Recent Advances on Modeling the Lateral Flow Immunoassay

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Abstract: The rapid immunochromatographic test strip, also called lateral flow immunoassay (LFIA), has recently attracted considerable research attention in the past decade because of its advantages when applied to a wide variety of point-of-care (POC) tests. This paper reviewed recent advances on modeling the LFIA and summarized their advantages and limitations. It is worth mentioning that there is a growing research interest on the general modeling issue for the LFIA system. In order to optimize LFIA performance for the purpose of quantification, it is of great importance to develop a mathematical model that allows us to simulate dynamic characteristics and also find out the effects of various design parameters in a both rapid and inexpensive way.

Keywords: Lateral flow immunoassay, Modeling, Convection diffusion reaction, Nonlinear state-space model, Stochastic dynamic model, Immunochromatographic assay.

1. INTRODUCTION

The lateral flow immunoassay (LFIA), which utilizes the specific interaction between antigens and antibodies as shown in Figure **1**, consists of a porous membrane or strip that is often made out of nitrocellulose [1-3]. In the past few years, LFIA has recently attracted considerable research attention because of its advantages such as ease of use, short analysis time, low cost, high sensitivity, good specificity, satisfactory stability when applied to a wide variety of point-of-care (POC) tests [4]. Owing to these attractive properties, the LFIA has been widely used in many fields including clinical diagnostics [5], food safety testing [6], environmental health and safety [7], agriculture [8], as well as some emerging areas such as molecular diagnostics and theranostics [9]. Many organizations and departments, such as World Health Organization (WHO), Food and Drug Administration (FDA) of the U.S., are concerning with the development of immunochromatographic strip techniques. In the report of WHO, immunochromatographic strip has been recommended for screening part of diseases, and its test quality standards have been revised in accordance with the standards of International Standards Organization (ISO) and the European Union (EU) .

Although the LFIA technology is widely used in a variety of fields, the continuing demand for quantitative result and sensitivity has presented great challenge for researchers since such a detection method suffers from several flaws including poor reproducibility for quantitative analysis and hook effects occurred when there is a high concentration of analyte in the sample [1, 3, 10]. Therefore, most immunochromatographic assays can only offer qualitative or semi-quantitative results observed directly by naked eyes at present which, in turn, significantly limit the application scope of these assays [11-15]. Thus, in order to optimize LFIA performance for the purpose of quantification, it is of great importance to develop a mathematical model that allows us to simulate dynamic characteristics and also find out the effects of various design parameters in a both rapid and inexpensive way. Furthermore, such a model could also enable us to optimize LFIA performance by providing insights into LFIA operation, [3, 16]. Therefore, a series of multidisciplinary approaches are needed for the lateral flow quantitative

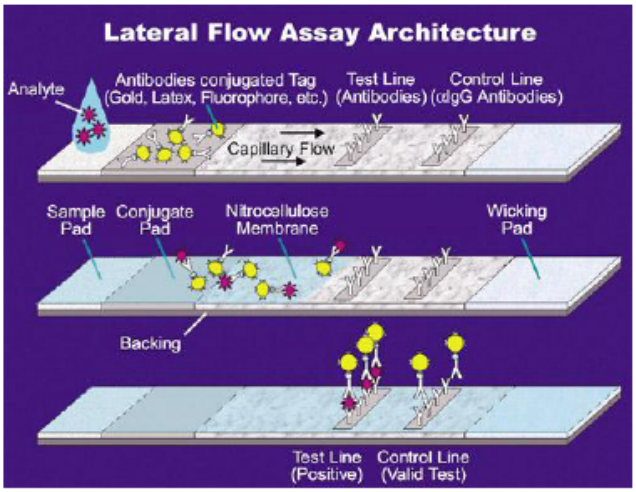

Figure 1: Lateral flow immunoassay architecture.

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assay development. Hence this motivates the review outlined in this paper, which will have great theoretical and practical significances in the areas of biomedical engineering and signal processing.

2. METHODS

Up to now, little research has been done on the general modeling issue for the LFIA system. In [16, 17], the convection diffusion reaction equations have been used to model the LFIA systems and the simulation has been carried out by using the COMSOL software. The model developed in [18] predicted the optimized location of test line on LFIA strip, sample volume and total reaction time that is needed to achieve the required sensitivity for different analytes. In [19], the effect of membrane pore size on lateral diffusion of protein molecules in a nitrocellulose membrane has been investigated. Very recently, in [3, 20, 21], a nonlinear state-space model for sandwich-type LFIA system has been developed via the Bayesian filtering theories. Furthermore, the expectation maximization (EM) algorithm is applied to the modeling of the nano-gold immunochromatographic assay (Nano-GICA) via available time series of the measured signal intensities of the test and control lines in [22]. The model for the Nano-GICA is developed as the stochastic dynamic model that consists of a first-order autoregressive (AR) stochastic dynamic process and a noisy measurement. Therefore, these methods described above will be classified and introduced as follows:

2.1. Convection Diffusion Reaction Model for the Lateral Flow Immunoassay

In [16,17], a mathematical model based on the convection diffusion reaction equations for sandwich assays is developed and exploited to study the performance of the LFIA device under various operating conditions.

