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Optimization of Enzyme and Mediator Concentrations in Chitosan-Based Membranes for Enhanced Performance of Second-Generation CGM Devices

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Abstract

Diabetes affects over 415 million people globally, emphasizing the need for effective glucose monitoring systems.

Continuous glucose monitoring (CGM) devices rely heavily on membrane performance, which must be selective and sensitive to glucose while maintaining long-term stability. This study optimizes glucose oxidase and mediator concentrations in chitosan membranes to improve CGM performance.

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Stability Analysis of Operating Range

The stability of enzymatic membranes was tested on screen-printed electrodes. The operational range, with glucose oxidase activity from 0.125 U to 15.625 U per electrode, spanned 0 to 1.5 mM.

Sensor performance was limited by glucose diffusion or mediator electron transfer, not by the enzyme's reaction rate. Adding a membrane to control glucose diffusion expanded the range, highlighting the mediator as the limiting factor. Five electrodes were prepared for each membrane composition, varying glucose oxidase and neutral red concentrations. After 21 days in phosphate buffer, the operational range remained stable, though higher mediator concentrations led to neutral red leaching.

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Results

- Cyclic voltammetry in PBS with and without mediators (Neutral Red and Nile Blue) revealed significant differences in electrochemical behavior.
- Neutral Red (NR) produced a higher current peak at 25 mV/s, indicating more efficient electron transfer compared to Nile Blue (NB).
- Glucose oxidase type VII supported a broader range of enzyme activities but did not significantly enhance overall sensor performance.
- The limiting factors were glucose diffusion and electron transfer through the mediator.
- Coating the electrode with a membrane expanded the operational range, highlighting the importance of optimizing mediator concentration over enzyme activity for improved CGM device performance.

Membrane Components

The membrane consists of a chitosan matrix cross-linked with glutaraldehyde. Glucose oxidase (types II and VII) was used as the enzyme, while neutral red acted as the electron mediator.

Bovine serum albumin (BSA) was added to stabilize protein concentration within the membrane. Variations in enzyme activity and mediator concentration were tested to optimize membrane performance for CGM applications.

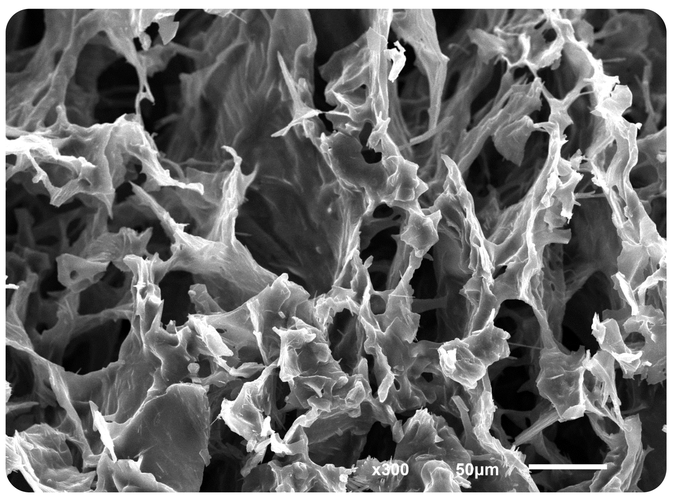
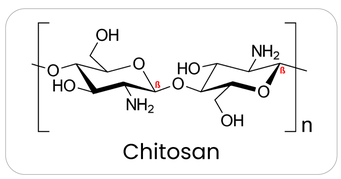
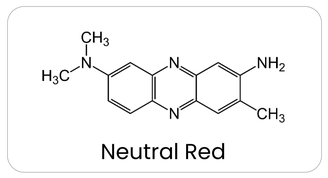


