

Optimizing the Enzyme Immobilization Layer of a Levodopa Biosensor

Fine-tuning the BSA and glutaraldehyde to tyrosinase ratio of an L-DOPA biosensor for Parkinson's disease by coupling mass transport, enzyme kinetics, and electrode dynamics in a 1D model.

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Introduction

Tyrosinase-based biosensors offer a promising way to detect L-DOPA, a key drug in Parkinson's disease management¹. In these sensors, L-DOPA diffuses into an immobilized hydrogel layer, where tyrosinase converts it into an electroactive product that reacts at the electrode surface to generate a measurable current. But getting a reliable signal depends heavily on how the enzyme is immobilized². Glutaraldehyde is commonly used to cross-link the enzyme in place², and BSA is added alongside it to protect the enzyme from denaturing². Too much of either, though, creates a

dense matrix that blocks diffusion and slows the sensor's response³, while excess glutaraldehyde also distorts the enzyme's structure and reduces its activity⁴.

This project uses a 1D COMSOL Multiphysics® model to explore that trade-off. By coupling species transport, enzyme kinetics, and electrode kinetics, we map how peak current changes across a range of glutaraldehyde and BSA ratios, validating simulation results against experimental data to optimize the immobilization layer for our SensUs 2026 biosensor.

Methodology

The tyrosinase biosensor is modeled as two coupled domains (Figure 1): an enzyme layer (BSA/glutaraldehyde/tyrosinase) and a diffusion layer extending to the bulk solution. Species transport follows the Electroanalysis interface, with L-DOPA diffusivity reduced inside the gel through a Mackie–Meares effective diffusion coefficient dependent on porosity and BSA tortuosity.

Enzymatic conversion of L-DOPA is described by Michaelis–Menten kinetics, with the catalytic rate constant scaled by factors accounting for GA-induced denaturation, enzyme retention, and BSA's scaffolding role. Electrode kinetics follow Butler–Volmer theory, with parameters derived from EIS and CV characterization of the bare electrode. A full-factorial sweep over BSA and glutaraldehyde ratios was run to identify the formulation maximizing peak current.

Results

The parametric sweep identified an optimal glutaraldehyde:BSA:tyrosinase ratio of 0.5:2:1, avoiding the diffusion restriction and enzyme denaturation caused by excess glutaraldehyde. This was supported experimentally: EIS diffusion measurements showed a much higher diffusion rate at a glutaraldehyde ratio of 0.5 compared to 1, confirming that lower cross-linking density improves mass transport through the enzyme layer.

The resulting biosensor exhibited superior sensitivity and reliability; consequently, it was awarded the Analytical Performance Award at the international SensUs 2026 competition across a field of 18 teams from 12 countries.

