A Computational Model for the Design of ElectroWetting On Dielectric (EWOD) Systems

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ABSTRACT

ElectroWetting On Dielectric (EWOD) is a unique method of moving small volume droplets in microfluidic biochips that relies on modification of surface wetting characteristics using electrical methods. Modeling and simulation of the EWOD phenomena can aid in the design of microfluidic devices using the EWOD effect. This paper presents a validated computational model of droplet motion coupled with biochemical species transport as well as surface and volume reactions. The model is applied to the problem of passive binding of proteins onto the dielectric surface. Ultimately, the results of these computations will be used to construct design rules for digital microfluidics.

Keywords: electrowetting, VOF, biochemistry, nonspecific binding, proteins.

1 INTRODUCTION

Microfluidics has enabled the development of integrated lab-on-a-chip (LoC) devices for use in a clinical diagnostics, high throughput screening, drug discovery, biodefense and environmental monitoring. Although most microfluidic devices are based on continuous flow of liquids in microchannels, there is an increasing interest in devices that rely on manipulation of discrete droplets using surface tension effects that are dominant at the small length scales associated with these devices. One such technique is ElectroWetting-On-Dielectric (EWOD), which is based on changing the wettability of liquids on a dielectric solid surface by varying the electric potential. This method offers advantages over conventional continuous-flow microfluidic chips, by way of significantly reduced sample size, as well as reconfigurability and scalability of architecture. The similarity of the EWOD system with digital microelectronic systems, has led to the term "digital microfluidics" [1]. The phenomenon of EWOD has been demonstrated for dispensing, cutting, and transport of tiny droplets [2, 3], and more recently, a proof-of-concept has been demonstrated for an integrated lab-on-a-chip system for clinical diagnostic applications [1].

While considerable attention has been focused on fabrication and demonstration, there has been little attention to modeling of droplet transport and bioassays in these systems. The present paper demonstrates the use of a CFD based model to describe droplet hydrodynamics and transport of biological species in EWOD systems, including reactions involved in bioassays and passive binding of proteins to the dielectric surface. The model is implemented in a commercial simulation software CFD-ACE+™ (ESI CFD Inc., Huntsville, AL) [4]. The eventual aim of this effort is to use the computational model as a rapid "virtual prototyping" tool for design analysis of electrowetting-based LoC devices.

Theoretical background of the droplet motion and protein surface binding chemistry is presented in Section 2. Two specific validation cases are presented in Section 3, and Section 4 presents a discussion of the role of passive protein binding in EWOD.

2 THEORY

2.1 Droplet Motion

The current model for droplet motion includes the equations governing the laws of conservation of mass and momentum (Navier-Stokes). For an incompressible, Newtonian droplet, these are written as follows:

$$\nabla \cdot \vec{V} = 0 \tag{1}$$

$$\rho \frac{D\vec{V}}{Dt} = \rho \vec{f} - \nabla p + \mu \nabla^2 \vec{V} \tag{2}$$

A single scalar variable defined as the volume of fluid, f, is used to characterize the volume fraction of each phase (gas and liquid) in each computational grid cell. Once the flow field and initial volume fraction distribution is known, a passive transport equation for f (Equation 3) can be solved to determine the time evolution of the droplet interface [5]:

$$\frac{\partial f}{\partial t} + \nabla \bullet \vec{V} f = 0 \tag{3}$$

Based on knowledge of the volume of fluid, the gas-liquid interface (i.e. droplet position and shape) is reconstructed using an upwind scheme with a Piecewise Linear Interface Construction (PLIC) method [6]. The effect of surface tension on droplet motion is incorporated by determining the net normal force on the gas-liquid interface (computed from the shape of the gas-liquid interface) [4]. The contact angle at the gas-liquid-solid interface is provided as a

boundary condition at the wall, where it is used to evaluate the force due to surface tension, which is added as a source term in the momentum equation. The electric field generated from the applied voltage is not considered in the present work, and the variation of contact angle with the applied voltage is directly applied from measured experimental data.

Generally, in most electrowetting-based biochips, the gap between the electrodes is considerably smaller the size of the chip. As a result, an analytical expression for velocity variation along the gap can be used. Based on this, a parabolic velocity profile based on the flow between two infinite parallel plates is assumed between the top and bottom surfaces of the device (i.e. between the electrodes). This assumption (also known as the Hele-Shaw approximation) has been validated previously [4], and leads to a significant reduction in computational time, with little loss in accuracy.

