Approaching single molecule sensing: predictive sweat sensor design for ultra-low limits of detection

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ABSTRACT

Sweat provides direct information of the real-time emotional and cognitive state of the subject, with applications ranging from situational awareness and mission effectiveness of armed forces to disease diagnosis for clinicians. Development of a broad class of human performance monitoring devices to quantify sweat biomarkers necessitates non-invasive, real-time monitoring of ultra-low concentrations (µM to fM) of hormones, proteins, and neurotransmitters. Field effect transistors are the predominant sensor approach whereby the gate electrode is modified with a selective bio-recognition element (BRE). However, FETs have diminished sensitivity in high ionic strength environments associated with sweat. Alternatively, BRE-modified photonic integrated circuits (PICs) have high sensitivity in high ionic strength fluids, low cost at the manufacturing scale, and enable a number of novel device concepts to achieve ultra-low levels of detection. One major technological challenge is to predict the limit of detection (LoD), or sensor response function, for a particular PIC geometry in a microfluidic chamber. LoD is highly dependent on analytic capture efficiency, fluid dynamics and affinity, analyte/light interaction, and analyte concentration. This work presents finite element simulations to emulate microfluidic BRE sweat sensors and provide a predictive limit of detection for different sensoring structures or elements. Specifically, the optimum mass transfer and kinetics for sensing approaching single molecule detection is discussed, including flow characteristics, biomarker size, adsorption and desorption kinetics, and sensor geometry. Key metrics include capture efficiency (molecules being captured over molecules entering channel), time to reach steady state, and temporal adsorption site occupancy to predict PIC system LoD. It is found that these systems are kinetically controlled, with capture efficiencies remaining below 1% even for $k_{ads}/k_{des}$ ratios of $10^3$. The need for adsorption kinetics measured for flow systems instead of stationary fluid systems is stressed, as these parameters are what need to be optimized to greatly increase analyte capture.

Keywords: biosensing, photonic sensing, sweat sensing, bio-recognition elements, fluid dynamics, microfluidics, finite element simulations, photonic integrated circuits

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INTRODUCTION

Sweat biomarker sensing provides direct information of the real-time emotional and cognitive state of the subject, with applications ranging from situational awareness and mission effectiveness of armed forces to disease diagnosis for clinicians. Development of a broad class of human performance monitoring devices to quantify sweat biomarkers necessitates non-invasive, real-time monitoring of ultra-low concentrations (µM to fM) of hormones, proteins, and neurotransmitters. For example, cortisol can be used to monitor the stress levels of a human subject, but exist in nM concentrations. Neuropeptide Y is a polypeptide which can be monitored as a sign of major depressive disorder, but exists in pM concentrations. Another challenge with sweat sensing is the fluid dynamics of sweat emerging from pores. Downstream sweat collection sites are undesired as molecules of interest can degrade; therefore, microfluidic channels...
compatible with collection directly from a pore site are desired to provide real-time monitoring of biomarkers. The unique flow characteristics at pore sites are a large design barrier for sensors, with sweat emerging from pores at 70 kN/m^2 at flows of 20-80 nL/min.\textsuperscript{7} The flow is pulsed, not continuous, and the rate is highly dependent on the activity level or underlying conditions of the subject. In addition, sweat droplets evaporate and the concentration of some biomarkers are dependent on the rate of secretion – for example, sodium ion concentration increases from 10 mM to 100 mM at high sweat rates.\textsuperscript{7}

