Computational Studies of Membrane-Based Test Formats

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ABSTRACT

Membrane-based test formats provide simple, accurate and cost effective diagnoses of acute and chronic conditions in general population health. The sensitivity of the immunoassay is determined by the complex biochemical binding kinetics associated with the analyte and receptor molecules in the system. In most flow-through diagnostic systems, the analyte-receptor binding is influenced by the transport of analyte molecules to the receptor surface, which in turn is governed by the flow of sample in the system. Thus, understanding the flow of sample and transport of analyte molecules in a membrane is a crucial step in designing an optimum assay. In the present work, this is accomplished with the help of computational models (CFD-ACE+ from CFD Research Corporation). Line sensitivity analysis and formation of immobile reagent are discussed in the context of a lateral-flow test strip.

Keywords: Rapid Diagnostics, Lateral-Flow, Simulation, Membrane, Quantitation

1 INTRODUCTION

Immunoassay is an analytical method which uses antibodies as selective biochemical reagents to detect and/or measure the amount of antigens in a given sample. These assays are highly sensitive to antigen and provide an analytical system capable of detecting very low levels of analytes. They are highly selective due to the high discriminatory capabilities of the antibodies and are inexpensive in terms of laboratory set-up and per sample cost when compared to conventional methods. However, improperly designed immunoassays may lose sensitivity and exhibit high variability. Besides, sample contamination can interfere with analyte-antibody binding, causing "false positive" results. Hence one needs to understand the fundamental physico-chemical process that occurs in the system to design an efficient assay. An immunoassay that uses membrane-based (lateral-flow) format is considered in this study.

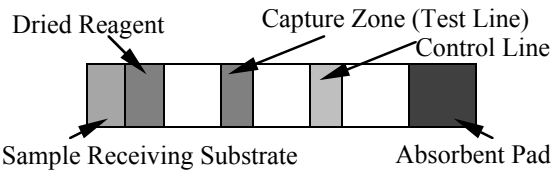


Figure 1. A Typical Configuration of Lateral-Flow Strip

As shown in Figure 1, a lateral-flow strip is a single step assay that requires only sample addition at one end of the device. Due to wicking (capillary) effects, the sample is drawn into the interstitial spaces of the membrane. While continuing along this flow path, the sample contacts the dried reagent, usually a labeled secondary antibody, which then migrates with the analyte to a capture zone containing immobilized antibodies. At this zone, the analyte-antibody complex binds to the immobilized antibody. Unreacted labeled antibody, along with antigen and antigen-antibody complex, continues to flow past the capture zone to the end of the assay indicator. In some systems, a control line is placed next to capture zone. This may be used for calibration purpose. This type of assay is commonly employed for pregnancy detection. Generally there is an absorbent material at the distal end of these devices to aid in drawing sample through the device. The challenge in designing a immuno-chromatographic or lateral flow system is to predict line sensitivity at test and control lines as a function of flow kinetics, reagent concentration, membrane characteristics and time.

The complex interdependence of the various physical and chemical processes occurring during a lateral flow assay cannot be easily resolved by simple analytical methods or be described by empirical rules. However, this is a natural application for Computational Fluid Dynamics (CFD) - a specialized branch of science dealing with fluid flow, heat and mass transfer. The entire performance of the device can be simulated virtually under a variety of operating conditions. This methodology enables a device designer to virtually test and evaluate various different design concepts in a rapid fashion without the need for excessive trial-and-error experimentation with multiple physical prototypes of the design. In the present work, a diffusion-based porous flow model is coupled with a reaction model to predict line sensitivity in a membrane based lateral flow test assay.

2 NUMERICAL METHODOLOGY

Flow through lateral-flow strips is similar to flow through a porous medium. The fluid is considered as a two-phase mixture: liquid reagent that partially or fully fills up the pores and air that occupies remaining space. Occasionally a third phase, solid products, may also be present that fill the pore spaces partially or fully. Each phase represents a continuum and its behavior can be predicted by conservation laws. However, a different set of governing equations will arise for each phase and there is no way of knowing *a priori* in which phase an arbitrary computational point lies and therefore, which set of equations is appropriate to solve. To achieve a single set of equations we need to use volume-averaged phase equation that includes the effects of each phase as a whole. Our objective is to solve only one set of governing equation without losing any critical information.

