METHOD FOR DETERMINING THE PARAMETERS AND THE TIME DELAY OF OSCILLATORY KINETIC ADSORPTION SYSTEMS

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Abstract— The understand of adsorption kinetics and apparent formation of mono molecular biotin and bis-biotin layers onto an evaporated thin gold film onto glass substrates remain unknown. Cell membrane permeation in some cases displays the phenomenon of overshoot and oscillation. Oscillation is an important and interesting phenomenon observed in biological and chemical systems. An adsorption kinetics model with time delay can be used in other to explain these phenomena. So, in this work it is proposed an estimation technique for the adsorption kinetics model parameters including the time delay. This technique avoids the non linear estimation problem using a recursive estimation algorithm. The proposed technique is evaluated under experimental tests.

Keywords— Adsorption Kinetics, Time Delay, Recursive Estimation, Oscillation dynamics.

Resumo— A compreensão da adsorção e da aparente formação de camadas mono-celulares de biotin e bisbiotin em filmes de ouro fino sobre substratos de vidro permanece desconhecida. A permeação em membranas, em alguns casos, resulta em comportamentos oscilatórios e com sobressinal. A oscilação é um penômeno importante e interessante obervado tanto em sistemas biológicos como em sistemas químicos. Um modelo para a adsorção com atraso pode ser utilizado para descrever este fenômeno. Desse modo, neste trabalho é prposta uma técnica de estimação para os parâmetros do modelo incluindo o atraso. Esta técnica evita o problema de estimação não-linear utilizando um algoritmo de estimação recursivo. A técnica proposta é avaliada em testes experimentais.

Palavras-chave— Adsorção, Atrso, Estimação Recursiva, Oscilação.

1 Introduction

Organic, enzymatic, proteins, whole cells or other biological materials, immobilized at solid substrates as thin films, are essential components in the design of assays for biosensors. Typically, such assays are composed as a stacked layer assembly. In general, these stacks must arrange in quite specific way, regarding coverage, homogeneity, orientation, robustness and chemical stability. Thiol based self-assembled mono-layers (SAM) are frequently employed as an initial layer. The connection to a supporting thin gold film relies on the chemical interaction (Karpovich and Blanchard, 1994; Laborde et al., 2013).

Apart from its various physiological functions in the cell metabolism, biotin is broadly used as a reagent for biochemical analysis. In connection with its high affinity and complex formation with Avidin, Streptavidin and Neutravidin, it also serves as an important linker molecule in the design of bio-chemical immuno-assays, especially when utilizing biotinylated antibodies (Laborde et al., 2013).

Adsorption and apparent formation of mono molecular biotin and bisbiotin layers onto an evaporated thin gold film onto glass substrates, as well as to colloidal silver particles and SAM functionalized gold surfaces have been reported previously (Morgan et al., 1992; Liu et al., 2011). The adsorption kinetics of free biotin onto the plain gold surface dissolved inwater, as well as its interactions with serum remained unknown. This is considered important, towards achieving a better understanding of non-specific binding mechanisms, especially associated with use of body fluids that are required in medical diagnostics (Laborde et al., 2013).

Cell membrane permeation in some cases displays the phenomenon of overshoot (Ohshima and Kondo, 1989). Also, it was observed oscillatory permeation of drugs into microcapsules with composite membranes (Ohara et al., 1985). Oscillation is an important and interesting phenomenon observed in biological and chemical systems (Ohara et al., 1985).

In (Ohshima et al., 1992) were proposed a model for solute transport through a cell membrane with a time delay in order to explain the phenomena of overshoot and oscillatory permeation. The importance of the time delay has been emphasized in various fields, such as biology, ecology and economics.

The time delay was introduced into the intracellular solute concentration based of the fact that within the cell there is a number of small particles or sites which are expected to trap or accumulate diffusing solutes. The introduction of the time delay leads to, under certain conditions, overshoot or oscillatory permeation (Ohshima and Kondo, 1989; Ohshima et al., 1991).