It is difficult to predict a limit of detection (LoD) and sensor performance due to these challenges, especially since sensor geometry can vary widely. Field effect transistors (FETs) are the predominant sensor approach whereby the gate electrode is modified with a selective bio-recognition element (BRE), such as a self-assembled monolayer from cysteamine and 4-formylphenyl boronic acid used to sense dopamine.\textsuperscript{8} However, FETs have diminished sensitivity in high ionic strength environments associated with sweat. Alternatively, BRE-modified photonic integrated circuits (PICs) have high sensitivity in high ionic strength fluids, low cost at the manufacturing scale, and enable a number of novel device concepts to achieve ultra-low levels of detection.\textsuperscript{9,10} Propagation of an optical wave (e.g. wavelength $\sim 1.55 \mu m$) in the BRE-modified waveguide is perturbed in the presence of captured analyte molecules in the form of an optical phase shift. The minute optical phase perturbation can be detected interferometrically from which the concentration of the captured analyte can be deduced. Efficient interaction of light with a target molecule adsorbed in the cladding layer on the surface of a sensing waveguide is critical, such that when a small amount of analytes have been captured, a large perturbation of the optical mode can be achieved yielding a good LoD and a high detection probability. Predicting the LoD for a certain sensing waveguide geometry within a microfluidic chamber is difficult since it is dependent on many different variables, such as capture efficiency (analyte captured/analyte flowing through channel), fluid dynamics, capture molecule affinity for the analyte of interest (e.g., Langmuir or Freundlich adsorption isotherm), analyte concentration, and the interaction efficiency of the analyte with light. Also, the kinetics of flow systems are highly dependent on the fluid dynamics, namely, the size of the diffuse layer (concentration gradient) near the capture site. Higher flow rates may transport more analytes to the surface, but not necessarily increase the capture efficiency due to a diminished boundary layer. The majority of benchtop laboratory sensing processes (such as enzyme-linked immunosorbent assays, or ELISA) require a stationary fluid sample, such that measured adsorption kinetics in the literature may not be completely applicable to flow systems.

In this work, COMSOL Multiphysics simulations are utilized to rationalize the effects of fluid dynamics and analyte transport in microfluidic channels, as the modelling software is able to couple the temporal fluid response with kinetics occurring at a sensing interface. Specifically, the flow rate through the channel, biomarker size, adsorption and desorption kinetics, and sensor geometries are varied. These design parameters can be manipulated and a limit of detection predicted for a given time scale, such that with a specified biomarker size, geometry, and kinetics, the sensor response is predicted.

**SIMULATION PARAMETERS**

The COMSOL MultiPhysics simulation is a finite element analysis software. An image of the simulation is shown in Figure 1. Fluid flow is in the y-direction and a channel height of 100 $\mu m$ is shown; 50 $\mu m$ and 10 $\mu m$ channel heights are also utilized to analyze the effect of fluid velocity on capture efficiency. Sensor widths of 2$\mu m$ were utilized as specified for PIC sensor arrays,\textsuperscript{10} and the height of the sensors is considered to be negligible as compared to the sweat channel. Active waveguide sensors are alternated – only the highlighted 2\textsuperscript{nd}, 4\textsuperscript{th}, and 6\textsuperscript{th} sensing strip are BRE-modified. The 1\textsuperscript{st}, 3\textsuperscript{rd} and 5\textsuperscript{th} sensing strips are non-active or reference waveguides. The active and reference waveguide pair constitute the sensing interferometer from which captured analyte induced optical phase perturbations can be converted to electrical signals for readout using photodetectors. Careful design of the interferometer minimizes impact of background technical noise such as thermal and shot noise which impact the LoD. A constant flow estimated from literature for a single pore is 80 nL/min.\textsuperscript{7} This sweat sensor array is considered part of a larger array on either side with symmetric flow conditions in the x-direction. The analysis done on a single array here can be extrapolated to predict the characteristics of an entire sweat sensing device with many of these arrays.

COMSOL Multiphysics software allows the user to specify the necessary physics of the system required for modeling. First, laminar incompressible flow through the channel is specified. This is a reasonable assumption considering the relatively low aqueous flow rates emerging from sweat pores – at no point is this flow expected to be turbulent, and creeping flow can be an oversimplification at higher velocities. For the fluid dynamic calculations, the software utilizes conservation of momentum (Navier Stokes, equation 1) and conservation of mass (continuity, equation 2).

\[
\rho \frac{du}{dt} + \rho (u \cdot \nabla)u = \nabla \cdot [-p I + \mu (\nabla u + (\nabla u)^T)] + F
\]  

(1)
\[ \rho \nabla \cdot \mathbf{u} = 0 \]  \hspace{1cm} (2)

where \( \mathbf{u} \) and \( p \) are the dependent variables of velocity profile and pressure profile respectively, \( \rho \) is fluid density (1000 kg/m\(^3\)), \( \mu \) is the viscosity of water at 25°C (8.9 \times 10^{-4} \text{ Pa} \cdot \text{s}) and \( F \) is gravitational force. Initially, the channel is considered to be at rest (\( \mathbf{u} = 0 \)), and an inlet velocity boundary condition specifies that sweat enters the channel at \( u_y = \text{flowrate} / \text{width of channel} \times \text{height of channel} \). An outlet pressure boundary condition of \( p = 0 \) is specified as the sweat is expected to be at atmospheric pressure once exiting the channel.