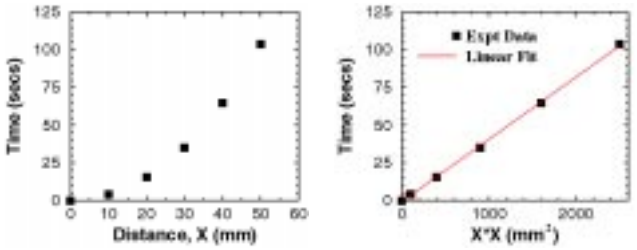
We assume that the flow is two-dimensional and there is no loss of reagent from the surface of the membrane through evaporation. Within the averaged volume, the density of the fluid is assumed constant and the viscous dissipation is neglected. To couple gas-phase and liquid-phase governing equations into a single transport equation, we will use a physical model that solves the transport of reagents as diffusion through "empty" pores due to a concentration gradient. This approach has been used successfully by many research scholars to analyze transport of moisture in soil and wood [1]. The "diffusion approach" is analogous to the "Washburn model" for liquid flow in capillary pores and can be applied to study the flow of reagents in a nitrocellulose membrane. In this model, the location of the front of the liquid is proportional to a diffusion constant based on the pore size, viscosity and surface tension.

The flow of antigen/antibodies in the nitrocellulose membrane is defined through transport equation

$$\frac{f\phi}{ft} = \mathbf{D}\ ^2\phi + S - \mathbf{U}\langle\ \ \phi \qquad (1)$$

where **D** is the diffusivity of the antigen/antibody, ϕ is the concentration of the antigen/antibody in KgMole/m^3, S is the source due to binding reaction, *t* is the time, and **U** is the velocity vector. The left side of the equation describes the transient variation in serum concentration at any given location on the membrane. The first term on the right side models the diffusion transport, the second term models the binding kinetics and the last term models the advection effects. To solve the above equation, we need to calculate convective liquid-phase velocity. This is done by solving the full-scale 2-D Navier-Stokes equations, which describes momentum and mass transport of the fluid [2] instead of the simplified Darcy's equation for fluid flow through porous medium. The only disadvantage of solving the full set of Navier-Stokes equation is that implementation of permeability and porosity of the medium is not straightforward. In the present work, these effects are incorporated into the diffusivity term, **D**. The above equation for species transport along with Navier-Stokes and continuity equations are solved to obtain the flow and concentration fields.

In Figure 2(a), a typical curve, obtained from flow visualization experiment, showing location of the diffusing front (distance from the sample receiving substrate) as a function of time is plotted for a lateral-flow test strip. The sample used in this particular experiment is 5% BSA, 0.1% sucrose and 0.2% surfactant solution and the membrane is made of 5 mm pore size, nitrocellulose polyether sulfone. This curve and the underlying physical phenomena, described by Washburn analogy, suggests that the front moves with the square root of time. When the data is processed and plotted as distance^2 versus time, we obtain a straight line as shown in Figure 2(b). From the slope of this plot, an effective diffusivity ($\mathbf{D}_e$) is calculated as 1/(2*slope). This parameter accounts for the effects of membrane characteristics and liquid properties on the net motion of the liquid front (or sample wicking rate). The sample motion, however, has both a diffusive component (which results in the slowing down of the front as it moves away from the inlet) and a convective component (which provides directionality of motion). From simple mass balance considerations, we can extract the diffusive component of sample transport from the effective diffusivity as follows: $\mathbf{D} = \theta\mathbf{D}_e(1-\phi)$, where θ is a calibration parameter.



(a) (b)

Figure 2. Processing of Raw Experimental Data to Calculate Sample Diffusivity

In the present work, reagents A diffuse from the inlet and react with dried reagent *B* (see Figure 1) to form a complex conjugate *AB*.

$$A + B \rightarrow AB\ ;\ S_1 = K_1[A][B] \qquad (2)$$

where S_1 is the production rate of the complex *AB*, K_1 is the reaction rate constant, and square brackets represent concentration.

This binding is modeled as an instantaneous reaction. As the reagent flow past the test line, the complex

conjugate binds to the immobilized antibody *X* to form the complex conjugate *D*. The following single-step reaction is modeled:

$$AB + X \rightarrow D;\ S_2 = K_2 [AB] [X] \quad (3)$$

where S_2 is the rate of production of *D*, and K_2 is the rate constant for this reaction.

