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An analysis of antigen–antibody binding kinetics for biosensor applications utilized as a model system: influence of non-specific binding

Zhanchi Chen, Ajit Sadana *

Chemical Engineering Department, University of Mississippi University, Mississippi, MS 38677-9740, USA

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Abstract

The influence of non-specific binding on the specific binding of antigen in solution to antibody immobilized on a biosensor surface is presented for first-, one and a half-, second-, and other order reactions occurring under external diffusion-limited conditions. Both single-step and dual-step binding of antigen to antibody is considered. For a half-order reaction the value of the ratio of non-specific binding to specific binding (α) does not affect the rate of specific binding since a single curve represents the binding curve for $\alpha = 0$ to 0.5. An increase in the α value leads to a decrease in the rate of binding and in the amount of antigen bound specifically to the antibody on the surface for first-, one and a half-, and second-order reactions. Also, an increase in the reaction order increases the sensitivity of the specific binding to the α value. An increase in the antigen concentration in solution increases the amount and the rate of specific binding for first-, one and a half-, and second-order reactions.

The introduction of non-specific binding leads to complexities in the specific binding of the antigen for the one and half- and second-order reactions as the antibody concentration on the surface is varied. When non-specific binding is present there is an optimum value of the antibody concentration on the surface that yields the maximum rate and amount of antigen specifically bound for one and a half- and second-order reactions. Though this optimum amount of antibody immobilized on the surface is the same for the one and a half- and second-order reactions, it is different for different α values. No such complexities are observed for first-order reactions.

The inclusion of non-specific binding in the analysis provides a more realistic picture of the binding of the antigen in solution to the antibody immobilized on the surface. The figures that show the numerically calculated binding rates for different orders when non-specific binding is present, represent the most useful part of the analysis for readers who are interested in constructing biosensors, and should assist in the control and manipulation of these interactions at the surface. These curves can be used to interpret a far from ideal binding of antigen to an immobilized antibody surface or vice versa. More-or-less all of the analysis should also be applicable to analytical systems that would not be classified as biosensors (that is, immuno and receptor assays).

Keywords: Non-specific binding; Antigen–antibody binding kinetics; Biosensor applications

* Corresponding author.

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1. Introduction

Sensitive detection systems (or sensors) are required to detect and distinguish a wide variety of substances. Sensors find application in the areas of biotechnology, physics, chemistry, medicine, aviation, oceanography, and environmental control. Lowe [1] emphasizes that the rapid ever increasing advances in health care represent a major driving force for the development of biosensors. He further indicates the application of sensors in agricultural, horticultural, and veterinary areas; besides they may be used in the detection of flavors, essences, and pheromones. Mathewson and Finley [2] emphasize that the exponential growth in interest in the development of biosensors has arisen due to their potential for ease of application in the detection of almost a limitless number of analytes in a wide variety of surroundings. Sensors should be reliable, simple, be rapid in their measurement, and should be able to detect low levels of analytes often in a mixture of similar substances. Furthermore, after possessing the above qualities to various degrees, the sensor should also not be unreasonably expensive.

Biosensors, as the name indicates, use biologically derived molecules as sensing elements. The main feature of the biosensor is the spatial unity of the biomolecules with a signal transducer [1]. Scheller et al. [3] emphasize the importance of providing a better understanding of the mode of operation of biosensors to improve their sensitivity, stability, specificity, and speed of response. The success of the detection scheme will be significantly enhanced if one obtains physical insights into the different steps that are involved in the 'sensing' process.

The solid-phase immunoassay technique provides a convenient means for the separation of reactants (for example, antigen) in solution. Such a separation is possible because of the high specificity of the analyte for the immobilized antibody. Obviously, external diffusion limitations play a role in the analysis of such assays. The influence of diffusion in such systems has been mentioned [4] and analyzed to some extent [5–11]. Eddowes [12] emphasizes that the chemical binding kinetics of the antigen to the immobilized antibody or vice versa, the equilibrium, and the mass transport limitations of the analyte to the surface would constrain the performance and sensitivity of biosensors. Furthermore, the binding constants for antigen–antibody reactions at interfaces and also for protein adsorption systems (which exhibit behaviour similar to antibody–antigen systems) are often of a temporal nature. This temporal nature of the binding constants and the inherent heterogeneities present in these systems may be described utilizing fractals [13,14]. Kopelman [15] indicates that surface diffusion-controlled reactions that occur on cluster or islands are expected to exhibit anomalous and fractal-like kinetics. These fractal kinetics exhibit anomalous reaction orders and time-dependent rate (for example, binding) coefficients.

It needs to be emphasized that in all of the above analysis, non-specific binding was not seriously considered. However, in the real situation, non-specific binding does exist and this kind of binding does not make any contributions to the signal obtained. Besides, it plays a derogatory role with regard to the sensitivity, stability, specificity, and response time of biosensors. For example, Eddowes [12] emphasizes the 'balance' inherent in the practical utility of biosensor systems. He estimates that though acceptable response times of the order of minutes or less should be obtainable at μM concentration levels, inconveniently lengthy response times will be found at nM or lower concentration levels. Scheller et al. [3] and Sadana and Sii [10] have emphasized the need for analyzing non-specific adsorption and for providing some factor that relates the extent of non-specific adsorption to the total adsorption (specific plus non-specific) adsorption. No serious discussion was however presented by either sets of authors.

