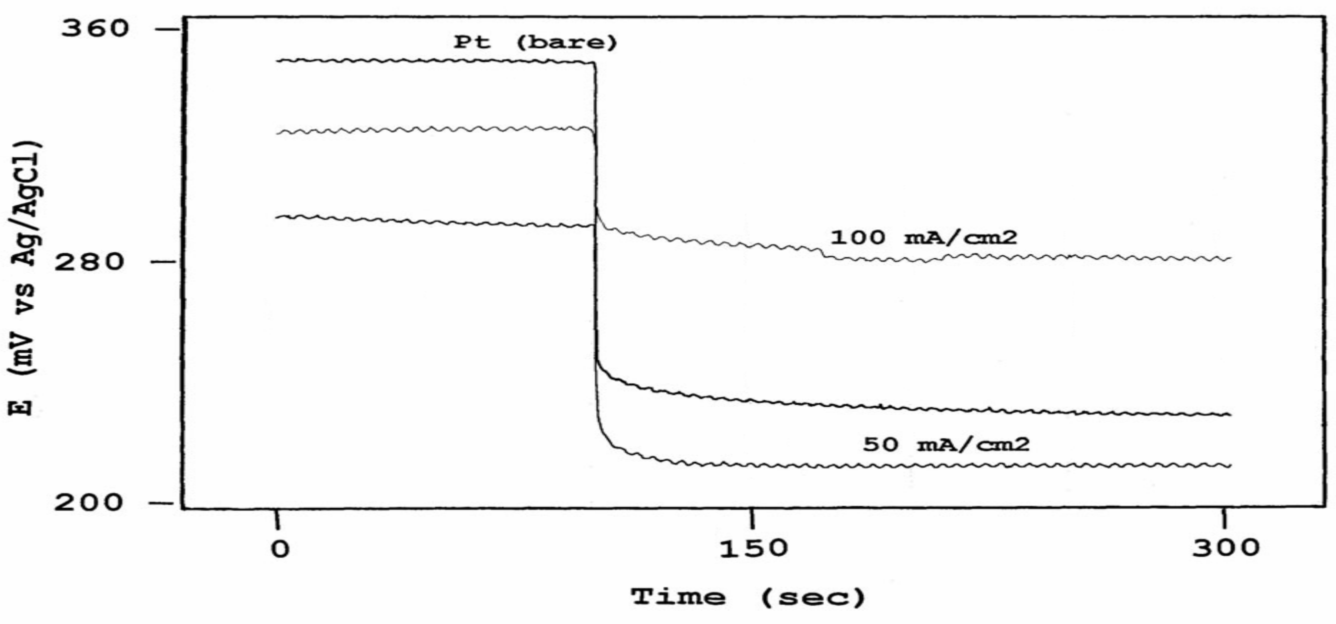
- 1st Peak Current Magnitude is Directly Proportional to Square Root of Scan Rate and is Associated with o-PDA Oxidation
- 2nd Peak Current is Independent of Scan Rate and Attributed to the PolymerisationProcess