The governing nonlinear partial differential equations for flow, mass transport and binding chemistry will then be solved iteratively in a coupled manner. These solutions will provide a detailed description of the physico-chemical processes occurring in the membrane during the assay. The performance of a typical assay will be evaluated based on incubation time, overall assay time, lower limit of detection and sample size.

3 RESULTS AND DISCUSSIONS

3.1 Model Validation

Numerical models were developed to simulate flow of various reagents (distilled water, distilled water with gold conjugates, BSA with gold conjugates) in various membrane types (Millipore, S&S) with various pore sizes (4 to 20 mm diameter). Bovine Serum Albumin (BSA) was used as a buffer solution and Human Chorionic Gonadotropin (HCG) solution (1mg/ml) was used as diffusion indicator. In Figure 3, the location of the reagent interface predicted by the numerical model at different time levels is plotted. The simulation results agreed well with experimental data.

3.2 Line Sensitivity Studies

The validated models were also used to study the effect of membrane porosity and placement of the capture lines on the line sensitivity. The results are shown in Figures 4 and 5. Some key observations from these studies are:

- Flow rate decreases as we move away from the sample receiving substrate. Hence, the placement of capture line relative to the origin has significant impact on assay sensitivity.
- The flow rate increases moderately as the pore size increases. This reduces the incubation time and hence the sensitivity of the developed signal at the capture line.
- If the binding reaction involves formation of immobile (solid-phase) reagents, the flow dynamics are significantly affected (reduced flow and reaction rate) due to filling of the pores.

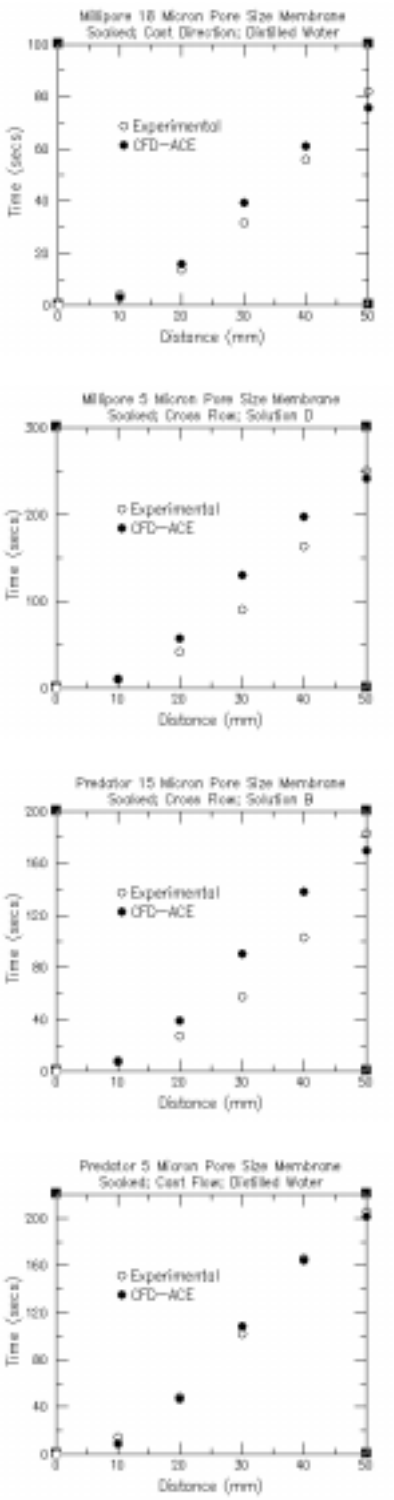


Figure 3. Location of Reagent Interface at Different Time Instants. Plots Show the Comparison of CFD-ACE Simulation with Experimental Data. Solution D is Distilled Water + 0.2% Surfactant, Solution B is 5% BSA + 0.1% Sucrose