Figure 1 illustrates a sketch of protein Neutravidin (NAv) adsorption steps, in presence of time

delayed kinetics: 1. Initial Biotin adsorption and film formation; 2.diffusion of NAv molecules to the Biotin film; 3. Irreversible NAv attachment onto the surface, with NAv Biotin complex formation; 4. Simultaneous (irreversible) NAv binding to uncovered fractions on the Gold surface and NAv-binding to excess Biotin 5. Delayed desorption of these weakly attached NAv-Biotin complexes over the time period, during which the molecules may change their shape (conformational change), undergo chemical surface reactions and/or change position through surface diffusion.

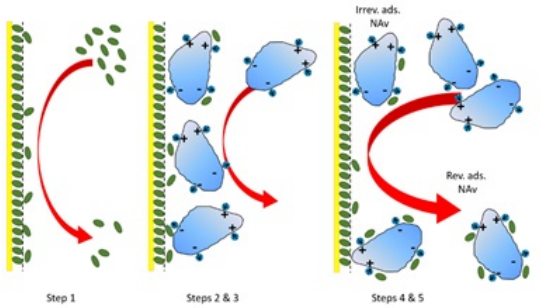

Figure 1: Sketch of the time delayed adsorption

Due to the time delay existence in the adsorption phenomena, in this work it is proposed an estimation method based on the adsorption kinetics model parameters including the time delay proposed in (Ohshima et al., 1992). The time delay is an important parameter because it is direct related with the oscillations observed on the permeation. The proposed method avoids the non linear estimation problem using a recursive estimation algorithm. The proposed technique is evaluated under experimental tests.

This work is organized as follows: in Section 2, the adsorption kinects model is developed; in Section 3, the parameter estimation method is formulated; in Section 4, the experimental setup used is described; in Section 5, the experimental results are presented based on two scenarios; finally, in Section 6, the conclusions are made.

2 Adsorption Kinetics Model

The accumulation of macro-molecules on a solid substrate or the air-solution interface may occur. Attached particles may require a certain time span for desorption from the interface, as a result of their conformational changes or other surface processes that affect the residence time. This accounts for a time delay in the temporal evolution of the solute concentration at the interface, possibly leads to overshoot and oscillation.

Consider adsorption of diffusing solutes onto a solid surface (area A) from the solution phase (volume V). There are N_m sites per unit area on the solid surface that are capable of adsorbing solutes. The solute concentrations in the solution phase and on the solid surface by $C(t)$ and $N(t)$, respectively. The initial conditions are given by

$$C(0) = C_0, \tag{1}$$

$$N(0) = 1. \tag{2}$$

The differential equations for $C(t)$ and $N(t)$ are

$$V\frac{dC(t)}{dt} = -JA, \tag{3}$$

$$A\frac{dN(t)}{dt} = +JA, \tag{4}$$

with

$$J = v_a - v_d \tag{5}$$

and

$$v_a = k_a C(t)\left[N_m - N(t)\right], \tag{6}$$

$$v_d = \begin{cases} 0, 0 \le t \le \tau \\ k_d N(t-\tau), t > \tau \end{cases}, \tag{7}$$

where v_a and v_d, respectively, denote the adsorption and the desorption velocities, J the net flux flowing from the solution phase into the surface, k_a, and k_d, the rate constants of adsorption and desorption, and τ the delay time.

Equation (7) states that during the time interval between $t = 0$ and $t = \tau$, only adsorption occurs and at the time instant $t = \tau$, in addition to adsorption, desorption starts.

As $C(t)$ and $N(t)$ satisfy the conservation relation

$$C(t)V + N(t)A = C_0 V, t \ge 0, \tag{8}$$

this implies

$$V\frac{dC(t)}{dt} = -A\frac{dN(t)}{dt}, t \ge 0. \tag{9}$$

Equations (3)-(7) subject to the initial conditions (1) and (2) can be rewritten as

$$\frac{\dot{C}(t)}{C_0} = \begin{cases} \frac{-\alpha R C(t)}{C_0}\left[1 - \frac{N(t)}{N_m}\right], 0 \le t \le \tau, \\ \frac{-\alpha R C(t)}{C_0}\left[1 - \frac{N(t)}{N_m}\right] + \frac{\alpha R}{K}\frac{N(t-\tau)}{N_m}, t > \tau, \end{cases} \tag{10}$$

