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Research Paper

Numerical Study of ACET Assisting Concentrator for On-chip Analytical Detection of Low-concentration Analytes

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Abstract :

Miniaturization has been widely applied in biosensing and immunosensing applications nowadays. Due to low Reynolds number flow, microchips usually suffer from mass transport and low detection yield. It should be noted that the antibody is immobilized on the surface of the biosensor, and the antigen is in the mobile phase. In this research, we propose an electrode structure to generate rotational flow near the biosensor and enhance the antibody-antigen binding process on the surface of the immune-sensor. Electrodes are excited at a proper voltage and frequency to generate an AC Electrothermal flow effect near the biosensor and concentrate the antigen to the immobilized antibodies. To study the AC electrothermal effect for an immunsensor, the physical equations, including electrostatic, fluid mechanics, temperature field, species concentration, and antibody-antigen binding reaction, is solved by the finite element method. By generating the electrothermal flow, the best location for the immunosensor is determined. The binding reaction is investigated both with and without applying the AC electrothermal effect. Based on the results with application of electrothermal flow (12-volt, 500 kHz), a rotational flow is induced and leads to a 9-fold improvement in antibody-antigen binding. The proposed system is studied for different Damköhler and Peclet numbers, and high throughput binding enhancement is observed for miniaturized and on-chip immunosensors.

Keywords: Analytical Detection, ACET, Miniaturization, Biosensor.

Introduction

In recent years, engineering fields such as electronics, biomedical engineering, fluid mechanics and etc, have faced with

miniaturization challenge [1]. Microfluidic science develops different biomechanical, biological, and biochemical analyses and widely employed to develop implantable and portable devices such as PCR, DNA hybridization, genomics, drug delivery, etc [2, 3]. An immunoassay system operates based on the antibody-antigen interaction, and offers extensive diagnostic capabilities in the fields of medicine and pharmacology. Immunoassay samples such as

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antibodies, antigens, enzymes, etc, are expensive and microdevices highly reduce the sample consumption [4]. In an immunoassay system, the goal is to deliver the samples to a sensor and increase the sample concentration on the sensor. Various sensors, such as Surface Plasmon Resonance (SPR), microcantilever and Quartz Crystal Microbalance (QCM) are used to detect the interaction between antibodies and antigens [5]. Surface plasmon resonance is a phenomenon that occurs where electrons in a thin metal sheet become excited by light that is directed to the sheet at a particular angle of incidence, and then travel parallel to the sheet [5]. Delivering mobile samples (i.e. antigens) onto immobilized antibodies on the sensor enables rapid surface reactions with high reaction efficiency [6].

The immune-sensing system in micron scale faces fundamental challenges, such as adaptability to various biological applications with highly conductive buffers, power consumption, portability, and compatibility. Immunoassay tests are employed in various formats for detecting antigens or antibodies [7]. Typically, one of these two substances is immobilizing on a solid substrate and traces the mobile analyte which present in the fluid phase (e.g., in ELISA tests) [8]. In microchannels, we are faced with laminar flow (low Reynolds number flow) and weak diffusion of analyte molecules to the sensor surface, which limits detection time and sensor sensitivity. In a standard immunoassay analysis, the following steps are generally carried out [8]:

Coating the Plate: The target protein must first be adhered to the plate's surface. Antigen (or antibody) is added in microgram quantities to the wells of an ELISA plate and given enough time to attach sufficiently to the well surface. Depending on the protein type, different buffers are used, and the concentration of protein must be optimized. If the concentration is too low, detection sensitivity decreases, while overly high antigen concentrations can lead to weak bindings that detach during subsequent washing steps, carrying away the antigen-antibody complexes, thereby reducing test sensitivity. Typically, common antigens do not require additional binding agents, but if a particular antigen fails to attach with conventional methods or forms weak bonds, specialized substances or techniques (e.g., glutaraldehyde or X-ray treatment) are used for binding.

1. Blocking Unbound Sites: Uncovered areas of the plate are blocked with a suitable protein

solution to prevent nonspecific binding in later steps. Neutral protein solutions, such as skim milk, bovine serum albumin (BSA), gelatin, or casein, are commonly used for blocking. Non-ionic detergents such as Tween 20 or Tween 80 are often added to enhance this process.

2. Adding the Test Solution: The test solution is added, and time is given for the two interacting substances to find and bind to each other.

