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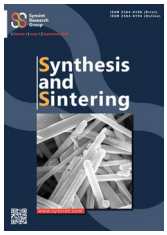

Research article

Comsol Multiphysics modeling of an electrochemical biosensor using carbon nanotubes for detecting urinary estrogen receptor

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ABSTRACT

A class of steroid hormones known as estrogens is essential for the health of the heart, bones, and reproductive system. Changes in estrogen levels have been connected to several health problems, such as endocrine disorders, metabolic syndromes, and cancer. In pharmaceutical applications, environmental monitoring, and medical diagnostics, biosensors that measure estrogen levels are essential. This study models estrogen detection biosensors based on urine liquid, horseradish peroxidase biorecognition, and carbon nanotubes (CNT) using Comsol Multiphysics. This study demonstrates that most interactions happen at the upper boundary of the concave pillars put inside the box. Besides, it shows that the velocity has the highest value between the concave pillars inside the box. The results demonstrate that the number of interactions (absorption and adsorption) rises with increasing the concave pillars' area, affecting the biosensor output.

KEYWORDS

Biosensors
Estrogen detection
Nanomaterials
Hormone monitoring
Electrochemical sensors

OPEN ACCESS

1. Introduction

Estrogen is an important hormone in both men and women, playing a role in breast development, the urogenital tract, cardiovascular, and central nervous systems [1, 2]. There are three types of estrogen in the human body including estrone (E1), estradiol (E2), and estriol (E3), each of which is made in a specific period; E1 is secreted after menopause, E2 is made during reproductive years of body and E3 is secreted during pregnancy [3]. In addition to estrogen, a protein called estrogen receptor (ER) exists inside the cells in different body tissues such as the breast, ovaries, prostate, etc. It is a type of nuclear receptor that gets activated by binding to estrogen. There are two types of ERs: ERα, and ERβ. ERα is in the uterus, liver, kidneys, and mammary glands and plays a crucial role in sexual development and reproductive function. It's also involved in various cellular processes such as cell growth and differentiation. ERβ is in the ovaries, prostate, lungs, gastrointestinal tract, and central nervous system. Similar to ERα, it has distinct roles in regulating cellular differentiation and proliferation. It often counterbalances the effects of ERα and is involved in protective roles against certain diseases. ERs can bind to estrogen, dimerize, and interact with DNA to regulate gene expression, although the specific genes and regulatory effects can differ for each of them. This binding regulates the transcription of various genes, leading to changes in cellular function [2]. Measurement of the concentration of ERs can help clinicians diagnose the level of cancer and make a better plan for the treatment.

Various techniques have been proposed to detect and measure estrogen, including gas chromatography, gas chromatography-mass spectrometry, and some biological methods like enzyme-linked immunosorbent assay (ELISA). Despite being trustworthy, traditional laboratory-based techniques are frequently unsuitable for routine or point-of-care testing because of their complexity, expense, and time commitment [4]. The demand for portable, user-friendly biosensors that can measure estrogen quickly and accurately in a variety of contexts, such as environmental monitoring and clinical diagnostics, is rising. Biosensors are analytical tools that monitor and quantify compounds by fusing a physicochemical detector with a biological

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component [5]. Molecule-based biosensors like electrochemical, optical, photoelectrochemical, and cell-based biosensors were designed to detect estrogen. They use biological recognition components such as aptamers, enzymes, or antibodies to bind estrogen molecules selectively and produce a signal that can be measured and is proportionate to the hormone concentration [4].