$$\frac{N(t)}{N_m} = \frac{1}{R}\left[1 - \frac{C(t)}{C_0}\right], \tag{11}$$

with

$$\alpha = k_a C_0 \tag{12}$$

$$R = \frac{N_m A}{C_0 V} \tag{13}$$

$$K = \frac{k_a}{k_d} C_0 \tag{14}$$

where R is the ratio of the possible maximum amount of solutes adsorbed on the surface to the total solute amount and K is the product of the

binding constant ($= k_a/k_d$) and C_0. Note that R and K are both nondimensional parameters.

In other to write the Equations (10) and (11) independently, the following relations were found and notation changes were made:

$$c\,(t) = \frac{C\,(t)}{C_0} \quad (15)$$

$$n\,(t) = \frac{N\,(t)}{N_m} \quad (16)$$

$$n\,(t) = \frac{1}{R}\left[1 - c\,(t)\right] \quad (17)$$

and with respect to the first derivatives

$$\dot{n}\,(t) = \frac{dn\,(t)}{dt} \quad (18)$$

$$\dot{c}\,(t) = \frac{dc\,(t)}{dt} = -R\dot{n}\,(t)\,. \quad (19)$$

Substituting Equations (15)-(18) in Equation (10), it possible to write for $\dot{c}\,(t)$ in Equation (20) and for $\dot{n}\,(t)$ in Equation (21).

3 Parameter Estimation Method

3.1 Preliminaries

The estimation problem is a non-linear problem if all the parameters (α, R, K, τ) need to be estimated as can be seen from Equation (10). Non-linear estimation problems are difficult to solve especially due to the local minimum solutions. Also, these problems do not have analytical solutions and are frequently non-convex problems (Ljung, 1999).

But the non-linear estimation problem can be turned into a linear estimation problem. This is true if it is possible to obtain an estimate for the time delay τ separately. So, just α, R, K need to be estimated and the problem can be written as linear estimation problem and it has a global minimum.

This way, two kinds of estimation algorithms can be used to solve the adsortion estimation problem: non-linear algorithms and linear algorithms. The linear algorithms were used in this work based on the reasons stated above.

In order to obtain an estimate for the time delay τ and turn the non-linear problem into a linear problem, it was performed a search procedure. The chosen value for τ is the one that results on the least quadratic residual error. This residual error is calculated by the difference of the experimental data $y(t)$ and the estimated data $\hat{y}(t)$ and given by

$$V(\tau) = \min_{\tau} \sum_{N} \frac{1}{N} \left|y(t) - \hat{y}(t|\tau)\right|^2 . \quad (23)$$

To performed the search procedure, the estimation algorithm proposed in this work is a two step algorithm for each value of the time-delay: first, the time-delay τ is chosen; second, the parameters (α, R, K) are estimated using this time-delay value.

3.2 Regression form

In order to formulate the estimation problem in the regression form as $y(t) = \varphi(t)\theta$, the discrete-time model equations were used. As the information available is the solute concentrations on the solid surface $n(t)$, the discrete-time model of the Equation (21) using Euler discretization method is developed and given in Equation (22).

It is necessary to point that it would be possible to use the solute concentrations in the solution phase $c\,(t)$ or the solid surface $n\,(t)$ as the experimental data. There is no limitation.

Assuming that the time-delay τ is known, Equation (22b) can be written as

$$n(t) = \varphi(t|\tau)\theta \quad (24)$$

where

$$\varphi\,(t) = \left[\begin{array}{cccc} 1 & n\,(t) & n\,(t)^2 & -n\,(t-\tau) \end{array}\right] \quad (25)$$

$$\theta^T = \left[\begin{array}{cccc} h\alpha & (1-h\alpha) & \alpha hR & \frac{h\alpha}{K} \end{array}\right] . \quad (26)$$