Electropolymerisation of o-PDA in Phosphate Buffer



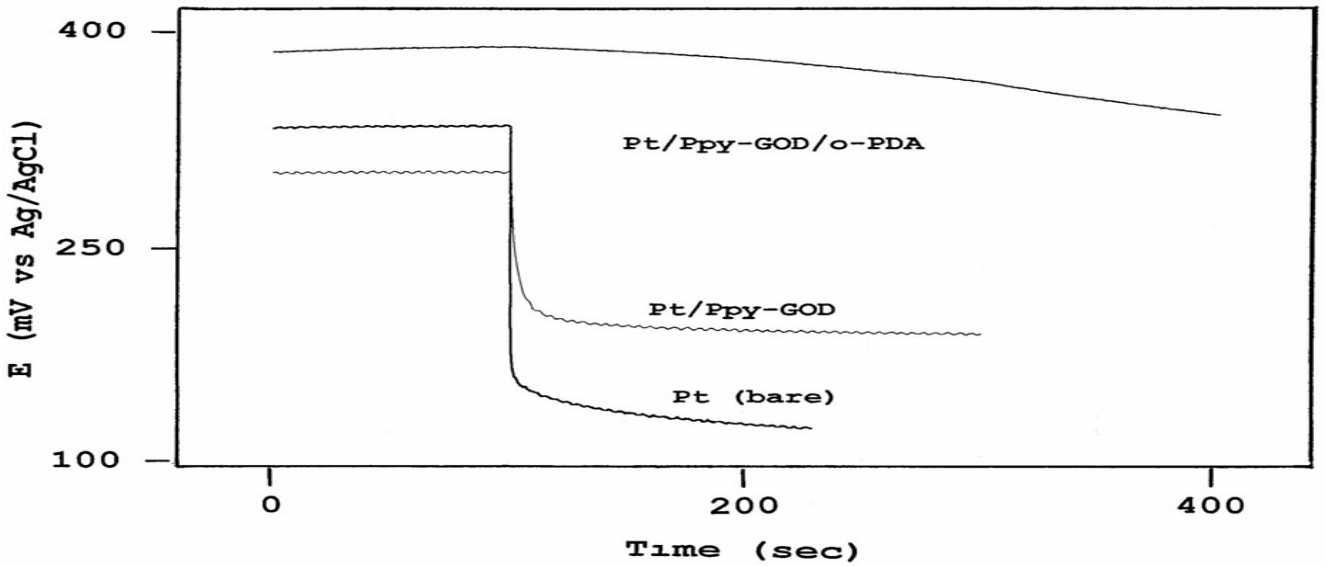
- Anodic Peak Currents Decreased Significantly with Repeated Cycle
- Current Magnitudes were Lower than in Chloride Solution, Possibly Due to Difference in Electroconductivity of the P-o-PDA films in the different Media
- Chosen as it Enabled Better Regulation of Film Thickness at Low Current Magnitude

Permeability of P-o-PDA Film by H_2O_2



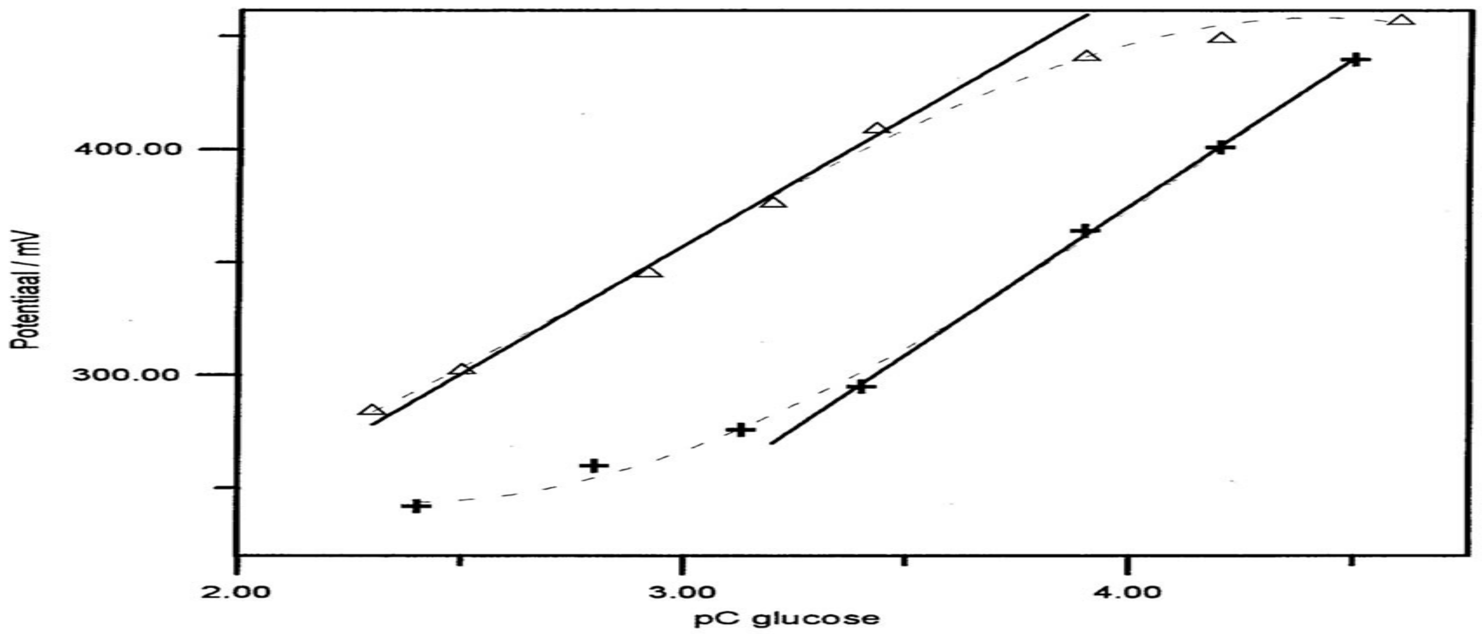
- P-o-PDA Films are Highly Permeable to H_2O_2 as Evident with Different Thickness
- Response Decreased with Increased Film Thickness
- Use of P-o-PDA as an Outer Layer in a Bilayer Arrangement will Enable Adequate Detection of Glucose via its Catalytic Product (H_2O_2)