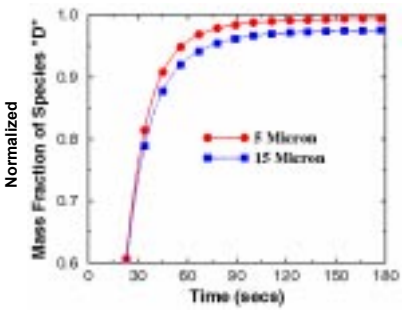


Figure 4. Effect of Pore Size on Developed Line Sensitivity

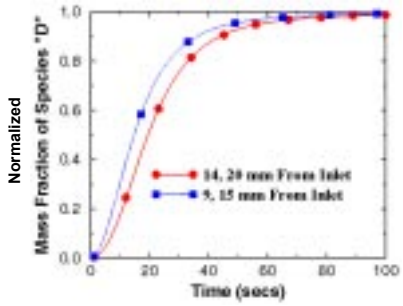


Figure 5. Effect of Test and Control Line Placement on Developed Line Sensitivity

3.3 Modeling of Immobile Reagent

In some lateral flow assays, the complex conjugate *D* may become immobile and will start filling up the pores. This, in effect, will cut off lateral-flow in this region. Modeling of this flow phenomenon is very challenging and was simulated by varying the flow resistance and reagent diffusion in the reaction zone. The results from this simulation are shown in Figure 6 for time t = 10, 30, 60 and 120 seconds. As time progress, more of *D* is formed; the viscosity increases and diffusion decreases rapidly. This is evident in Figures 6 (a), (c), (e) and (g) where the contour plot of *AB* is shown at time t = 10, 30, 60 and 120 secs. Velocity vector plot (Figures 6 (b)) indicates that at time t = 10 seconds, the flow is uniform. As more and more *D* forms, the flow starts going around the reaction zone as illustrated in Figures 6 (d), (f) and (h). At time t = 120 sec (Figures 6 (g)), concentration of *AB* is highest in the reaction zone due to the back diffusion.

3.4 CONCLUSIONS

These results clearly indicate how computer simulations can provide a fundamental understanding of microfluidics and biochemical kinetics in a membrane-based system, which is essential in developing a successful membrane-based immunoassay. They also show how the models can give valuable insight into the dynamics of reagent flow, eliminate guesswork from device design and avoid pitfalls due to counter-intuitive phenomena.

Simulations show that the line sensitivity at the test and control sites is significantly affected by time, concentration of reagents, pore size and placement of test and control lines with respect to the sample receiving substrate. Line sensitivity (concentration of conjugate) increases with decreasing pore size and with the distance between the test line and sample receiving substrate. These simulations demonstrate the applicability of multi-physics computational modeling techniques for the design and analysis of membrane-based test formats.

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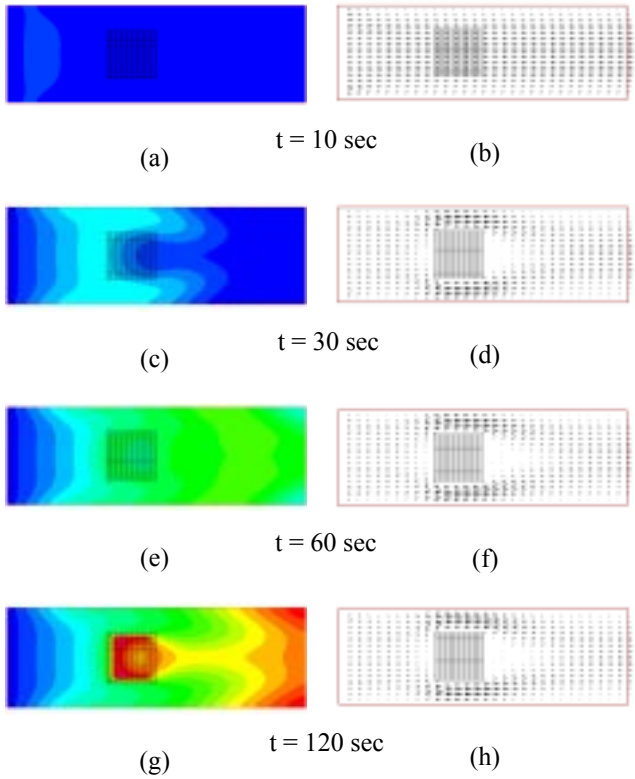

Figure 6. Modeling of Immobile Specie in a Lateral Flow System. Concentration of Complex Conjugate (AB) and Velocity Vectors are Shown at Various Time Levels.

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