The constrained least-square problem is formulated as

$$\hat{\theta} = \min_{\theta} \frac{1}{2} ||A\theta - B||_2^2 \quad (27)$$

$$subject \quad to \quad \theta(1) + \theta(2) + \theta(3) = 1 \quad (28)$$

where

$$A = \sum_{N} \varphi(t|\tau)\varphi^T(t|\tau) \quad (29)$$

$$B = \sum_{N} \varphi(t|\tau)n(t). \quad (30)$$

So, the proposed estimation algorithm steps are:

1. Set the initial value for τ;
2. Estimate the parameters (α, R, K) using Equation (24);
3. Calculate the residual error $V_i(\tau)$ using Equation (23);
4. Store α, R, K, τ and $V_i(\tau)$ for this iteration i;
5. Go to step 1 until $\tau = N$;
6. Search the least absolute value of $V_(\tau)$ for all iterations;
7. Get the corresponding parameters α, R, K and τ.

$$\dot{c}(t) = \begin{cases} -\alpha(R-1)c(t) - \alpha c^2(t), & 0 \le t \le \tau, \\ -\alpha(R-1)c(t) - \alpha c^2(t) + \frac{\alpha}{K} - \frac{\alpha}{K}c(t-\tau), & t > \tau \end{cases} \tag{20}$$

$$\dot{n}(t) = \begin{cases} \alpha - \alpha(R+1)n(t) + \alpha R n^2(t), & 0 \le t \le \tau \\ \alpha - \alpha(R+1)n(t) + \alpha R n^2(t) - \frac{1}{K}n(t-\tau), & t > \tau \end{cases} \tag{21}$$

$$n(t+h) = \begin{cases} h\alpha + [1 - h\alpha(R+1)]n(t) + \alpha h R n^2(t), & 0 \le t \le \tau \\ h\alpha + [1 - h\alpha]n(t) + \alpha h R n^2(t) - \frac{h\alpha}{K}n(t-\tau), & t > \tau \end{cases} \tag{22}$$

4 Experimental Setup

A disposable, polymer SPR-biochip has been employed for these investigations, upon appropriate initial surface treatment and functionalization. Chip fabrication technology and associated optical set-up, are described in detail elsewhere (Laborde et al., 2013; Filho et al., 2010). The device is loaded into a separate optical instrument, denoted as the SPR reader. It houses all components, as a microfluidic flow cell for transporting the diluted serum or the analyte solutions to the sensing chip surface, as well as optical and electronic components for optical monitoring and recording the temporal variations of the SPR signal that originate from the NSB phenomenon.

The immunoassay under consideration in the present work utilized NeutrAvidin (NA) mediated attachment of biotinylated monoclonal antibodies (MAB's) against Dengue 2 (DENV2). Briefly, NeutrAvidin is the deglycosylated form of Avidin and does not contain carbohydrates. The NeutrAvidin has a lower, nearly neutral isoelectric point (pI) at pH 6.3 when compared to Avidin (with pI of 10) and Streptavidin, thus being less prone to non-specific binding effects, including those from the serum.

The NA protein forms a tetramer, with four identical domains for Biotin binding, therewith acting as a molecular bridge between biotinylated pathogen specific MAB's above, and the substrate beneath. NeutrAvidin films also can be attached directly onto the pre-cleaned gold film surface, out of an admitted diluted NA solution, and without use of any molecular linker, with the molecular anchoring mechanism to gold not yet clearly identified. The diluted serum is then admitted to the functionalized surface.

The surface functionalization and cleaning protocol, applied in this work, was taken from (Filho et al., 2010), and is illustrated in the insets to Figure 2. Briefly, an atomically clean surface of the 50 nm thin gold layer is an essential precondition for efficient surface functionalization of the disposable optical SPR-biochip.

The polycrystalline gold film was sputter deposited at ambient temperature under high vacuum conditions. Organic and an-organic surface contaminations on the surface were initially removed by storage over a period of 3 hrs in hot

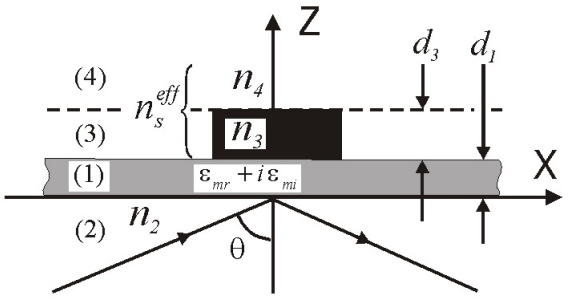

Figure 2: Illustration of the four layers model used to describe the SPR sensor: (1) the gold film, (2) the semi-infinite substrate, (3) a bio-/chemical sensor element, and (4) a semi-infinite environmental medium, which is usually the analyte solution.