3. Signal Amplification: Primary amplification systems are applied to enhance test sensitivity. Systems such as biotin-avidin, biotin-streptavidin, and lectin-ligand interactions are commonly used for this purpose.

4. Sensor Activation: The sensing system is activated for measurement and detection. Various detection methods can be employed, with sensors like SPR, QCM, and cantilevers offering the highest accuracy.

Therefore, the antigen-antibody (Ab-Ag) interaction mechanism includes the Ag coating, blocking, and Ab binding. Biological molecules typically exhibit high reaction rates but have very weak molecular diffusion. Therefore, the dimensionless Damköhler number describes the reactive and transport behaviors; on the other hand, it expresses the ratio of the biomolecule reaction rate to its mass transport rate as shown in Eq.1 [9]:

$$Da = \frac{k_{on}\theta_0}{D/h} \quad (1)$$

In this relationship, k_{on} represents the reaction rate, θ_0 denotes the number of available receptor sites for Ab-Ag binding, D is the molecular diffusion coefficient, and h is the characteristic length (height of the channel). The Damköhler number is crucial for understanding the relative importance of reaction rates versus transport rates (such as diffusion) in biological and chemical processes. When $Da \gg 1$, it indicates mass transport limitation, whereas $Da \ll 1$ signifies reaction rate limitation. In the case of Immunoassays-on-a-Chip, the Damköhler number is typically much greater than 1, and the system is Transport-Limited [9]. Therefore, electrokinetic flows are able to enhance the concentration process, improve mass transfer, and link Lock-and-Key processes [10].

In reference [11], a DNA concentration example is designed using a combination of AC Electroosmosis (ACEO) flow and

Dielectrophoresis (DEP) force. The ACEO force is responsible for generating flow, while the DEP force is responsible for capturing DNA onto the reaction area. As a result of the ACEO flow, a volumetric force is applied to the fluid, generating a stirring mechanism around the sensor. This ultimately improves the binding process. According to Surface Reaction relations, the time required to detect the pathogen by the sensor is reduced [12].

Due to ease of implementation, lack of moving parts, and compatibility, the electrokinetic method is an efficient mechanism for fluid manipulation in microscales. An AC electric field interacting with polarized particles and fluids can induce fluid and/or particle motions inside a microchannel. The AC electrokinetic mechanism is broadly categorized into three forces, including: Dielectrophoresis (DEP), AC Electroosmosis (ACEO), and AC Electrothermal (ACET) flows [13]. In most cases, ACEO and ACET flows generate similar effects, but their principles are entirely different. ACEO flow occurs due to ion motion in the Electric Double Layer (EDL) at the interface between the electrode and the electrolyte. It produces flow inside microchannels due to fluid viscosity [14]. At high frequencies, there is insufficient time for charge relaxation; therefore, no ions cover the electrode surfaces, and the electric charge of the EDL becomes negligible. As a result, the ACEO flow doesn't form inside the channel [15]. Given that in fluids with high electrical conductivity, the ACEO flow loses its effectiveness, the ACET flow could be a suitable alternative for such scenarios. In reference [16], the concentration process of Biotin, which is in the solid phase, and Streptavidin, labeled with fluorescence, was investigated by applying the AC electrothermal force. In reference [17], it was shown that when the electrical conductivity of the solution exceeds 0.14 S/m, no ACEO flow is generated at any frequency.

In this research, the mass transport and antibody-antigen reaction will be studied to increase the efficiency of immunosensors. For this purpose, the physical equations will be coupled, and the system will be simulated by FEM. The behavior of the system will be investigated in passive and active modes, and the effect of different parameters on the binding reaction will be studied.

Theory

The ACET effect describes fluid movement

induced by a thermal gradient in the presence of an AC electric field. When an electric field is applied to a fluid with electrical conductivity σ , the Joule Heating phenomenon occurs according to the energy balance equation [18].

$$k\nabla^2 T + \frac{1}{2} \langle \sigma E^2 \rangle = 0 \quad (2)$$

In this relationship, T represents the temperature, and k denotes thermal conductivity. It should be noted that at this scale, thermal convection can be neglected compared to thermal diffusion [19]. When a non-uniform electric field is applied to the system, spatial variations in heat generation occur in the fluid (i.e., local thermal gradients). This thermal gradient induces spatial variations in the local electrical conductivity and the local dielectric permittivity of the fluid.