2.2 Species Transport

Transport of biomolecules occurring within the bulk is due to both convective transport (driven by the flow field) and diffusive transport (diffusion rate of the species in the solution). The mass conservation equation for each biomolecule species in the bulk solution can be written as:

$$\frac{\partial \boldsymbol{C}_i}{\partial \boldsymbol{t}} + \boldsymbol{V} \cdot \nabla \boldsymbol{C}_i = \boldsymbol{D}_i \nabla^2 \boldsymbol{C}_i + \boldsymbol{S}_i \qquad (4)$$

where $\boldsymbol{t}$ = time, $\boldsymbol{D}_i$ = i^{th} species diffusivity, $\boldsymbol{C}_i$ = molar concentration of the i^{th} species in solution, and $\boldsymbol{S}_i$ is the reactive source term coupling the surface reaction to transport.

Biochemical reactions occurring at the surface are accounted for in the conservation equation by balancing the diffusive flux to the surface with the rate of reaction at the surface [5]. This can be written as:

$$-D_i \frac{\partial C_i}{\partial \boldsymbol{n}} + R_i^{production} = \boldsymbol{0} \qquad (5)$$

where $\boldsymbol{D}_i$ is the diffusion coefficient of the i^{th} species, **n** is the direction normal to the reactive wall oriented into the control volume. At non-reactive boundaries, the standard zero flux condition for species applies along with the no slip condition for fluid flow. The VOF method in has also been coupled with species transport and chemistry. In the current implementation, species transport is not allowed across the liquid interface. This condition is enforced by imposing a zero flux condition normal to the liquid interface:

$$\frac{\partial \boldsymbol{C}_i}{\partial \boldsymbol{n}} = 0 \qquad (6)$$

where $\boldsymbol{n}$ is the normal to the liquid interface pointing into the gas phase.

3 VALIDATION

3.1 Validation of VOF Model

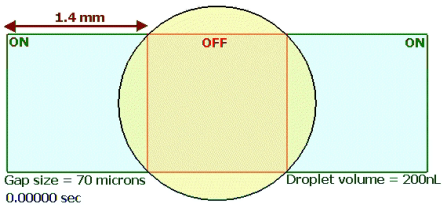
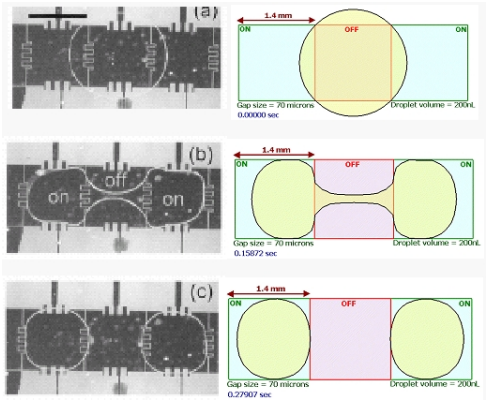
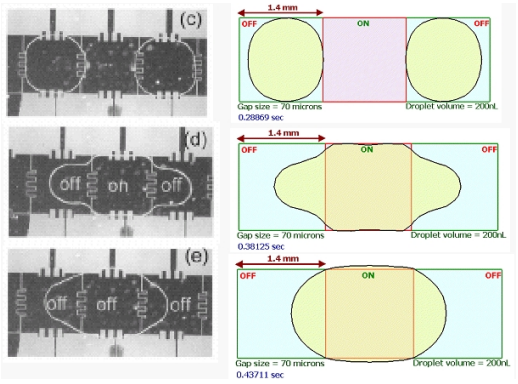


Figure 1: Initial position of droplet (top view)



(a)



(b)

Figure 2: Comparison of experiment (left) [2] and simulation (right) for (a) droplet splitting, and (b) droplet merging, using EWOD.

In order to validate the computational model, a droplet cutting and merging simulation was performed in accordance to the experiments of Cho et al [2]. The computational domain consisted of electrode patches of 1.4 mm x 1.4mm, with a liquid droplet of volume 0.2μL stationary over the electrode patch. Identical to the

experimental setup, the gap size is 70 µm. The initial position of the droplet is shown in Figure 1. The initial contact angle for all electrode patches (under no activation) is 117 degrees. For cutting the droplet, a voltage is applied at the two electrode patches on either side of the droplet. This activation results in a decrease in contact angle, which was experimentally measured to be 90 degrees. Accordingly, these contact angles are prescribed at the electrode patches indicated by "OFF" and "ON" respectively in Figure 1.