Permeability of PPy-GOx/P-o-PDA Bilayer by Ascorbic Acid



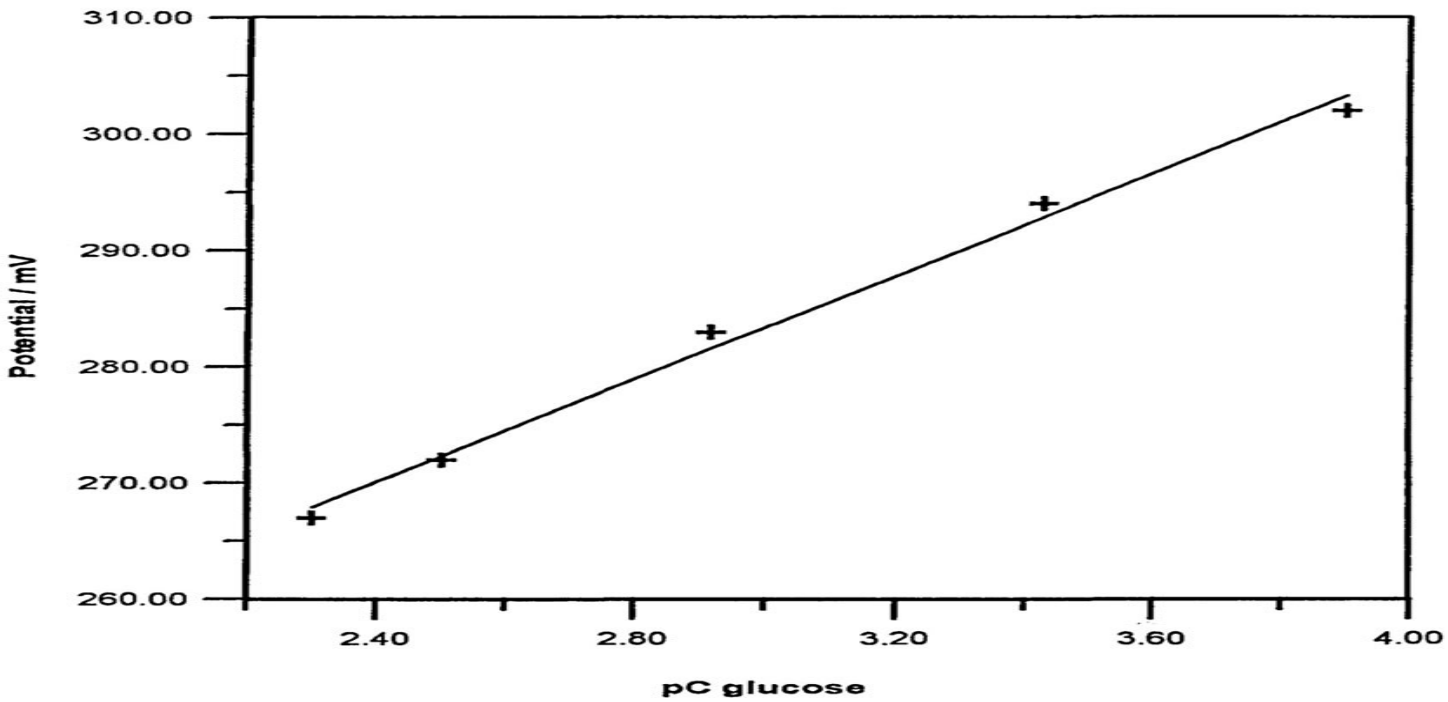
- Bilayer Arrangement of Pt/PPy-GOx/P-o-PDA Eliminate Ascorbic Acid Interference
- Use of Pt/P-o-PDA-GOx Electrode Gave Low Sensitivity (27 mV/dec) and Long Response Time for Potentiometric Sensing of Glucose
- Use of a Pt/PPy-P-o-PDA-GOx Electrode (Q = 30mC/cm2) Gave High Sensitivity (84 mV/dec); Ascorbic Acid Interference as for Pt/PPy(0.1M)-GOx Electrode