$$\begin{aligned} \nabla \varepsilon &= \left(\frac{\partial \varepsilon}{\partial T} \right) \nabla T \\ \nabla \sigma &= \left(\frac{\partial \sigma}{\partial T} \right) \nabla T \end{aligned} \quad (3)$$

Additionally, the gradients in electrical conductivity and dielectric permittivity result in the generation of mobile space charges ρ within the fluid [19].

$$\begin{aligned} \rho &= \nabla \cdot (\varepsilon \vec{E}) = \nabla \varepsilon \cdot \vec{E} + \varepsilon \nabla \cdot \vec{E} \\ \frac{\partial \rho}{\partial t} + \nabla \cdot (\sigma \vec{E}) &= 0 \end{aligned} \quad (4)$$

This leads to the fluid experiencing a volumetric force f_E .

$$f_E = \rho \vec{E} - \frac{1}{2} |\vec{E}|^2 \nabla \varepsilon \quad (5)$$

The interaction of this force and the viscosity of the fluid induces fluid motion. The time-averaged force exerted on the fluid is expressed by Eq.6 [20]:

$$\langle F_{et} \rangle = -\frac{1}{2} \left(\frac{\nabla \sigma}{\sigma} - \frac{\nabla \varepsilon}{\varepsilon} \right) \cdot \frac{\varepsilon |\vec{E}|^2}{1 + (\omega \tau)^2} - \frac{1}{4} \nabla \varepsilon |\vec{E}|^2 \quad (6)$$

In this relationship, $\tau = \varepsilon / \sigma$ represents the charge relaxation time. For an aqueous solution at a temperature of 293 K, we have [21]:

$$\frac{1}{\varepsilon} \frac{\partial \varepsilon}{\partial T} = -0.004 \Rightarrow \frac{\nabla \varepsilon}{\varepsilon} = \frac{1}{\varepsilon} \frac{\partial \varepsilon}{\partial T} \nabla T = -0.004 \nabla T \quad (7)$$

$$\frac{1}{\sigma} \frac{\partial \sigma}{\partial T} = 0.02 \Rightarrow \frac{\nabla \sigma}{\sigma} = \frac{1}{\sigma} \frac{\partial \sigma}{\partial T} \nabla T = 0.02 \nabla T \quad (8)$$

$$\text{giving } \langle F_{et} \rangle = -0.012 \nabla T \cdot \frac{\varepsilon |E|^2}{1 + (\omega \tau)^2} + 0.001 \nabla T \cdot \varepsilon |E|^2 \quad (9)$$

The first term in Equation (9) represents the Coulomb force, while the second term represents the dielectric force. At low frequencies, $1/(1+(\omega\tau)^2)$ tends to 1; therefore, the Coulomb force is much stronger than the dielectric force. As the frequency increases, the Coulomb force weakens until, at very high frequencies, the dielectric force becomes dominant. The crossover frequency between these two forces can be derived from $f_c = \sqrt{1/2\pi\tau}$, which highly depends on the fluid properties. If the applied frequency is much smaller than the crossover frequency, and also the applied frequency is large enough to neglect ACEO flow, then we are able to claim that a dominant ACET force has been generated [22,

23].

By considering a pair of embedded electrodes at the bottom of microchannel and exciting the electrodes with sinusoidal ac electric potential, an electric field will form from positive electrode towards the negative electrode (as shown in Figure 1a). Due to the ionic strength of the medium fluid, in presence of the electric field, the fluid will start to heat up and temperature gradient will induce inside the channel (Figure 1b). Based on the joule heating equation, the generated temperature rise is dependent on the amplitude of electric field and also the ionic strength of the fluid. As discussed earlier, the generated temperature gradient will induce an electric charge, and as a result, an electrothermal flow induces inside the channel. The velocity, arrow, and streamlines of the generated ACET flow are shown in Figure 1c.