The biochemical reactions of the LFIA signal pathway can be summarized as follows [16]:

1) If the sample contains the target analyte A, the analyte A interact with the particulate color particle conjugate P to form particle-analyte complexe PA,

$$A + P \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PA$$

2) The free analyte A and the particle-analyte complexe PA interact with the immobilized ligands of type R to form the complexes when migrating into the membrane by the capillary action,

$$A + R \underset{k_4}{\overset{k_3}{\rightleftharpoons}} RA$$

$$PA + R \underset{k_6}{\overset{k_5}{\rightleftharpoons}} RPA$$

3) Finally, unbound particulate conjugate P may bind to the complex RA to form the complex RPA,

$$P + RA \underset{k_8}{\overset{k_7}{\rightleftharpoons}} RPA$$

In the above, references [16, 17] assumed that the first-order reversible interactions.

The concentrations of the free target analyte ($[A(x,t)]$), the particle–analyte complex ($[PA(x,t)]$), the free particles ($[P(x,t)]$), the ligand–analyte complex ($[RA(x,t)]$), and the ligand–analyte–particle complex ($[RPA(x,t)]$) are described by the convection–diffusion–reaction equations as follows:

$$\frac{\partial [A]}{\partial t} = D_A \frac{\partial^2 [A]}{\partial x^2} - U \frac{\partial [A]}{\partial x} - (F_{PA} + F_{RA})$$

$$\frac{\partial [PA]}{\partial t} = D_P \frac{\partial^2 [PA]}{\partial x^2} - U \frac{\partial [PA]}{\partial x} - (F_{PA} - F^1_{RPA})$$

$$\frac{\partial [P]}{\partial t} = D_P \frac{\partial^2 [P]}{\partial x^2} - U \frac{\partial [P]}{\partial x} - (F_{PA} + F^2_{RPA})$$

$$\frac{\partial [RA]}{\partial t} = F_{RA}$$

$$\frac{\partial [RPA]}{\partial t} = F_{RPA}$$

In the above, D_A and D_P are the molecular diffusion coefficients of the analyte and the particles, respectively. F_{PA}, F_{RA}, F^1_{RPA} and F^2_{RPA} are the rates of formation of the particle–analyte complex (PA), the ligand–analyte complex (RA) and the complex RPA, respectively. RA and RPA exist only in the capture zone and are equal to zero elsewhere.

Then, the model was used to study the performance of the LFIA device under various operating conditions by using the COMSOL software. It should be mention that, based on the above model, Ragavendar and Anmol [18] predicted the optimized location of test line on LFIA strip, sample volume and total reaction time that is needed to achieve the required sensitivity for different analytes on a case to case basis. Therefore, this model can be used as a design tool to optimize the LFIA strip construction and reagent development processes.

2.2. Nonlinear State-Space Model for the Lateral Flow Immunoassay

In [3, 20, 21], a nonlinear state-space model is considered that consists of the biochemical reaction system equations and the observation equation. The system state equations describe the dynamics of the concentration distribution subject to stochastic disturbances, and the system measurements are determined in terms of an observation equation containing measurement noises.

According to the biochemical reactions of the LFIA signal pathway and the general form of dynamic balance equations or kinetic models, the nonlinear model for the LFIA consists of a pair of equations as follows

$$\frac{dx}{dt} = SV(x(t)) + G(t)w(t) \tag{1}$$

$$y(t) = g(x(t)) + L(t)v(t) \tag{2}$$

where $x(t)$ is the vector of state variables which are concentrations of antibodies, antigens or complex material; $y(t)$ is the measurement process; $SV(x(t))$ with S being a stoichiometric matrix that describes the biochemical transformation in a biochemical network and $V(x(t))$ being the vector of reaction rates (usually the vector of nonlinear function of the state) [23]; $G(t)$ and $L(t)$ are arbitrary time-varying matrices independent of $x(t)$ and $y(t)$; $g(x(t))$ is the measurement model function; $w(t)$ and $v(t)$ are system noise and measurement noise, respectively.

In order to obtain the nonlinear model for lateral flow immunoassay biochemical networks from discretely obtained measurements, it is usually essential to formulate the discrete-time analogue as follows [23]:

$$x(k+1) = x(k) + SV(x(k)) + w(k) \tag{3}$$

$$y(k) = g(x(k)) + v(k) \tag{4}$$

Especially, the nonlinear model can be described in detail as follows:

$$\begin{cases} x_1(k+1) = x_1(k) - k_1x_1(k)x_2(k) + k_2x_3(k) - k_3x_1(k)x_4(k) + k_4x_5(k) + w_1(k) \\ x_2(k+1) = x_2(k) - k_1x_1(k)x_2(k) + k_2x_3(k) - k_7x_1(k)x_3(k) + k_8x_6(k) + w_2(k) \\ x_3(k+1) = x_3(k) + k_1x_1(k)x_2(k) - k_2x_3(k) - k_5x_3(k)x_4(k) + k_6x_6(k) + w_3(k) \\ x_4(k+1) = x_4(k) - k_3x_1(k)x_4(k) + k_4x_5(k) - k_5x_3(k)x_4(k) + k_6x_6(k) + w_4(k) \\ x_5(k+1) = x_5(k) + k_3x_1(k)x_4(k) - k_4x_5(k) - k_7x_2(k)x_5(k) + k_8x_6(k) + w_5(k) \\ x_6(k+1) = x_6(k) + k_5x_3(k)x_4(k) - k_6x_6(k) + k_7x_2(k)x_5(k) - k_8x_6(k) + w_6(k) \end{cases}$$

$$y(k) = k_9(x_3(k) + x_6(k)) + v(k)$$

where x_1, x_2, x_3, x_4, x_5 and x_6 are the concentration of A, P, PA, R, RA and RPA, respectively. And, k_1, k_3, k_5, k_7 and k_2, k_4, k_6, k_8 are the association and dissociation rate constants, respectively.

When the association and dissociation rate constants in the vector $V(x(k))$ are denoted by $\theta = \left[k_1, k_2, \cdots, k_9\right]^T$, the model (3)-(4) can be rewritten in the following more compact form:

$$x(k+1) = f(x(k), \theta) + w(k)$$

$$y(k) = g(x(k), \theta) + v(k)$$

where $x(k)$ is the vector of state variables at the time point k, $f(.,.)$ is a nonlinear function with θ being a parameter vector to be identified. $w(k)$ and $v(k)$ denote the zero-mean uncorrelated Gaussian noises with covariance matrices Q_k and R_k, respectively. $y(k)$ is the measurement data from experiments at the time point k.

Finally, Bayesian filtering theories such as the extend Kalman filter [4], Particl filter [20], hybrid extend Kalman filter and particle swarm optimization algorithm [21], are applied for joint state and parameter estimation of the lateral flow immunoassay model.

2.3. Stochastic Dynamic Model for the Lateral Flow Immunoassay

Different from the above problems, the focus of the paper [22] is on the new research issue of gaining deep insight into the relationship between the signal intensities of the test and control lines of the nano-gold immunochromatographic assay (Nano-GICA). The model is viewed as a stochastic dynamic model, which consists of the first-order autoregressive (AR) stochastic dynamic process and the noisy measurement.

The measured data from the signal intensities of the Nano-GICA system are often contaminated by measurement noises.

$$y_i(k) = x_i(k) + v_i(k), \quad i = 1, 2, \cdots, n, \quad k = 1, 2, \cdots, m,$$

where $y_i(k)$ is the measurement data of the ith value of test and control lines at time k, $x_i(k)$ is the ith actual value of test and control lines at time k, $v_i(k)$ is the measurement noise, n is the number of states

($n = 2$ in this system including the signal intensities of the test and control lines) and m is the measurement time points. $v_i(k)$ is assumed as a zero mean Gaussian white noise sequence with covariance $V_i > 0$.

The Nano-GICA containing n states is modeled by the following stochastic discrete-time dynamic system:

$$x_i(k+1) = \sum_{j=1}^{n} a_{i,j} x_j(k) + w_i(k), \quad i = 1, 2, \cdots, n, \quad k = 1, 2, \cdots, m,$$

where $a_{i,j}$ represents the relationship and degree amongst the value of test and control lines. $a_{i,j} > 0$ means the j th state positive stimulating the i th state and, similarly, $a_{i,j} < 0$ stands for the j th state negative repressing the i th state, while a value of zero indicates that j th state does not influence the transcription of i th state. $w_i(k)$ is a zero mean Gaussian white noise sequence with covariance $W_i > 0$, and $w_i(k)$ and $v_i(k)$ are mutually independent.

After specifying the model structure, the expectation maximization (EM) algorithm [22] is applied to handle such a system identification problem via available time series of the measured signal intensities of the test and control lines. By using the EM algorithm, the model parameters, the actual signal intensities of the test and control lines, as well as the noise intensity can be identified simultaneously. Therefore, we could be well guided to choose a good feature parameter for the purpose of quantification.

3. CONCLUSION AND FUTURE WORK

In this paper, we have reviewed recent advances on modeling the lateral flow immunoassay. Up to now, the modeling issue can be castigated into three methods, which are, 1) Convection diffusion reaction equations; 2) nonlinear state-space model; 3) stochastic dynamic model for the LFIA system. Especially, we have summarized their advantages and limitations.

It is worth mentioning that the existing results on the issue of modeling the LFIA system have largely focused on the chemical reaction kinetics without considering the various uncertainties, time-delays, random factors and state-variables constraints in the biochemical reaction networks between the antigens and the antibodies. However this is not always the case in practice and significant differences exist widely within the LFIA systems. Therefore, there still exist many problems on the modeling issue to gain further insight into device operation. Especially, a series of multidisciplinary approaches are needed for the lateral flow quantitative assay development.

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