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Enhanced capture efficiencies of antigens in immunosensors

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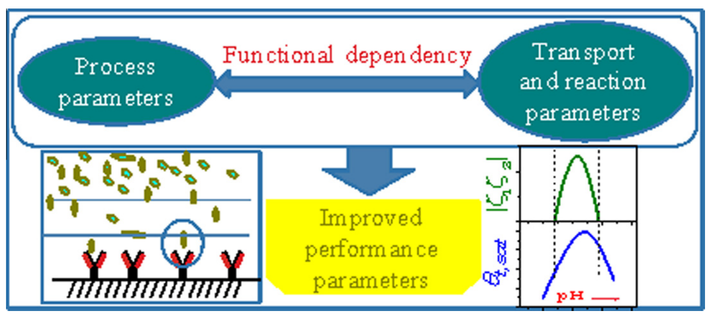
HIGHLIGHTS

- Higher capture efficiency is an important performance parameter for immunosensors.
- Experiments were conducted to understand the effect of the process parameters.
- Models were used to extract the dependencies of the transport and reaction parameters.
- Simulations were used to find the optimal conditions for the highest antigen capture.
- Effects of pH and the underlying mechanisms of antigen capture were explained.

GRAPHICAL ABSTRACT



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ABSTRACT

Higher capture efficiencies with faster response times are important performance benchmarks for heterogeneous immunosensors which are utilized in recognitions of analytes for disease detection. As the capture involves transport of antigens from the bulk solution to the surface immobilized antibodies and the subsequent binding, the transport and reaction parameters play a critical role. While there are reports on the effect of individual process parameters (temperature, extent of mixing, pH) on the antigen/antibody interaction, we are not aware of any studies on the combined effect of these process parameters, and moreover, there are limited studies on the effect of these process parameters on the reaction and transport parameters. Knowledge of the effect of the process parameters on the transport and reaction parameters is necessary to understand their relative contributions to and the mechanism involved in the capture of the antigens. In this work, we have made an attempt to quantify the transport and reaction parameters and studied their dependencies on the process parameters. Experiments were conducted with three systems – BSA/anti-BSA, PSA/anti-PSA and CRP/anti-CRP, to quantify the captured antigen. The dependencies of the process parameters on the transport and reaction parameters were obtained from the experimental data using a diffusion–convection–electromigration–reaction model. Utilizing these dependency functions, the process parameters were modeled to find the optimal conditions for the highest capture efficiencies. Specifically, the influence of pH on maximizing the antigen capture and the underlying mechanism of the electrostatic interactions based on the surface charges and zeta potentials were elucidated.

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Nomenclature

C	concentration of antigen in the solution at any time (mol/m^3)
C_b	bulk concentration of antigens (mol/m^3)
D_{eff}	diffusivity of the antigen in the solution (m^2/s)
D_0	pre-exponential factor in the Arrhenius dependency of diffusivity (m^2/s)
E_a	activation energy for diffusion (KJ/mol)
e	elementary charge (C)
F	faraday constant (A s mol^{-1})
K_{on}	forward rate constant (M^{-1} s^{-1})
K_{off}	reverse rate constant (s^{-1})
K_D	dissociation rate constant (M)
k_B	Boltzmann constant (J K^{-1})
N	Avogadro's number (mol^{-1})
n_∞	number concentration of antigens in bulk solution (m^{-3})
R	universal gas constant (J mol^{-1} K^{-1})
T	the absolute temperature (K)
t	time (s)
t_{sat}	time taken to reach $\theta_{t,sat}$ (s)
v_x	convective velocity in x-direction (m/s)
x	spatial distance (m)
z	the charge on the antigen surface
ε_0	permittivity of the medium (F m^{-1})
ε_r	relative permittivity of the medium
ζ	zeta potential of biomolecules (mV)
ζ_1	zeta potential of antigens (mV)
ζ_2	zeta potential of antibodies (mV)
θ_t	the surface density of the antigen-antibody bound complex at any instant t (mol/m^2)
θ_{max}	the maximum surface density of the immobilized antibodies (mol/m^2)
$\theta_{t,sat}$	the saturated amount of θ_t (mol/m^2)
λ	Debye length (m)
ν	kinematic viscosity (m^2/s)
Φ	potential due to formation of electrical double layer (V)
ω	angular velocity (rad/s)