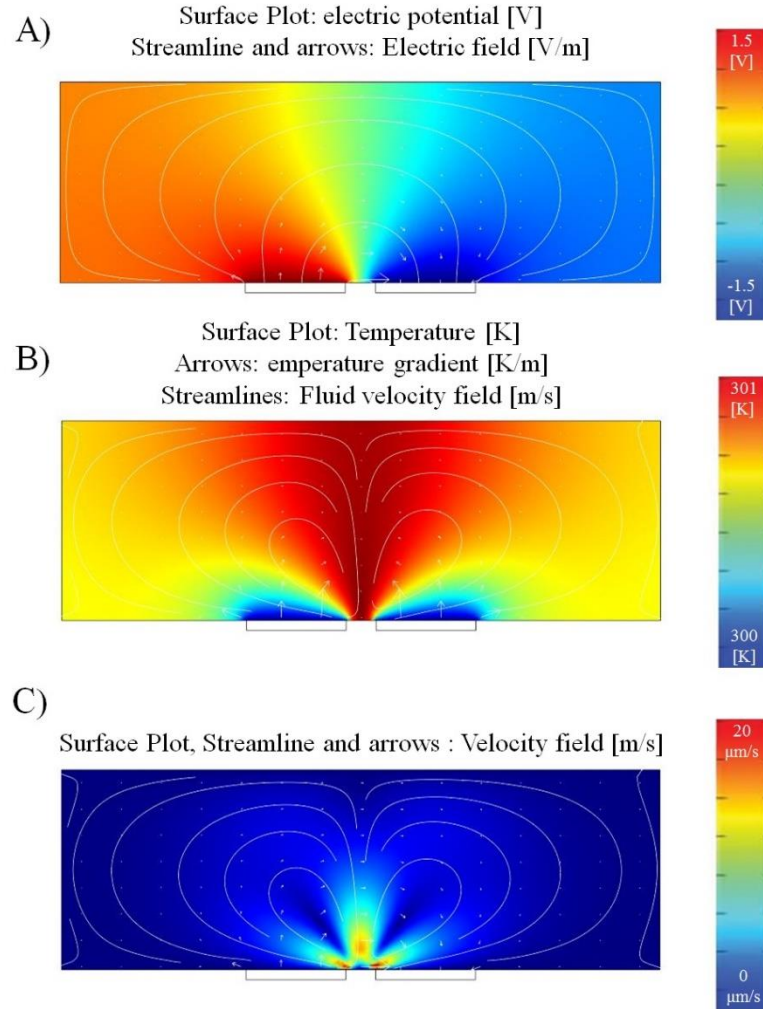


Fig. 1. ACET simulation results for a pair of embedded electrodes; a) electric potential; b) Temperature; C) Fluid velocity field

As shown in Figure 1, the size of the electrodes was the same. To generate the most applicable ACET fluid, efficient electrode structures can be designed. Figure 2 illustrates the geometrical parameters of the microchannel dimension and electrode structures for assisting the concentration process in microfluidic chips. In our simulation study, we used the Taguchi method for optimization [24]. Taguchi developed a special design of orthogonal arrays to study the entire parameter space with a small number of experiments only. The experimental results are then transformed into a signal-to-noise (S/N) ratio. It uses the S/N ratio as a measure of quality characteristics deviating from or nearing the desired values [24].

The optimized geometrical dimensions are listed in Table 1. These dimensions will be used for the on-chip immune-sensing enhancement by ACET flow.

Table 1. Geometrical dimensions of design

Symbol	Description	Value (μm)
W_{Channel}	Microchannel width	120
N.E	Narrow Electrode	10
C.E	Center Electrode	20
g	Gap between two electrodes	10
G	Gap between two pairs of electrode structures	80

Results and Discussion

As shown in Figure 3, to generate a non-uniform electric field, some pieces of electrode structures were used in the microchannel bottom to create ACET flow and concentrate the biosensor. After exciting the electrodes and generating ACET flow, the biosensor is located at the proper place of the microchannel roof, and a three-dimensional simulation is carried out.