Figure 1. SEM Micrograph of Chitosan-Based Membrane



Chitosan



Neutral Red

Wearable Device Prototype Development

Electrochemical properties

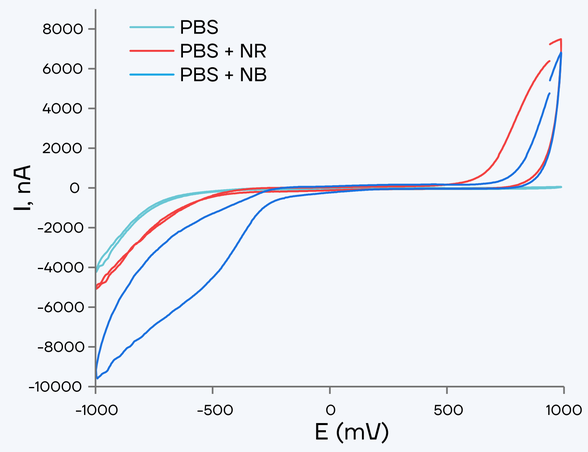


Figure 2. Cyclic Voltammetry of Various Solutions

Chronoamperometric Response of Sensor Without Diffusion-Limiting Layer

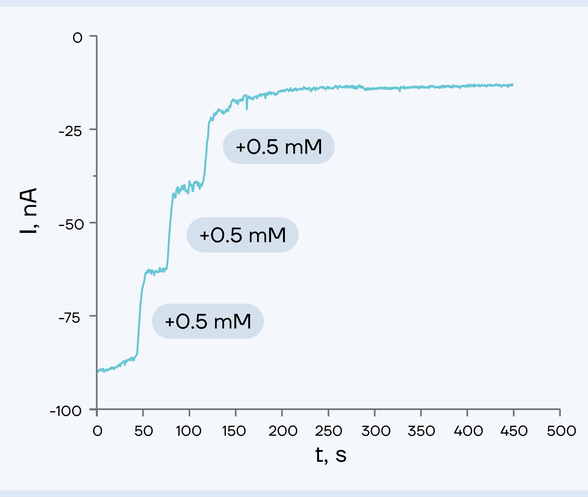


Figure 3. Amperometric responses with successive glucose injection at low concentration. The applied potential was 0.4 V vs. Ag.

Transition from Screen Printed to Nafion-Protected Microelectrodes

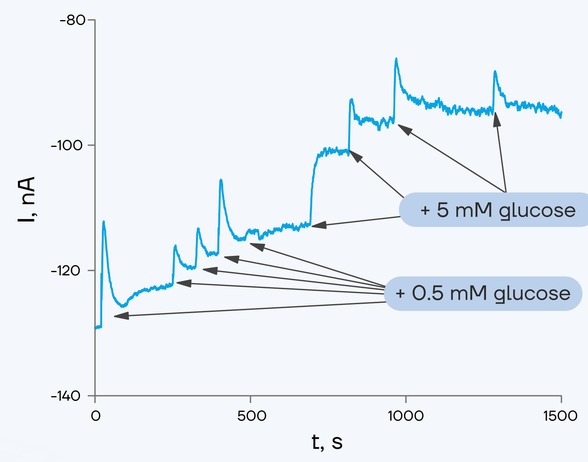


Figure 4. Amperometric responses with successive glucose injection at low concentration. The applied potential was 0.4 V vs. Ag

Discussion

1. This study aims to develop a domestically manufactured device by optimizing the concentrations of glucose oxidase and mediators in chitosan membranes.
2. These efforts contribute to the development of competitive, locally manufactured CGM (continuous glucose monitoring) systems for effective diabetes management.
3. Further research will focus on refining membrane composition and mediator properties in order to improve sensor accuracy.
4. A functional operational range for glucose detection within physiological limits was achieved.

Refference

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2. 2. Arlyapov V. A. et al. On the Development of Reagent-Free Conductive Nanocomposite Systems for the Modification of Printed Electrodes when Producing Glucose Biosensors //Nanobiotechnology Reports. – 2022. – Т. 17. – №. 1. – С. 106-117.