The glucose enzyme electrode developed by Clark and Lyons in the 1960s introduced the idea of biosensors and launched the field of biosensor technology. Biosensors have evolved in complexity and range of applications over the years. Initially, immunoassays were the main approach used for detecting estrogen; these were then improved upon by electrochemical, optical, and piezoelectric biosensors [6]. A bioaffinity sensor was created by Murata to detect estrogen by immobilizing the estrogen receptor on a gold electrode. The binding of estrogen altered the sensor's electrochemical response, indicating its effectiveness in evaluating interactions with the estrogen receptor [7]. A fluorescence resonance energy transfer (FRET)-based biosensor was developed to detect ER ligands, crucial for menopause treatment. The biosensor, with a modified ER ligand-binding domain (LBD) flanked by cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP), enhances the FRET signal during ligand binding, distinguishing agonists from antagonists. It stabilizes within 30 minutes and is ideal for high-throughput ligand screening, with potential applications for other nuclear receptors like the androgen receptor [8]. A receptor-hormone binding was used for selective detection and employs electrochemical impedance spectroscopy (EIS) for high sensitivity. Modified electrodes with covalently bonded estrogen receptors were characterized using EIS and XPS, successfully detecting estrogen at 10^{-6} M concentration [9]. ELISA was the method used by early estrogen biosensors, was accurate but time-consuming, and required significant sample preparation. A sandwich-type electrochemical biosensor was developed to detect estrogen. It was a chitosan-coated platinum electrode with an immobilized antibody (anti-17-β estradiol) on it [10]. Since then, developments in materials science and nanotechnology have accelerated the creation of biosensors that are quicker and more sensitive [5]. A fungal biosensor was validated using genetically modified Aspergillus nidulans with a human estrogen receptor, for detecting estrogen activity in cow samples. It performed well across various matrices and showed high accuracy for pregnancy diagnosis in mares and cows, with a detection limit of 1 ng/l for 17β-estradiol, offering a sensitive, cost-effective, and high-throughput alternative to traditional methods [11]. An ultrasensitive enzyme sensor for detecting 17β-estradiol was developed using L-lysine electropolymerized on a glassy carbon electrode modified with citric acid@graphene and laccase cross-linked with glutaraldehyde. The sensor showed a detection limit of 1.3×10^{-13} M and high selectivity for 17β-estradiol, performing well in human urine analysis, indicating strong potential for clinical and biological applications [12]. Various mechanisms can be utilized in a biosensor, as previously discussed.

Existing biosensors for the detection of estrogen still confront difficulties despite great progress, including low sensitivity at low concentrations, restricted selectivity in intricate biological matrices, and stability and repeatability problems. Furthermore, a lot of the gadgets on the market now are not user-friendly or appropriate for point-of-care uses. Innovative approaches to biosensor design that incorporate cutting-edge materials and cutting-edge detection strategies are needed to overcome these constraints [13].

This study aims to design an electrochemical estrogen-detection biosensor that overcomes the limitations of current technologies, including increasing sensitivity, specificity, repeatability, and stability of the biosensor, developing an interface that is easy to use and appropriate for non-expert users. By fulfilling these goals, this biosensor can be offered for a range of uses, such as pharmaceutical research, environmental safety monitoring, and clinical diagnostics, ultimately improving health outcomes.

2. Methodology

Comsol Multiphysics version 6.2 was used to design this biosensor. It is a computer-aided engineering platform that uses finite element analysis to provide a range of analysis and solution capabilities. It encompasses a broad spectrum of applications across different physics domains and can handle complex scientific and large-scale engineering challenges. It simplifies finite element programming and excels in solving mixed multiphysics phenomena simultaneously [14].

2.1. Comsol Multiphysics

First, the model environment is configured according to the design requirements, including setting global parameters and functions. Next, the geometry is designed and optimized to meet these requirements as closely as possible. After establishing the geometric structure, the material for analysis is specified, either from predefined options or custom selections. This material is then assigned to the relevant geometric domains. The study's physics are incorporated to support or drive the analysis, with constraints defined based on boundary conditions and design specifications. Finally, a mesh is generated, which can be either predefined or customized as needed.

The choice of simulation type, or study, depends on the specific settings required for each study. After computation, results can be visualized using various plotting options, including 3D, 2D, and standard graphical plots for numerical analysis.

Comsol Multiphysics operates in three stages: pre-processing, solution, and post-processing. Pre-processing involves creating the finite element model and setting up the environment parameters [15]. The solution stage includes mesh division and equation solving. Post-processing focuses on visualizing and interpreting results. Internally, Comsol Multiphysics compiles a set of partial differential equations (PDEs) that represent the entire model. The software employs a finite element approach to solve these PDEs, utilizing various numerical solvers for finite element analysis, with adaptable meshing, feedback, and error management.