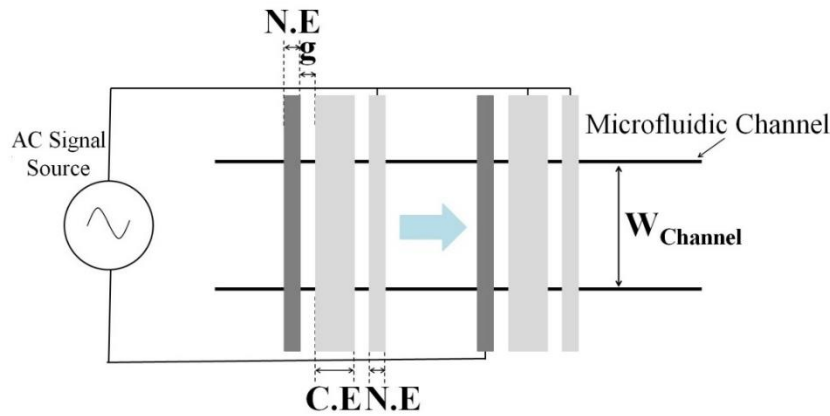


Fig. 2. Geometrical dimensions

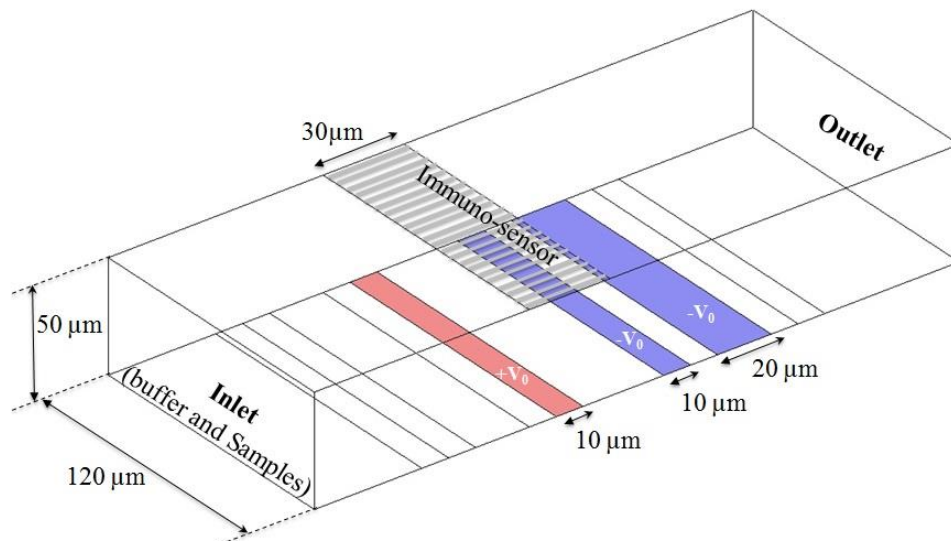


Fig. 3. On-chip microfluidic concentrator, including electrode structures and immune-sensor location

The electrode structures were biased in two conditions: passive (without applying electrothermal force) and active (with electrothermal force), and the results were extracted and discussed here. To calculate the AC electrothermal force, the electric field inside the microchannel can be solved using Laplace's equation, as described in Equation [25]:

$$\nabla^2 \varphi_e = 0 \quad (10)$$

$$\vec{E} = -\nabla \varphi_e \quad (11)$$

When the electric signals $+V_0 \times \sin(\omega t)$ and $-V_0 \times \sin(\omega t)$ are applied to the electrodes, and other boundaries are electrically insulated ($\partial \varphi / \partial y = 0$). Therefore, a non-uniform electric field is generated inside the channel. The field is stronger near the narrow electrode.

Figure Electric Potential and Electric Field

According to Joule's law, applying a non-uniform electric field to a fluid with high electrical conductivity results in the generation of local heat inside the channel, which alters the electrical properties of the fluid. The amount of heat and the thermal gradient generated within the fluid can be calculated using the energy equation:

$$\rho_m c_p \vec{u} \cdot \nabla T + \rho_m c_p \frac{\partial T}{\partial t} = k \nabla^2 T + \sigma |\vec{E}|^2 \quad (12)$$

In this relationship, ρ_m represents the fluid density, c_p is the specific heat capacity of the fluid, u is the velocity vector of the fluid, T is the temperature, k is the thermal conductivity of the fluid, σ is the electrical conductivity of the fluid, and $\vec{E}$ is the applied electric field. By considering that the fluid velocity in the microchannel is very small, it is a good approximation to assume that the temperature at the inlet and outlet of the channel is equal to the ambient temperature [26]. As a result, the generated thermal gradient leads to changes in the electrical and dielectric properties of the fluid, as described by the mentioned equations. The fluid flow inside the microchannel can be analyzed using the continuity and Navier-Stokes equations [27].