30 % hydrogen-peroxide solution. Afterwards, the surface was rinsed with de-ionized water, nitrogen dried and again rinsed with 100 % ethanol. After chip mounting into the instrument and flow cell attachment, the sensing surface remains without contact to ambient air, is repeatedly exposed to concentrated hypo-chloride solution and finally rinsed with triple distilled water and PBS solution. To attach the linker layer, 1 mM sulfonated Biotin was dissolved in ethanol (p. A grade) and the optical chip kept for 3 hr in the solution to form a self assembled monolayer on the thin gold film. NeutrAvidin (manufactured by Pierce Inc.), was admitted as a diluted solution with PBS at 0.01-0.1 mg/mL concentration. It is important to mention that only fresh prepared protein solutions and materials were used in these investigations. Aged samples, or those maintained for longer period at ambient temperature consistently lead to substantially lower coverage values. Pathogen free human serum and blood samples were provided from an unknown, healthy donor from a local hospital.

5 Experimental Results

In this section are presented the experimental results using the proposed estimation method to obtain the adsorption kinetics model parameters. In this work, the parameters are estimated using ex-

periment testes where the solute concentration on the solid surface $N(t)$ was measured.

Optical polymeric SPR-biochips have been employed in the present work. The sensing spot is covered with a magnetron sputtered 50 nm thin, optically semi-transparent gold film. The chip fabrication technology method details are described in (Thirstrup et al., 2004). The SP-output signal provides the adsorption induced (effective) refractive index variation with time $\Delta_{neff}(t)$ from which the surface coverage can be extracted (Laborde et al., 2013).

5.1 Experiment 1

The experiment data (concentration of the NAV-coverage) is shown in Figure 3. This data was pre-treated in order to remove the drift and the mean values. This was done to get unbiased parameters estimates (Ljung, 1999).

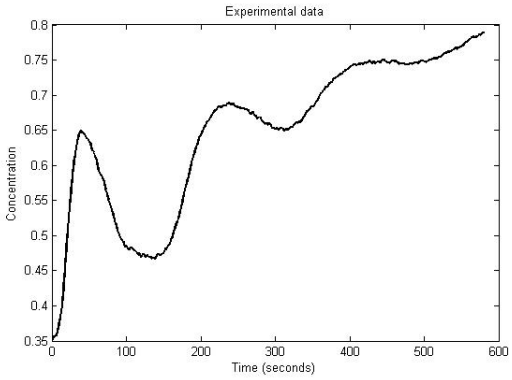

Figure 3: Experimental data - solute concentration on the solid surface $N(t)$

As said in the previous Section, it was performed a search procedure in order to keep the problem linear in the parameters. In this example, the time-delay range was chosen based on the total experiment test time, that was, $\tau \in [0, 577]$ seconds.

For each time-delay value in the range, the parameters (α, R, K) were estimated and the residual error $V_i(\tau)$ were calculated. Follow in Figure 4, the absolute residual error $|V(\tau)|$ versus the time-delay.

From Figure 4, it is possible to determine the value for the time-delay that results on the least absolute residual error. For this case, the time-delay value was $\tau = 553$ seconds and $|V(\tau)| = 2.402e^{-9}$. But, in this example, the time-delay value need to be close, not exactly, the oscillations period due to physical restrictions. Considering this assumption, the time-delay value is $\tau = 222$ seconds and $|V(\tau)| = 7.107e^{-4}$.