2.2. Geometry

This biosensor measures the levels of estrogen in the urine of postmenopausal women. Estrogen levels directly correlate with the risk of developing cardiovascular-related diseases. As it was mentioned before, the most potent estrogen during the reproductive years is E2 which is the analyte in this study. Hence, the biorecognition element for this biosensor is the enzyme horseradish peroxidase which has strong binding specificity for E2 [16]. The surface substrate that was used for coating the receptors is carbon nanotubes (CNT) for its exceptional properties [17]. CNTs have high tensile strength, fast electron transfer kinetics, and a large surface area, are

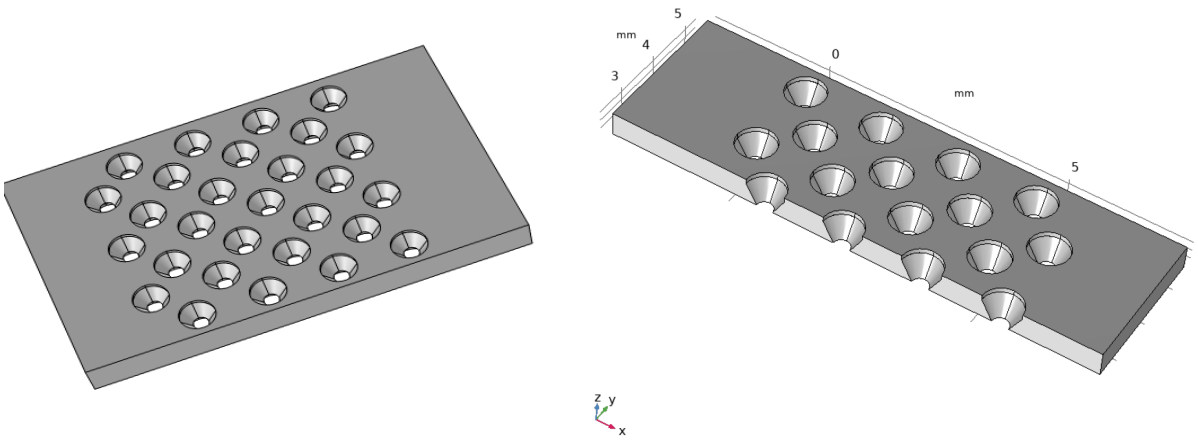

Fig. 1. The geometry of the biosensor, a) whole biosensor and b) computational geometry.

lightweight, highly biocompatible, chemically inert, and help in protein immobilization. This project aims to determine the surface adsorption of the CNT with the concave pillars at an adjusted angle [18].

The geometry that was designed is composed of a rectangular box with a group of concave pillars enclosed within it [19]. This geometry is a symmetrical shape that can be divided into two sections. The analysis of one of these sections is enough as it requires less time and memory and has less complexity of computations. The whole and simulated geometry are demonstrated in Fig. 1. Fig. 2 demonstrates the mesh structure of the geometry. As it shows, the reacting CNTs and the edges of the box have more small mesh elements, and the modeling there should have more accuracy [20].

This model is a three-dimension model. The rectangular box has the following dimensions 10×6×1 mm and a set of concave pillars enclosed within the box and acts as the reacting surface. The selected physics are the transport of diluted species, surface reactions, and laminar flow for determining the surface adsorption for the chosen material with the concave pillars angled at 30 °. The study for the model is time dependent and the materials are CNT and urine.

2.3. Materials

In this paper, the reaction rate between CNTs and urine samples of the human body was simulated on Comsol Multiphysics. There are three liquids from the human body including blood, urine, and saliva that are easy to access. The characteristics of these three samples are shown in Table 1 [19, 21–23].

2.4. Mathematical model

A surface reaction mechanism is employed where analyte molecules (A_m) adsorb and desorb from surface sites (S_s) on the substrate. The parameters involved in this process are listed in Table 2 [19].

The adsorption process can be represented as:

$$A_m + S_s \rightleftharpoons S_s \tag{1}$$

It's possible to convert the adsorbed analyte into a quenched state, which does not contribute to the sensor signal:

$$A_m + S_s \rightleftharpoons Q_s S_s \tag{2}$$

C_{Am} is the concentration of the analyte in the stream and the adsorption rate is given by:

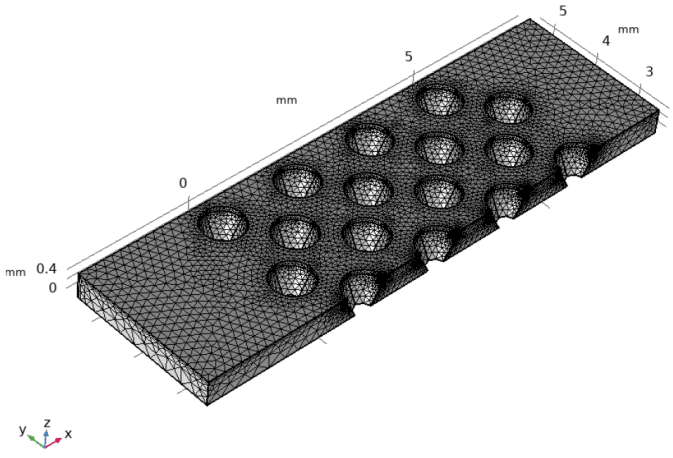

Fig. 2. A normal mathematical mesh applied to geometry.

Table 1. Physical properties of blood, saliva, and Urine.

	Blood	Urine	Saliva
Dynamic viscosity	0.00035 Pa.s	0.001 Pa.s	0.0015 Pa.s
Density	1050 kg/m^3	1030 kg/m^3	1048 kg/m^3
Coefficient of thermal expansion	0.0003 1/K	0.00021 1/K	0.00021 1/K
Bulk viscosity	0.0004 Pa.s	0.025 Pa.s	0.003 Pa.s
Electrical conductivity	100 µS/m	2.2 S/m	0.5 S/m
Heat capacity (at constant pressure)	3617 J/(kg.K)	4178 J/(kg.K)	3760 J/(kg.K)
Thermal conductivity	0.5 W/(m.K)	0.56 W/(m.K)	0.51 W/(m.K)
Speed of sound	1570 m/s	1537 m/s	1559 m/s

$$r_{ads} = k_{ads} C_{Am} \quad (3)$$

For the surface-adsorbed species concentration $C_{Am}S_S$, the desorption rate is linear:

$$r_{ads} = k_{ads} C_{Am} S_s \quad (4)$$

The mass transport of the analyte in the stream is described by the equation:

$$\frac{dC_{Am}}{dt} + \Delta(-D_{Am}.\Delta C_{Am}) + u.\Delta C_{Am} = 0 \quad (5)$$

where D_{Am} is the diffusion coefficient, C_{Am} is the species concentration, and u is the velocity vector (m/s).

Table 2 outlines the key parameters that define the operation of the biosensor, which are used as inputs in the Comsol Multiphysics model for CNT analysis. The forward rate constant represents the rate of the forward reaction (conversion from reactants to products), while the backward rate constant characterizes the rate of the reverse reaction (conversion from products back to reactants). The adsorption rate constant describes the rate at which analytes adsorb onto the surface.

3. Results and discussion

After conducting a stationery and time-dependent study for the transport of fluid species and surface reaction, laminar flow physics respectively, the following results were obtained.

Fig. 3 demonstrates the concentration of liquid in different areas of geometry. Following the color legend, it is seen that the reaction occurs at the upper boundary of the concave pillars. This is because of the flux at this region that allows the analyte-enzyme (Estradiol-horseradish peroxidase) reaction to take place as well as the effect of the Gaussian pulse controlling analyte flow. The blue area denotes no interaction which can be attributed to insufficient concentration of surface substrate and high inlet velocity that does not give the sample enough time to interact with the sensing surface. When the injection pulse amplitude was increased, an upward shift in concentration was expected. Instead, it diminished, which can be explained by an error in the configuration of the model since in ideal conditions the higher the pulse amplitude the higher the concentration. Another justification may be the unnecessarily large sample volume that when compared to lower values, the concentration seems less.

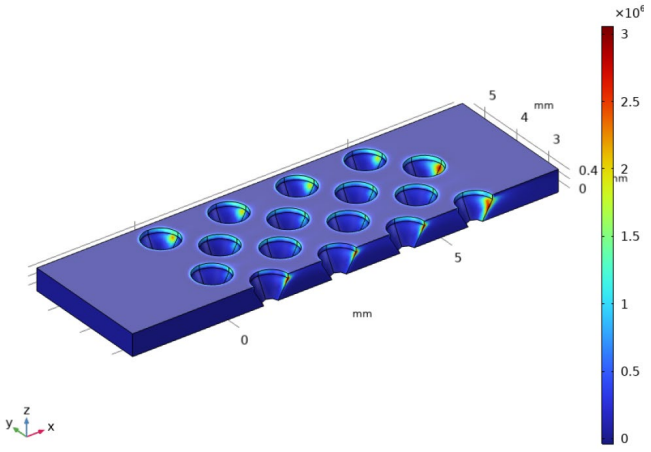

Fig. 3. 3D concentration plot at the time stamp 50 s with urine injection pulse amplitude of 200.