$$\rho_m \left(\frac{\partial \vec{u}}{\partial t} + \vec{u} \cdot \nabla \vec{u} \right) - \mu \nabla^2 \vec{u} + \nabla p = \vec{f}_{Bulk} \quad (13)$$

$$\nabla \cdot \vec{u} = 0 \quad (14)$$

In this equation, p represents the pressure inside the microchannel, μ is the dynamic viscosity of the fluid, and $\vec{f}_{Bulk}$ represents the bulk

force applied to the fluid. The boundary conditions for pressure are zero pressure at the inlet and outlet ($p=0$), and zero pressure gradient at the walls of the microchannel ($n \Delta p = 0$). In microfluidic channels, the fluid is completely laminar, and the velocity at the walls follows the no-slip condition ($\partial u / \partial x = 0$, $\partial u / \partial y = 0$ at the walls). All the electrokinetic effects influence the formation of the bulk force $\vec{f}_{Bulk}$. However, because the fluid has a high electrical conductivity and the excitation frequency of the system is much higher than the frequency of the fluid, the ACEO and DEP forces can be neglected, and the ACET force becomes the dominant force in the system. The biological fluid, PBS (Phosphate Buffered Saline), was considered the carrier buffer inside the microchannel. The fluid properties are shown in Table 2.

Table 2. Material properties and actuation

Symbol	Description	Value
D	Diffusion coefficient of analyte	$1 \times 10^{-11} \text{ m}^2 \cdot \text{s}^{-1}$
U_0	Average Inlet Velocity	$0.1 \text{ mm} \cdot \text{s}^{-1}$
ρ_m	Viscosity of fluid	$10^{-3} \text{ N} \cdot \text{s} \cdot \text{m}^{-2}$
μ	Density of fluid	$10^3 \text{ kg} \cdot \text{m}^{-3}$
σ	Electric conductivity of Fluid	0.48 [S/m]
V_0	Electric Potential	Variable
$\omega = 2\pi f$	AC signal Frequency	Variable
ϵ_r	Dielectric constant of Fluid	80.2
c_p	Heat capacity of fluid	$4.184 \text{ [kJ/(kg} \cdot \text{K)]}$
k	Thermal conductivity of fluid	$0.598 \text{ [W/(m} \cdot \text{K)]}$
$T_{ambient}$	Ambient temperature	300.15 [K]
C_{in}	Analyte concentration at the inlet (1 fM)	$10^{-12} \text{ [mol} \cdot \text{m}^{-3}]$
θ_0	Total Surface concentration of AB ligand	$1.7 \times 10^{-8} \text{ [mol/m}^3]$
k_{on}	Association rate constant	$10^8 \text{ [m}^3 \cdot \text{mol}^{-1} \cdot \text{s)]}$
k_{off}	Dissociation rate constant	0.02 [1/s]

In on-chip immunoassay systems, the reaction occurs between the antibody (which is immobilized on a solid surface) and the antigen (which is in the moving phase). As discussed earlier, the physics of electrostatics, fluid mechanics, and heat are used to analyze the AC electrothermal flow. To analyze the reaction and

binding process between antibody and antigen, molecular transport and diffusion equations, as well as the binding reaction equation, need to be solved according to the Eqs. 15 and 16 [28].

$$\frac{\partial C}{\partial t} + \nabla \cdot (-D \nabla c) = R - u \cdot \nabla c \quad (15)$$

In this relation, c represents the concentration of the input analyte (antibody or antigen), which is 1 nanomolar ($1 \mu\text{M}/\text{m}^3$) in this study. D represents the molecular diffusion coefficient of the analyte (equal to $10^{-11} \text{ m}^2/\text{s}$), and R is the reaction rate in the bulk fluid, which in this analysis is zero because no reaction occurs in the bulk fluid and the reaction only occurs at the surface of the sensor. In the binding region, the ligand-antibody concentration is R_t , and part of these antibodies that successfully react with the analyte present in the driving phase have a concentration B . The constants related to the binding and dissociation rates have been measured using the surface plasmon resonance (SPR) method and are available as reference values. Therefore, the binding reaction equation is given by Eq.16 [23].

$$\frac{\partial B}{\partial t} = k_{on} c(R_t - B) - k_{off} B \quad (16)$$

Where c is the concentration of analyte at the binding site, k_{on} is the binding rate constant, and k_{off} is the dissociation rate constant, which represents the breakdown of the bond between antibody and antigen. The required parameters for the FEM processing are provided in Table 2.