Figure 2(a) shows a comparison of experimental data [2] and model predictions when cutting a liquid droplet by activating the electrodes shown by "ON". The reduction in contact angle leads to a "necking" behavior, followed by complete splitting of the droplet. The model shows excellent comparison with experiment, not only with regard to the droplet breakup characteristics, but also with respect to the time required for splitting the droplet (~0.27s for the experiment, as compared to 0.28s predicted by the model). Similarly, the model is also able to predict the merging of the droplets when the electrode voltages between "ON" and "OFF" positions are reversed. The model also captures the lack of a necking state during the merging process.

3.2 Glucose Assay Validation

The computational framework described above has been used to simulate a colorimetric enzymatic assay for glucose based on the Trinder's reaction [6]. Glucose present in the droplet is oxidized to gluconic acid by glucose oxidase, while generating hydrogen peroxide. The peroxide reacts further 4-amino antipyrine (4-AAP) and N-ethyl-N-sulfopropyl-m-toluidine (TOPS) to form quinoneimine. This product is violet colored and can be detected colorimetrically at 545nm. The reactions are as follows:

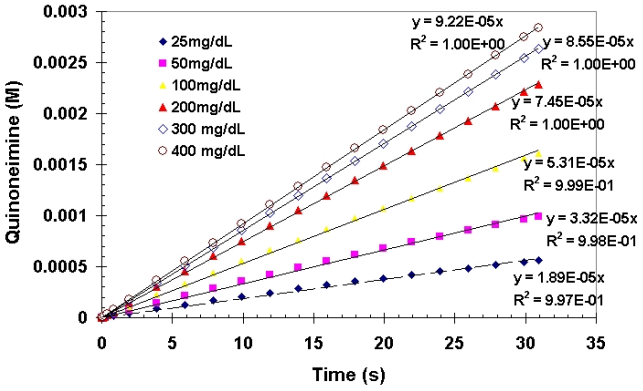
$$\text{Glucose} + H_2O + O_2 \rightarrow \text{Gluconic acid} + H_2O_2 \qquad (7)$$

$$2H_2O_2 + \text{4-AAP} + \text{TOPS} \rightarrow \text{Quinoneimine} + 4H_2O \qquad (8)$$

The glucose oxidation reaction is assumed to follow Michaelis-Menten kinetics, while the quinoneimine formation varies with the amount of hydrogen peroxide formed [6]. As a result, the rate expression for the formation of quinoneimine can be written as:

$$\frac{d}{dt}[\text{Quinoneimine}] \propto V_{\max} \frac{([\text{Glucose}]/DF)}{K_M + ([\text{Glucose}]/DF)} \qquad (9)$$

where V_{max} and K_M are the maximum reaction rate for the Michaelis-Menten reaction, while and the Michaelis constant respectively. The experimental protocol involves merging of two droplets containing the sample and reagents respectively, followed by mixing the sample and reagents in the merged droplet. The mixing is achieved by physically moving the droplet across three electrodes by pulsing the voltage at a frequency of 8Hz. The dilution factor (DF) of the sample in the assay mixture determines the actual concentration of glucose reacting in the assay. Since experimental data is reported only after complete mixing is achieved, the same case is simulated in this validation case. The merged droplet is held steady and is assumed to have all reagents and sample completely mixed. The gap between the electrodes is 475microns. The volume of the merged droplet is 2.0µL for DF = 2, and 2.1µL for DF = 3.



(a)

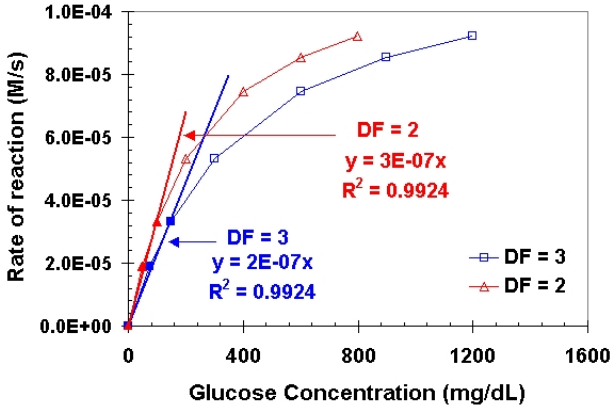


Figure 3: (a) Time variation of quinoneimine concentration (DF = 2); (b) Calibration curve for glucose assay for various dilution factors.