Figure 1: Sweat channel simulation, with arrows denoting direction of fluid flow. The sensing elements where adsorption/desorption are represented as 2 µm width strips in the middle of the channel. The second, fourth, and sixth highlighted elements are active, while the rest are inactive.

The \( yz \)-planes also have a symmetric boundary condition, where there is no penetration and vanishing shear stresses such that the flow is continuous with adjacent sensing arrays. Next, transport of dilute species is specified to calculate the distribution of particles/molecules in the channel, utilizing Fick’s First Law with convection, and conservation of mass equations (Equations 3 and 4) which provides a link to the adsorption/desorption interactions at the sensing surface.

\[
\nabla \cdot (-D_i \nabla c_i) + \mathbf{u} \cdot \nabla c_i = R_i \\
N_i = -D_i \nabla c_i + \mathbf{u} c_i
\]

where \( N_i \) is the flux of a specified analyte, \( c_i \) is the concentration of the analyte (only one in this case), and \( R \) is the reaction rate of the analyte at surfaces where reaction can occur. The inlet boundary condition is set for a constant concentration of 5 \times 10^{-9} \text{ M} for cortisol\(^{11} \) or 3.5 \times 10^{-13} \text{ M} of Neuropeptide Y\(^6 \) typical concentrations in sweat. Two analytes are considered in this simulation: Neuropeptide Y and cortisol, chosen due to their prevalence in sweat and the range of molecule size and concentration in sweat. The molecule size is expressed in the sensor response due to the differences in diffusion coefficients; the larger Neuropeptide Y has a diffusion coefficient of \( \sim 1.3 \times 10^{-10} \text{ cm}^2/\text{s} \) in 0.1M phosphate buffer saline solution\(^{12} \) while the smaller cortisol molecules have a diffusion coefficient of \( 2.8 \times 10^{-6} \text{ cm}^2/\text{s} \) in a theoretical model.\(^{13} \)The reaction term is utilized in this simulation to specify the adsorption and desorption rate of the target analyte, such that the flux towards the surface of the sensing strip is equal to the net adsorption to the surface. The “reactions” considered are adsorption of the analyte (A) to the surface where B is the empty site and C is the adsorbed molecule, with associated rate constants \( k_{\text{ads}} \) and \( k_{\text{des}} \) specified. The capture event that renders C immobile and unable to diffuse back into
solution is considered with a secondary equation (Equation 5), of high reaction constant such that the kinetics of capture are dependent only on the adsorption and desorption events. \( k_{\text{ads}} \) and \( k_{\text{des}} \) are the forward and reverse rate constants for the immobilization of the analyte (A) onto an empty site (B). \( k \) is the rate constant for the immobilization of C\( \rightarrow \) D. Figure 2 shows the effect of increasing \( k \) to 1000 s\(^{-1}\), where it has no effect on the sensor response.

\[
A + B \leftrightarrow C \rightarrow D
\]  
(5)

Figure 2: Capture efficiency over time varying the surface reaction rate for immobilized captured analyte (Equation 5).

The value of the rate constants is normally considered relative to the other; \( k_{\text{ads}}/k_{\text{des}} > 10 \) is practically irreversible, whereas \( k_{\text{ads}}/k_{\text{des}} \sim 1 \) is close to an equally reversible reaction. This simulation considers a range of these ratios and their effect on capture efficiency. The adsorption at the sensor surface is similar to a Langmuir isotherm, the likes of which is characterized by the work of Umpleby et.al. when considering molecularly imprinted polymers for capture of analyte. One molecule can occupy one site and only a monolayer of molecules is considered.\(^\text{14} \) Typically, horseradish peroxidase (HRP) (MW = 44 kg/mol) is a common capture molecule used for cortisol\(^\text{15} \) and dopamine\(^\text{16} \) sensing. The surface density will change with the application and immobilization process, but this simulation will use a surface coverage of 50 pmol/cm\(^2\) as estimated from Raghu et al.\(^\text{16} \). Au(111) surfaces functionalized with thiols can also be used for DNA sensing, and had similar surface coverages of 12 pmol/cm\(^2\),\(^\text{17} \) suggesting some universality to the simulation parameters. In the end, the fluid mechanics is coupled with the transport of our dilute analyte, which is dependent on the adsorption rate at the surfaces used for capture of the molecule. Parameters of concern are the time required to reach the maximum site density, and the capture efficiency. The capture efficiency (molecules captured/molecules entering the channel) is a particular metric which we propose gives extra insight into sensor performance, especially for sweat sensing environments, where the analyte concentrations vary with sweat rate and flow is noncontinuous.