According to the results, applying a relatively large voltage of 12 V to the excitation electrodes leads to twisting at the interface between the actuation electrodes, which expands as the voltage increases. The created twisting and rotation flow effect results in a significant portion of the analyte passing through the narrow region near the immunosensor. It also increases the possibility of contact between the analyte and the antibody. Meanwhile, another part of the analyte moves in a vortex inside the twist. It should be noted that the input velocity of the fluid and analyte at the channel entrance is $100 \mu\text{m}/\text{s}$, but with electrode excitation and the creation of electrothermal force, the average velocity of the fluid near the sensor surfaces reaches $900 \mu\text{m}/\text{s}$. The improvement factor coefficient is defined as $\text{BEF} = B/B_0$. B represents the antigen bound due to the application of electrothermal AC flow, and B_0

represents the antigen bound without the application of electrothermal flow. According to Figure 4, the excitation of the electrodes leads to the creation of a twist effect with a large radius at the interface between the electrode units, which increases in range as the voltage increases. The generated twist results in a significant portion of the analyte passing through the stream near the immunosensor, thereby increasing the chance for contact between the analyte and the antibody. According to the figures related to the concentration profile of the immunosensor in passive and active states, exciting the electrodes with a voltage of 9 V and a frequency of 500 kHz leads to a 4-fold improvement in antibody-antigen binding. With the application of 12 V, an increase in the BEF of nearly 9 times is observed.

The maximum flow velocity and the maximum temperature increase generated in the system are listed in Table 3. The fluid flow velocity increases with the voltage, which follows a fourth-order dependence on the voltage according to the electrothermal force.

Table 3. ACET flow results and related binding enhancement for different electric potentials

Variable	Value		
Applied Electric Potential	5	9	12
Maximum Velocity flow	250 $\mu\text{m}/\text{s}$	780 $\mu\text{m}/\text{s}$	1 mm/s
Maximum Temperature Rise	3	6.5	11
BEF	2	4	9

To further investigate the performance of the designed system, the effect of the Damköhler number and Peclet number is examined to evaluate the effect of K_{on} , D , θ_0 , inlet fluid velocity U_0 , and microchannel dimension. For small Da numbers, the reaction rate limits the binding rate due to low reaction speed, while for large Da numbers, the binding rate is limited by the slow diffusion of the analyte to the immunosensor. The dimensionless Peclet number represents the ratio of the fluid velocity rate to the molecular diffusion rate ($Pe = uh/D$) [29]. As shown in Figure 5, a and b, with the increase in inlet velocity, the improvement factor in the system decreases. It should be noted that the microfluidic devices have low initial fluid velocity, which makes the proposed device an optimal choice for high-throughput immune-sensing applications.

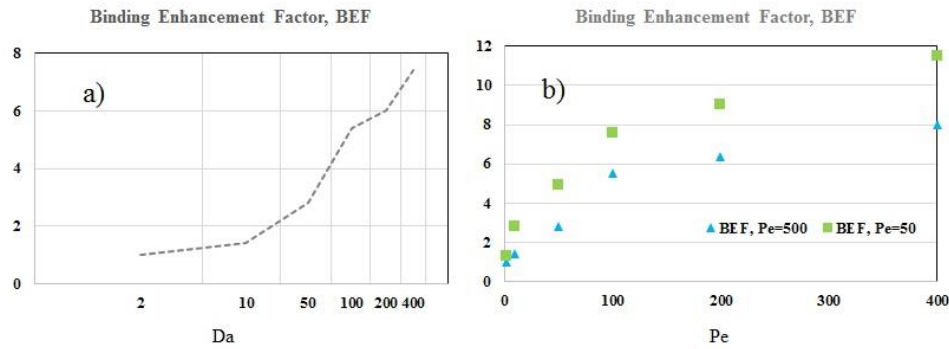


Fig. 4. Binding reaction for a) without ACET concentrator; b) with ACET concentrator