Figure 3(a) shows the variation of concentration of quinoneimine formed as a function of time for various concentrations of glucose (DF = 2). The computational model is qualitatively able to reproduce the linear increase observed experimentally. If the optical path length and the extinction coefficient for quinoneimine are available, then a quantitative comparison can also be made, in the linear range of the calibration curve. The initial rate of reaction (calculated from the slope of the curves in Figure 3(a) are plotted as a glucose concentration. The results are shown in Figure 3(b) for DF = 2 and DF = 3.The assay exhibits a linear variation up to glucose concentration of 100 mg/dL for DF = 2 and 150 mg/dL for DF = 3 (compared with 100 mg/dL and 300 mg/dL respectively in experiments). The

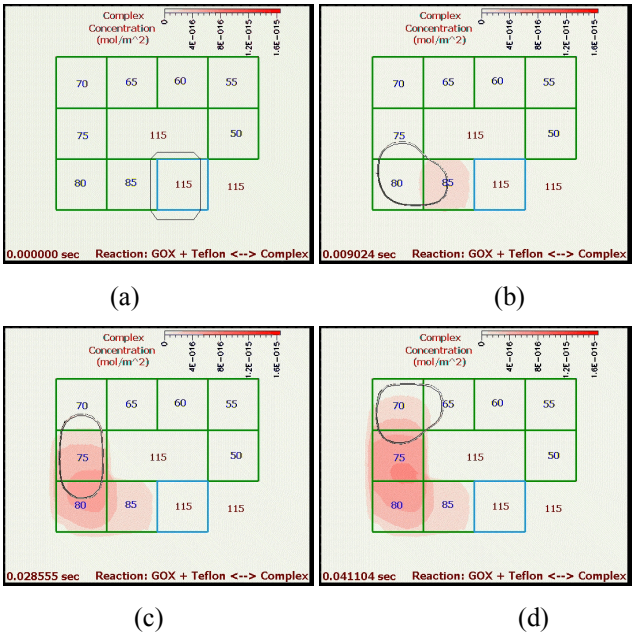
sensitivity ratio (DF = 3 / DF = 2) is equal to the ideal ratio 1.5 (as compared to 1.64 in experiments).

4 PROTEIN BINDING IN EWOD

4.1 Role of Passive Binding

Nonspecific adsorption of biomolecules is an undesirable phenomenon that affects all microfluidic devices. The passive binding of proteins and other molecules from aqueous solutions onto the Teflon or PDMS material used in EWOD devices can occur either due to hydrophobic interactions or electrostatic effects as a result of applied voltage. This can change the contact angle and thereby adversely affect the performance of the device [7]. In addition to potential loss of biomolecules from the solution, it can also affect the limit of detection when dealing with very low concentrations of analytes. Similar problems are also encountered in other electrically actuated surface phenomena such as electroosmotic flow, where binding of proteins and other molecules to the surface can significantly change local zeta potential. This can change the properties of the electrical double layer, causing serious degradation in performance. Here we will demonstrate the use of the computational model to characterize protein binding in electrowetting-based biochips.

4.2 Nonspecific Binding in EWOD systems



(a) (b)

(c) (d)

Figure 4. Time variation of droplet position and concentration of complex in an EWOD system.

The candidate system chosen for the study was the binding of the enzyme glucose oxidase to Teflon. Glucose oxidase is used commonly in microfluidic biochips for measurement of glucose. The nonspecific binding of the enzyme to Teflon is modeled as a reversible reaction that follows mass action kinetics (Equation 10).

$$\text{Glucose Oxidase} + \text{Teflon} \underset{K_d}{\overset{K_a}{\rightleftharpoons}} \text{Complex} \qquad (10)$$

The adsorption (K_a) and desorption (K_d) rate constants used in the model are 3.9 x 10^5 $(M.s)^{-1}$ and 1.1 x 10^{-8} s^{-1} respectively [5]. Figure 4(a) shows a Teflon lined parallel plate channel where a droplet is moved by varying changing the contact angle using the applied electric field. The numbers indicate the contact angle at each electrode. Figure 4(b)-(d) show the time varying position of the droplet and the concentration of the complex formed as a result of the binding of glucose oxidase to the Teflon surface. The adsorption is greater at the trailing edge of the droplet due to the additional time available for glucose oxidase to contact the surface. The amount of complex formed increases linearly with time [Figure 4(b)]. Such a quantitative estimate of nonspecific binding can be used to design strategies for minimizing the same. These include controlling the contact angle at the surface by adjusting the time, bias and magnitude of applied voltage [7].

5 SUMMARY

This paper presents an integrated computational model for simulating droplet transport, biochemical species binding, and nonspecific protein binding phenomena that has been validated against experimental data. The model was then applied to the problem of passive binding of protein in an EWOD system. The model presented in this paper can be used to provide valuable insights in efforts to optimize performance in EWOD-based devices.

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