With $\tau = 222$ seconds, the estimated parameters using Equation (24) are $\alpha = 0.0005$,

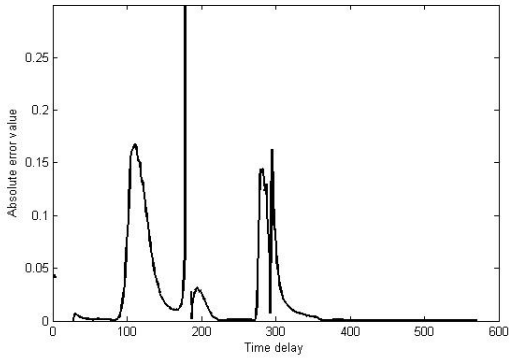

Figure 4: Absolute residual error $|V(\tau)|$ versus the time-delay

$R = 37.705$ and $K = 0.075$. Folow in Figure 5 the experiment test data and the estimated data with $\tau = 222$ seconds and with $\tau = 553$ seconds for comparison. It seems that the physical restriction for the time-delay did not compromised the model prediction too much.

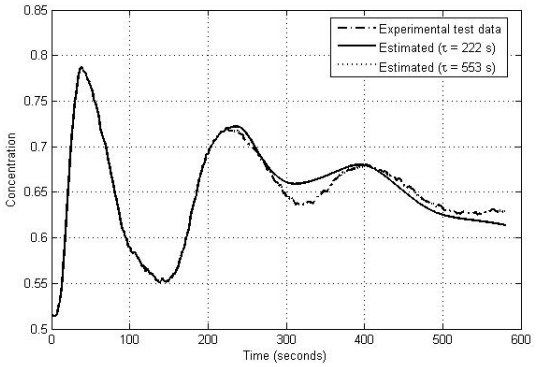

Figure 5: Experiment test data and estimated data with $\tau = 222$ seconds and with $\tau = 553$ seconds

From Figure 5, the optimum time delay value result in good fit result. It almost undistinguished. Even with physical constraint, the constrained time delay also have a good fit.

5.2 Experiment 2

The adsorption model under consideration aimed at describing the kinetics of complex molecules at the solid-liquid interface. In an attempt to explore the limitations of the model, the theory has been tentatively applied to small molecule adsorption at the solid-gas interface.

Under certain experimental conditions, binding of CO and its oxidation to CO_2 on Platinum, in presence of oxygen, also leads to periodic kinetic features, which are connected to spatio-temporal self-organization and a catalytic surface reaction.

Figure 6 exhibits a comparison of the numerically calculated time series with time delay model using the data from (Ertl, 1995). The constrained delay (or surface residence) is 84 seconds and close to the time period of the oscillation. Again, in this example, the time-delay value need to be close, not exactly, the oscillations period due to physical restrictions. The model parameters are $\alpha = 0.046$, $R = 0.089$ and $K = 0.639$.

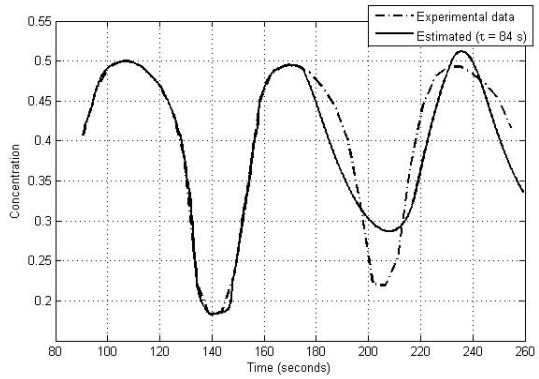

Figure 6: Experiment test data and estimated data with $\tau = 84$ seconds

It is important to mention that the model/concept proprosed in (Ohshima et al., 1992) is suited to replace the non-linear dynamics system approach and rate equations. It should be rather considered as a supplement to gain further information on the adsorption system. The time delay and associated surface residence time, respectively, can be understood as the period available to adsorbed molecules to diffuse, react or re-organize, before desorbing or disappearing from the surface.

6 Conclusions

In this work it was proposed an estimation method for the adsorption kinetics model parameters including the time delay proposed in (Ohshima et al., 1992). The technique avoids the non-linear estimation problem using a recursive estimation algorithm. The time delay selection criteria is based on the quadratic of the residual error. The experiment tests shown the capabilities of the proposed method. Apart from kinetic constants, the model also provides information on the surface residence time, which is otherwise difficult to determine directly.

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