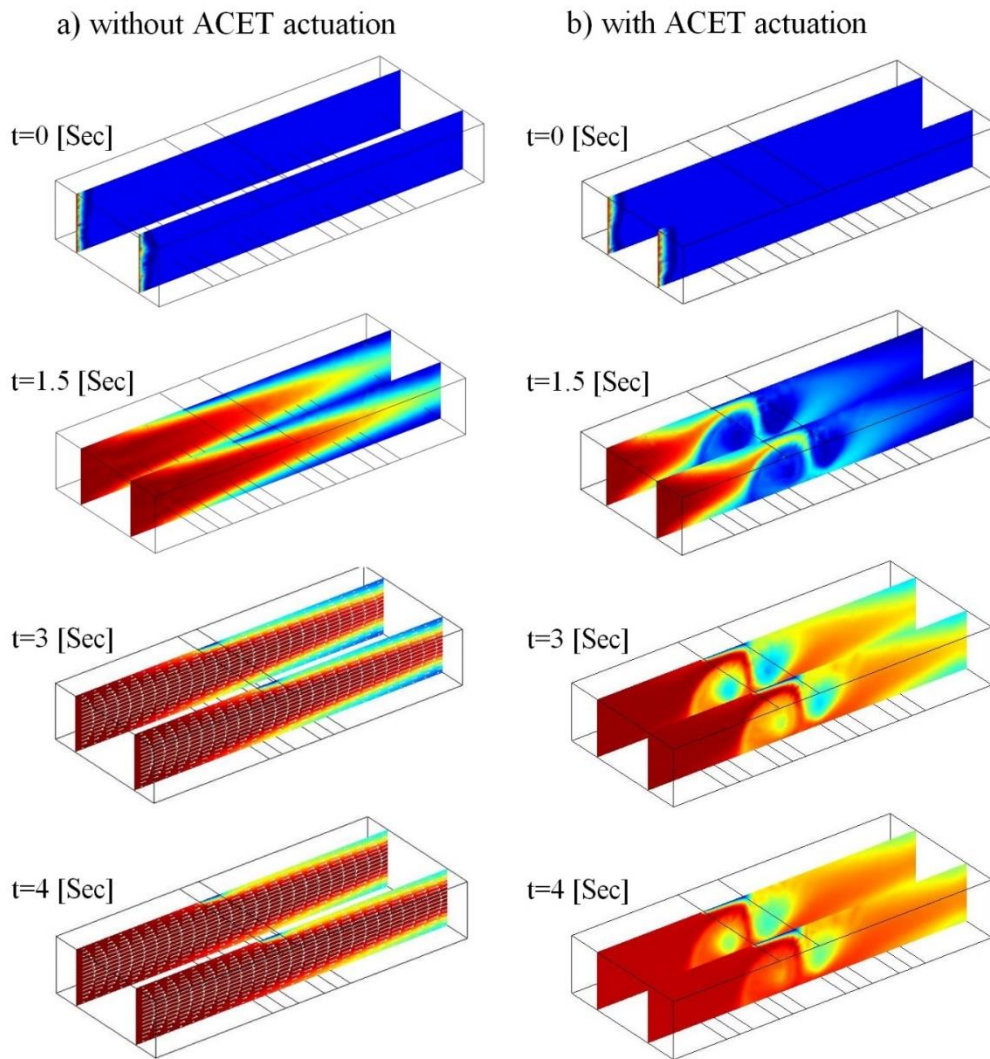


Fig. 5. Binding enhancement factor for a) different Damkohler numbers; b) different Peclet numbers

Conclusion

In this study, a finite element simulation of a microfluidic-based immunoassay system was fully conducted. The process of concentrating the immunosensor was performed using AC

electrothermal flow actuation. In the presence of a biological fluid with high electrical conductivity, the electrode structures were excited. As a result of the generated thermal effects, a rotational flow was formed inside the channel. By studying the

behavior of the rotational flow and the influencing parameters (including the electrical conductivity of the fluid, as well as the voltage and frequency of the electric field), an appropriate location for the reactive surface area (the immunosensor location) was selected. Then, by creating an AC electrothermal flow near the surface of the immunosensor reaction, a narrow region, like a constriction, was formed. Consequently, the analyte (biological buffer mixed with antigen) is forced to pass through the constricted area adjacent to the reaction surface, thereby increasing the rate of binding and dissociation of the antibody-antigen complex. The simulation of the concentrator system was employed in both passive and active modes. According to the results obtained, with a 12 Volt actuation of the electrodes and a biological fluid conductivity of 0.5 S/m, an improvement factor of 9 was achieved. The maximum AC electrothermal flow generated near the biosensor was calculated to be in the range of 1 mm/s (with 12 volts). Based on the obtained results, the microfluidic-based immunosensor systems are characterized by large Damköhler numbers and small Peclet numbers, and our proposed concentrator structure is highly efficient for large Damköhler numbers and small Peclet numbers. The reactive behavior of the immunosensor was significantly improved due to the optimized AC electrothermal flow.

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