**SIMULATION RESULTS & DISCUSSION**

First, the effect of channel inlet height is considered for sensing cortisol. Decreasing the channel height will increase the fluid velocity due to the specified boundary conditions, and increase the convective transport of analyte to the capture surface (Figure 3). Velocity profile slices are shown, with max flow in the center of the channel due to the no-slip boundary conditions decreasing fluid velocity to zero at the walls. It can be seen from Figure 3 that capture efficiencies peak at very short time scales before a slow decline – the capture efficiencies are almost constant at \( \sim 0.0375\% \) of molecules entering the channel for longer time scales. Capture efficiencies are low due to the low kinetics of biorecognition element sensing. Typical BRE sensing setups utilize immobile liquids and can take hours to produce a positive result,\(^\text{18} \) and it’s not clear whether these same kinetics can be considered for flow systems. Diffuse boundary layers are a function of the inlet velocity, and will also affect the sensor response. Figure 3 also shows that the strips are nearly equal in response other than the small differences in the rise to the maximum, with the first strip having a slightly quicker rise due to it being the first strip contacted with analyte. The responses are nearly equal because of the velocities considered here for the flow geometry; it takes less than a second for the 0.4mm/s flow to reach the end of the channel, and so the temporal differences
between each strip is negligible. Due to this result, the first strip is considered as a point of comparison for all future systems.

Figure 3: Left: Velocity profile of the chamber, showcasing a max flowrate of 0.4 mm/s for the 50 um channel height. Right: Capture efficiencies for each strip (molecules captured/molecules entering channel). A steady decline at longer time scales (top) is compared to the short time scales where capture efficiency is maximized.

Figure 4: Channel height comparisons for capture efficiency (left) and fraction of total sites occupied (right) over time. Low time scales show the capture efficiency ramp up before the steady decline, and higher time scales show concentration over time, with saturation occurring at 40+ hours. Surface concentration data is normalized to the max surface concentration $C_s$.

Channel height was varied from 10 um, 50 um, and 100 um, and this corresponds to maximum flow velocities of 2 mm/s, 0.4 mm/s, and 0.2 mm/s for this geometry, which correspond to different capture efficiency and possibly surface concentration behavior (Figure 4). Figure 4 shows that the concentration behavior over the long time scales is practically constant for channel height, suggesting the system is kinetically limited. The capture efficiencies at the short time scales of interest show the 10 um channel height increasing the quickest, while the 100um channel ramps up the lowest, before
reaching the steady, constant decline from ~0.037% capture efficiency. A capture efficiency of 0.037% constitutes only one molecule being captured for every 2703 molecules that enter the channel at these longer time scales. One interesting observation from Figure 4 is that the peak in capture efficiency does not change with increasing fluid velocity/decreasing channel height, which is one reason why the surface concentration of captured analyte over time is practically the same for all three channel heights. Red arrows on the fraction of captured sites over time are used to highlight 10% of saturation and 50% of saturation, which correspond to ~2 hr and ~7.5 hr, times that may be of more interest to PIC sensing.

Figure 5: Capture fraction over time for Neuropeptide Y (black, left axis) compared to cortisol (red, right axis). Neuropeptide Y has extremely low capture amount even after 40 hrs. Surface concentration data is normalized to the max surface concentration Cs.

Size and concentration of the molecule is also an important consideration, since important biomarkers in sweat exist at picomolar concentrations, such as neuropeptide Y (Figure 5). The size of the molecule will affect the diffusion constant of the molecule, and therefore affect the adsorption kinetics since mass transport and kinetics are coupled. Neuropeptide Y is also nearly 8 times the size of cortisol, so should have some effect on the sensor response. Figure 5 shows that even after 40 hours, the low concentration of NPY in the inlet combined with its large size leads to only ~0.02% of sites occupied, while cortisol is approaching saturation.