Table 2. Description of the parameters.

Parameter	Description	value
k_ads	Adsorption constant	0.041 m/s
k_des	Desorption constant	0.44 mol/(m^2.s)
kf	Forward rate constant	3.2 × 10^{-7} mol/(m^2.s)
kr	Reverse rate constant	4.1 × 10^{-8} mol/(m^2.s)

A 3D voltage plot of 2D vertical slices is shown in Fig. 4. As it shows, the velocity has the highest value between the concave pillars i.e. sensing surface. This is due to the obstruction of flow by the pillars in conjunction with fluid flow mechanics that dictate acceleration with abate volume and increased pressure.

To observe the flow of input liquid in the biosensor environment, a plot of streamlined flow was obtained showing the total flux. Fig. 5 depicts the liquid flux in the geometry. It shows that the average value was zero because of inbuilt no flux functions in the Comsol Multiphysics program for the transport of diluted species and laminar flow.

4. Conclusions

In summary, the simulation of biosensors in Comsol Multiphysics is a surfier method of investigating the parameters and constraints of such systems without the cost of building a physical device. The ability to adjust parameters, variables, and components while interacting with the software broadens understanding of factors that affect the output. Though this project aimed to investigate the interactions between the analyte and recognition element and reduce computation time by adjusting the dimensions of reactive surfaces, it is important to note that improvements can be made through the substitution of materials with better-suited properties. The above model shows that the side of the sensor with a larger area had higher interaction compared to the side with a smaller area. Through this study, the assumption of optimizing the physical properties of the sensor for more sensitive detection has been partially disproven and the importance of chemical properties was highlighted.

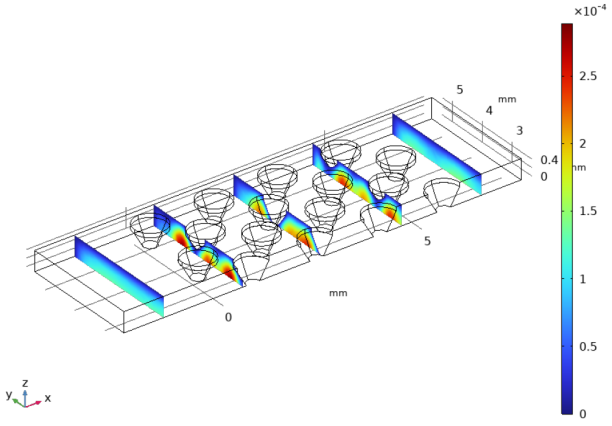

Fig. 4. 3D plot of input urine velocity in vertical slices.

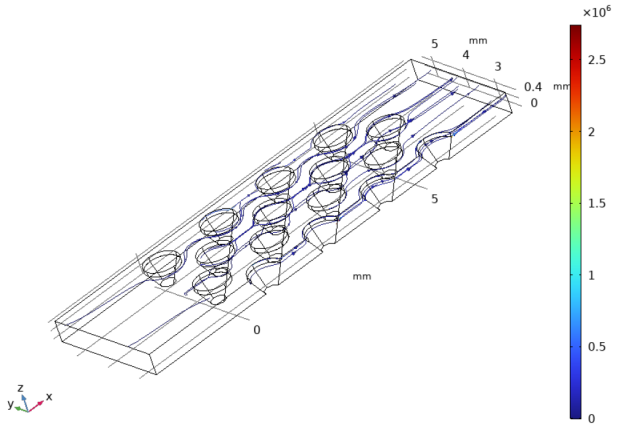

Fig. 5. Plot of streamline total flux of input urine and concentration.

CRediT authorship contribution statement

Lenah Serai Wangare: Validation, Data Curation, Visualization, Writing – original draft.

Thamsanqa Mafika: Conceptualization, Methodology, Investigation, Writing – review & editing.

Muneebah Ally Said El-Kitany: Methodology, Investigation, Writing – review & editing

Shahla Azizi: Project administration, Supervision, Writing – review & editing.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare no competing interests.

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