1. Introduction

Heterogeneous immunosensors are a class of biosensors providing reliable and specific means of recognition of analytes for disease detection. These immunosensors rely on the capture of target antigen molecules in solution by the antibody molecules immobilized on a solid surface. An efficient immunosensor must achieve high capture efficiency (defined as the ratio of the number of antigens captured to the maximum available antibodies) in minimum time, for which faster transport and optimal reaction conditions are required. Efforts towards obtaining optimum surface conditions through physical and chemical modifications for improved immobilization of antibody molecules have been reported [1]. Formation of the antigen/antibody complex involves the transport of antigens from the bulk solution to the surface, and the subsequent binding of the antigens with the immobilized antibodies. Three primary mechanisms which contribute to the transport of antigens are convection, diffusion, and electromigration depending on the design of the system.

Several experimental and modeling studies have been reported to describe the transport and reaction processes [2–9]. Earlier reports described simpler two-compartment models [2] and the reaction–diffusion models [3] for non-flow systems. Subsequently, the need of miniaturization led to the development of flow systems and hence convection–diffusion based models were proposed to understand the effect of the transport and reaction parameters [4,5]. Flow systems were simulated and variations of the parameters, such as density of binding sites, analyte concentration, flow rate, geometry of multiple surface capture sites, and surface density on the capture efficiency were investigated [6–8], and also implemented for the indirect and competitive formats of immunoassays [9,10]. The major process parameters influencing the transport-reaction phenomena include the temperature, the extent of mixing, and the pH of the solution containing the antigens. While increasing temperature and extent of mixing is known to enhance the reaction rate [11–13], comparatively lesser attention has been given in the literature to investigate the effect of pH which would affect the surface charge on the antigens and antibodies thus affecting the transport due to electromigration. The effect of pH was studied for amperometric immunosensors, where the best pH value was selected based on the highest oxidation current obtained from the experimental results; however, no fundamental understanding in terms of the effect of pH on the surface charges and transport properties of the antigen molecules has been discussed [14,15]. In another work, the pH was optimized for obtaining the maximum peak current for the optimized immunoassay to detect the analyte molecules [16], but the role of pH was not discussed. The effect of charge variation was reported to induce electrokinetic flow in microfluidics based sensors and the effects of surface heterogeneity and surface charge of antibodies were investigated in a direct format assay for the model system of IgG and Protein A [17]. Though the effect of variable charge present on the incoming antigens and the application of external electric field driven flow have been described, the variation in zeta potential of the immobilized molecules on the sensor surface were not investigated there. In another work [18], the slip and non-slip models for fluid flow have been compared and the effect of the variation in surface charges were considered along with the application of external potential field; however, no experimental evidences were presented for the observations. Thus, while there are a few reports on the optimization of the pH in immunosensors and some simulation studies on the flow of charged antigens, a deeper understanding of the effect of pH in conjugation with the effect of temperature and the extent of mixing on the transport phenomena is needed to obtain conditions to enhance the capture efficiency of antigens.

While in the literature, there are separate studies on the effect of the process parameters (temperature, extent of mixing, pH) on the antigen/antibody interaction, to the best of our knowledge there are no studies on the combined effect of the process parameters, and more importantly, there are limited studies on the effect of these process parameters on the transport and reaction parameters. Knowledge of the effect of the process parameters on the transport and reaction parameters is needed to understand the relative contribution of these parameters and thus the mechanism involved in the capture of the antigens. Hence, we have attempted to quantify the transport and reaction parameters and studied their dependencies on the process parameters. In our previous works, we have studied the effect of surface modification via nanotexturing utilizing site directed proteins to maximize the number density of properly oriented functional antibodies [19], and further determined the optimal surface conditions for the highest antigen