Potentiometric Response of PPy-GOx/P-o-PDA Bilayer Biosensor



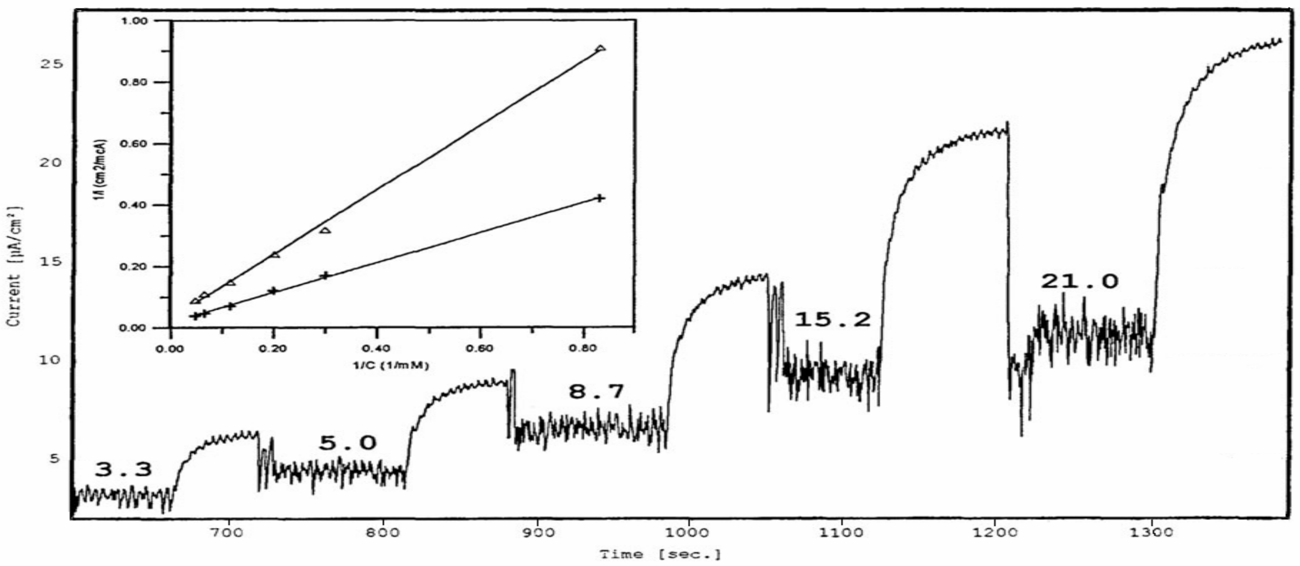
- Gave Very Sensitive Potentiometric Response to Glucose (about 100 mV/dec) and Not Affected by Ascorbic Acid and was Highly Stable with Time
- Significantly Influenced by Hydrodynamic Conditions (Electrolyte Stirring Rate)
- Responses in Stirred Solutions (Δ)were More Sensitive than in Stagnant Solutions (x)