REFERENCES

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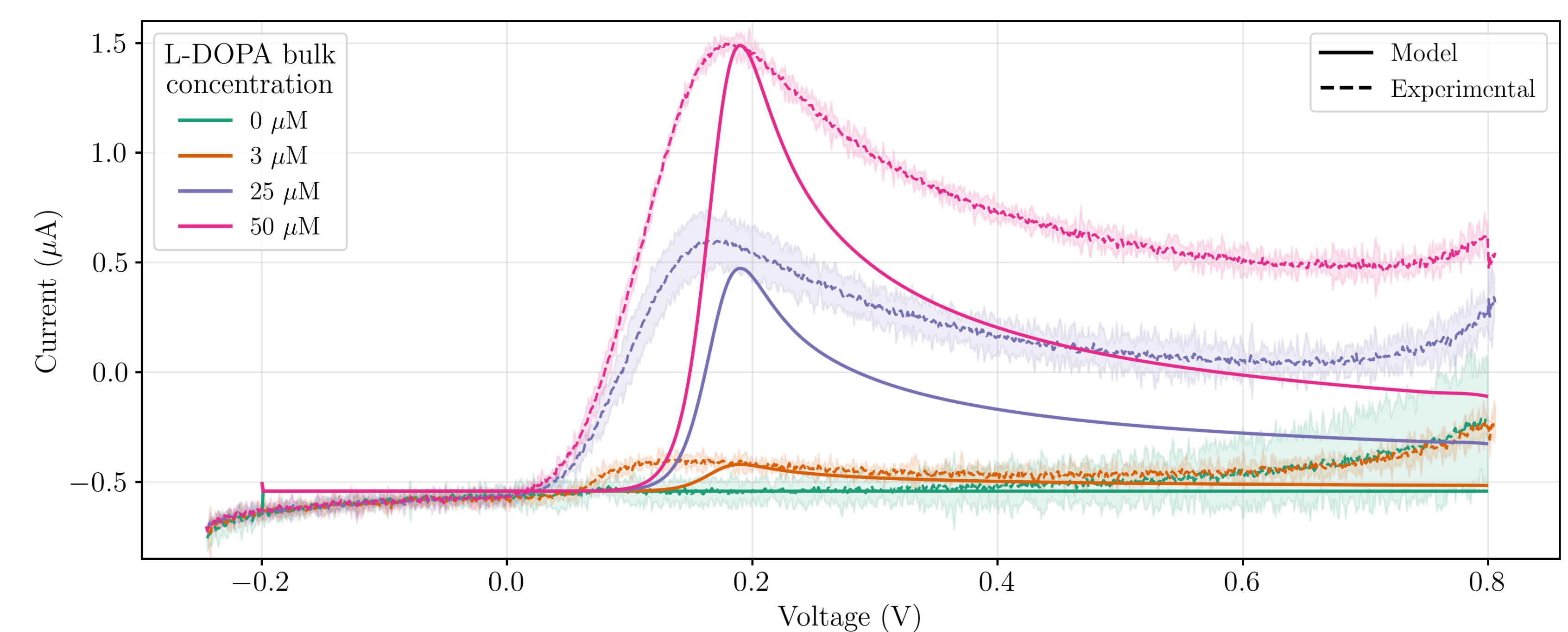
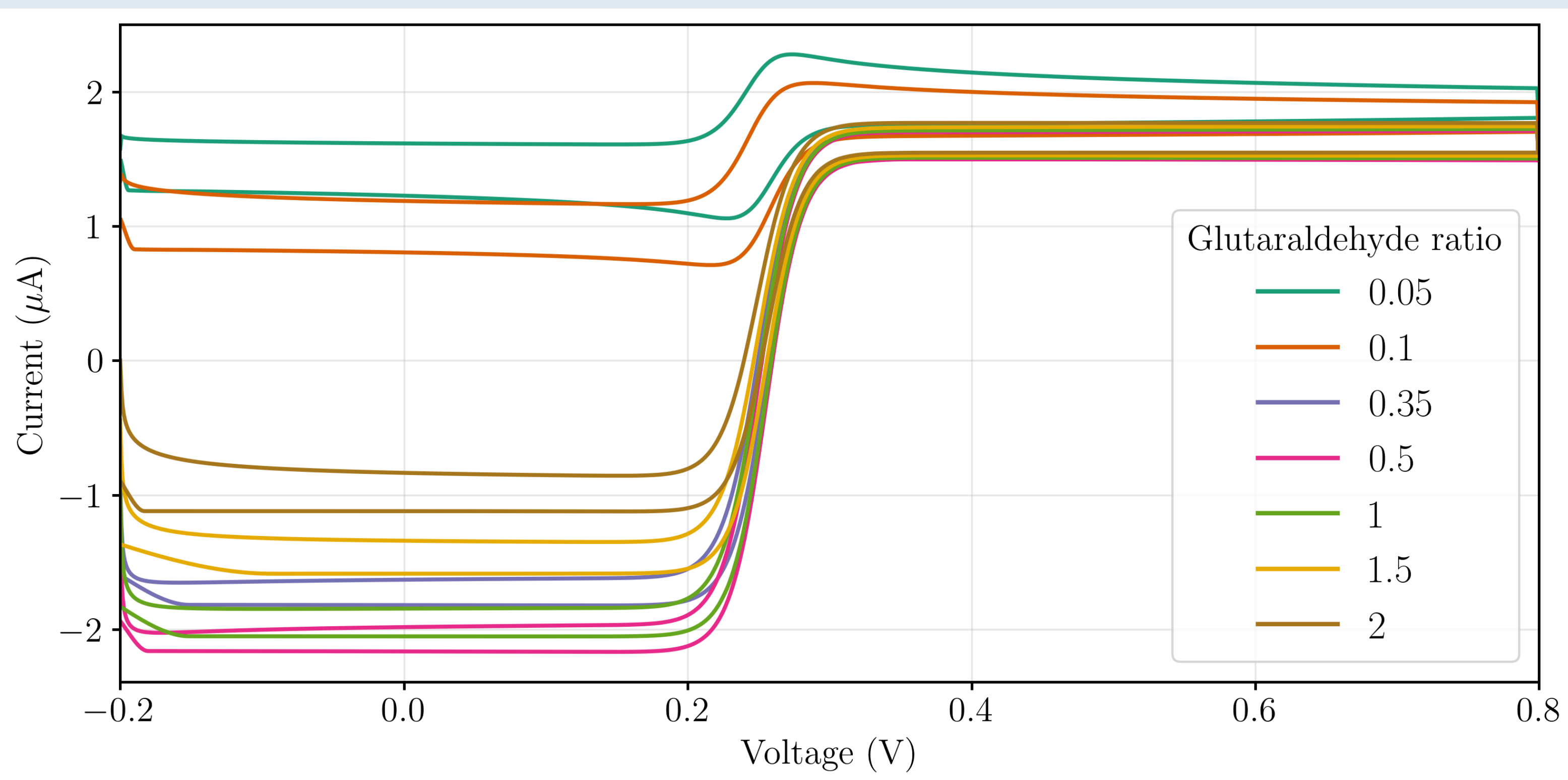


FIGURE 2. Baseline cyclic voltammetry of the bare electrode in PBS at varying L-DOPA bulk concentrations, comparing experimental data and the corresponding COMSOL modeling data (bulk diffusion and electrode kinetics only, no enzyme layer). Both show the anodic peak current increasing with L-DOPA concentration, validating the model's baseline diffusion and electrode kinetics before introducing enzymatic functionality.