Figure 6: Adsorption/desorption kinetics effect on capture efficiency and fraction of sites occupied over time. The rate constants are equal in both figures. Left shows the actual values of k_ads and k_des while the right shows the ratio. Surface concentration data is normalized to the max surface concentration C_s. The k_ads and k_des are similar in both plots – the ratios are highlighted on the right to showcase the effect of the ratio versus the effect of the individual values of k_ads and k_des.

The difference in slope is also immediately observable – cortisol is easier to capture and its higher concentration leads to fast sensor response as compared to NPY. Another unconsidered effect of the large molecules is proximity of other
molecules on the capture sites. In this simulation we assume a constant site density based off of capture materials typically used; for NPY, its large size (MW = 4254 as compared to cortisol MW = 363) will most likely contribute to steric interference with adjacent molecules, lowering the effective site density and further decreasing sensor response.

Next, the effect of kinetics is considered. An increase in the ratio of adsorption and desorption kinetics can increase the capture efficiency, but it’s important to remember kinetics are highly dependent on the system in question. Realistic kinetics for these systems are normally measured with a stationary fluid, yet the mass transport in flow systems are dependent on a fluid diffuse layer near the capture sites that depends on the fluid mechanics.

Figure 6 shows the effect of increasing the ratio, and how it’s not simply the ratio which affects the capture efficiency and fraction of sites occupied over time, where the red curve represents the baseline realistic kinetics used for all previous results. For example, increasing the $k_{\text{ads}}$ to 50 m$^3$/(mol-s) from 5 m$^3$/(mol-s) greatly increases the capture efficiency by a factor of 10 and saturation occurs in nearly 5 hrs as compared to the 40+ hours required for the baseline kinetics. Yet, the ratio of this system is lower than the blue curve ($k_{\text{ads}}/k_{\text{des}}=10^{15}$), suggesting it is not simply the ratio that promotes good sensor resposivitiy. The higher $k_{\text{ads}}/k_{\text{des}}$ ratio shows that decreasing the desorption kinetics while keeping the adsorption kinetics constant can decrease the time required to reach it’s maximum capture efficiency, but still give the same surface concentration temporal response on longer scales. It’s also important to note that capture surface saturation is not a qualifiier for a sensing event – certain systems may only require 1% of sites occupied, for example, which is achieved quickly with an elevated $k_{\text{ads}}$. It can be argued, then, that the fraction of sites occupied is the more valuable variable, but capture efficiency is important for the dynamics of sweat sensing, where the concentration of entering analytes isn’t constant.

Finally, the sensor geometry is varied, with a decreasing channel height towards the sensor area (Figure 7). Since the capture efficiency is achieved more quickly at smaller channel heights and higher flow velocities, the geometry was varied to attempt to amplify this effect. As shown in the figure, the capture efficiencies for this modified geometry are similar to that of a simple 10 um height channel, and the fraction of sites occupied over time is unchanged as compared to normal flow channels. This result reinforces the idea that this system is kinetically controlled, yet the geometry presented here may need to be investigated further with more accurate sweat sensing dynamics than that presented in this work. For example, a continuous flow was assumed here, yet sweat is pulsed out of pores. A noncontinuous flow may find the “reducer” or Venturi geometry of Figure 7 favorable, as sweat will need to accumulate at the sensor site in order to drive flow through. Or, a separate geometry where there is an increase in channel size above the sensing sites to achieve increased interaction time with the sensing sites.

Figure 7: Capture efficiency and fraction of sites occupied over time for the modified geometry system (Venturi) which leads to an increased flow velocity over the sensing area. The new system is compared to the 50 um and 10 um height channels from the previous figures. Surface concentration data is normalized to the max surface concentration $C_s$. 

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CONCLUSIONS & FUTURE WORK

COMSOL Multiphysics simulations were utilized to analyze the effect of flow systems with adsorption and desorption kinetics, and their effect on capture efficiency and the temporal response of the fraction of capture sites filled. Low capture efficiencies are observed for these systems (<0.1%), with a magnitude increase observed with a increase in \( k_{\text{ads}} \). The system is also kinetically controlled, with minimal change in sensor response with a magnitude increase in fluid velocity. Yet, capture efficiencies may not be the best metric of value for PICs, as only a small fraction of sites may need to be filled for detection. Future work can be done in this area regarding measurement of \( k_{\text{ads}} \) and \( k_{\text{des}} \) for flow systems instead of stationary flow systems, as the diffusion layer towards the sensor sites is affected by fluid dynamics.

REFERENCES