Potentiometric Response of P-o-PDA/PPy-GOx Bilayer Biosensor



- Less Sensitive Responses than Obtained with Pt/PPy-GOx/P-o-PDA Biosensor
- May be Related to Better Retention of GOx in Pt/PPy-GOx/P-o-PDA Bilayer and the Nature of Polymer Closest to the Sensing Medium
- Best Potentiometric Response for Glucose and Minimum Ascorbic Acid Interference was Obtained with a Pt/PPy-GOx/P-o-PDA Bilayer Arrangement

Amperometric Response of PPy-GOx/P-o-PDA Bilayer Biosensor



- Amperometric Response was Less During Stirring than in Stagnant Solution
- Convectional Diffusion of Glucose to the Surface is Not a Limiting Step of Electrode Process, Otherwise Response Should Increase in Proportion to the Stirring Rate
- Increase in Solution Stirring Increases Transport of H_2O_2 from Film to Solution and, Hence, Lowers $[H_2O_2]$ at Electrode Surface, Resulting in Less Current Response

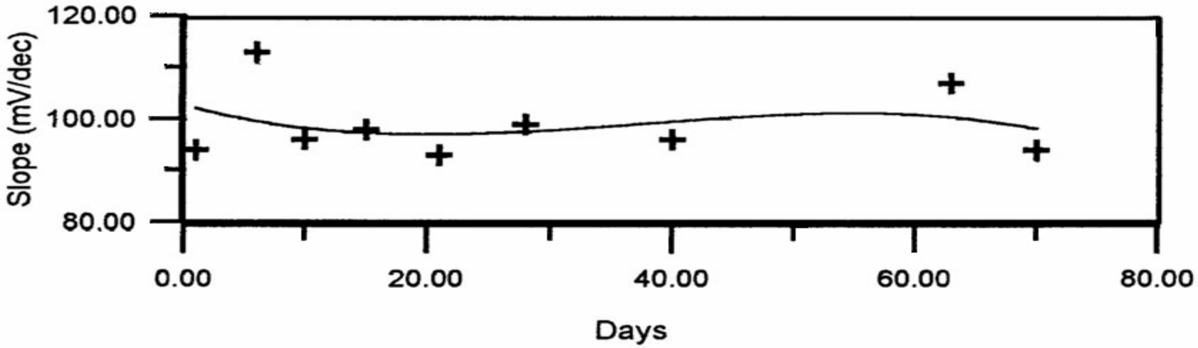
Analysis of Amperometric Response

- According to Lineweaver-Burke Equation:

$$1/i = (K_m/i_{max})(1/C) + 1/i_{max}$$

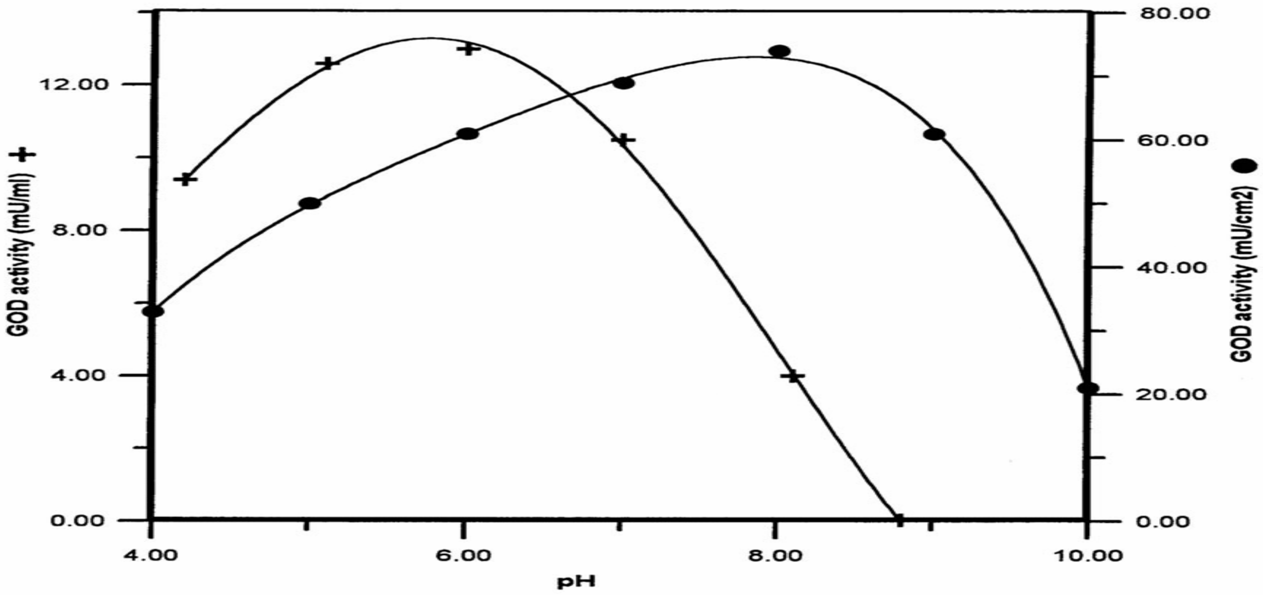
- Plot of 1/i vs 1/Cglucose Should Give Straight Line with a Slope Equal to (K_m/i_{max}) and Intercept Equal to $(1/i_{max})$
- From this, Achievable imax is Higher for Stagnant Solution (56 mA/cm^2) than for Stirred Solution (33 mA/cm^2); Consistent with the Associated Transport Processes
- However, Michaelis Constant (K_m) is Less for Stagnant Solutions (27 mM) Compared with Stirred Solutions (34 mM)
- Both were Much Higher than for Non-Immobilised GOx (usually about 7 mM)
- Explained by the Fact that the Lineweaver-Burke Equation is True for Rate of Catalytically Controlled Enzymatic Reaction
- For Stagnant Solution the Enzymatic Reaction may be Partly Mass Transport Controlled (Mixed Kinetics), So Calculated Km from Slope will only be "Effective K_m", Rather than True K_m

Stability of Potentiometric Detection of Glucose with PPy-GOx/P-o-PDA



- Bilayer Arrangement Gave Good Response to Glucose, Experienced Little or No Interference from Ascorbic Acid and Maintained High Stability over 70 days

GOx Activity in Solution and PPy-GOx/o-PDA Bilayer



- Measurement of GOx Activity in Solution Reveals its Maximum Activity at pH ~5.5 and was Nearly Completely Inactivated at pH 8.8
- Activity of Immobilised GOx in Pt/PPy-GOx/P-o-PDA Shows its Maximum Activity at pH ~ 8 and was Still Active (~ 25 % of Maximum) at pH ~ 10
- Evidently Immobilisation of GOx into PPy Film Results in Improved Stabilization and Extended Lifetime of the Biosensor

Conclusions

- Formation of P-oPDA Film Over PPy-GOx Layer Provided a Versatile Approach for Removal of Ascorbic Acid Interference on Glucose Detection
- Stirring of Solution (Hydrodynamics) Enhanced Potentiometric Response, but Decreased Amperometric Response due to Diffusion of H_2O_2 from Surface into Solution
- Additional P-oPDA Layer Improved the Containment and Retention of GOx
- Stability and Activity of GOx in the Bilayer Arrangement was Improved and Extended from pH 8.8 to pH 10.0

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