Scheller et al. [3] have indicated some methods by which this non-specific adsorption may be minimized; for example, by coating the surface with hydrophilic material which exhibits a low interfacial energy. Sadana and Sii [10] indicate that the ratio of the non-specific adsorption to the total adsorption should be carefully examined, and if it is negligible (say less than 5–10%) then it should be explicitly stated. Furthermore, Nygren [16] has analyzed the spatial distribution of antibody-coated colloidal gold particles over an antigen-coated surface by electron microscopy. This author noted that the non-specific as well as the specific binding for this case exhibits a

spatial distribution. Thus, non-specific binding should be considered in the development of an appropriate model.

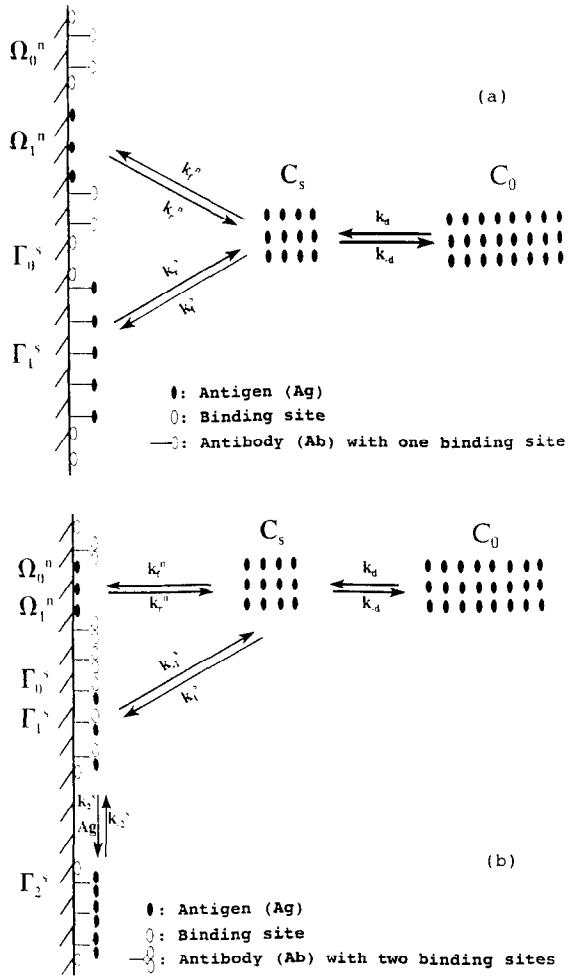

Fig. 1. (a) Elementary steps involved in the binding of the antigen to the antibody covalently attached to the fiber-optic surface for the first-order reaction. Γ_0^s and Ω_0^n are the total concentration of the specific binding sites on the antibody and of the non-specific binding sites on the fiber-optic surface, respectively. (b) Elementary steps involved in the binding of the antigen in solution to the antibody covalently attached to the fiber-optic surface for the second-order reaction. Γ_0^s and Ω_0^n are the total concentration of the specific binding sites on the antibody and of the non-specific binding sites on the fiber-optic surface, respectively. Γ_1^s and Ω_1^n are the concentration of the antibody which is bound to one antigen due to specific binding and of the filled non-specific binding sites on the fiber-optic surface, respectively. Γ_2^s is the concentration of the antibody which is bound to two antigens due to specific binding.

In this manuscript, we will analyze theoretically in some detail the influence of different parameters on the binding of antigen in solution to antibody immobilized on the fiber-optic surface. The theoretical model will include both specific as well as non-specific binding. The analysis should provide fresh physical insights into first-, second-and other order antigen–antibody reactions occurring on the fiber-optic biosensor surface under external diffusion-limited conditions. The analysis to be presented is of a general enough nature, and more-or-less all of the treatment can presumably be extended to apply to analytical systems that may not be classified as biosensors (for example immuno and receptor assays).

In general, the treatment may also be extended to apply to any receptor–ligand or analyte-receptorless (protein adsorption) systems. The characterization of a solid surface for antibody–antigen, receptor–ligand, and for analyte-receptorless (in general, any biochemical reporter molecules immobilized on the surface) systems is of immediate importance [17]. One word of caution on extending the idea from antigen–antibody systems to, for example, ligands and cell surface receptors is the importance of reduction-of-dimensionality kinetics. Axelrod and Wang [18] have indicated the importance of reduction-of-dimensionality kinetics, wherein reactions between ligands and cell-surface receptors can be enhanced by non-specific adsorption followed by two-dimensional diffusion to cell-surface receptors.

2. Theory

Fig. 1a describes the steps that are involved in the binding of the antigen in solution to the antibody covalently attached to the surface (specific binding), and to the fiber-optic biosensor surface (non-specific binding). External diffusion limitations play a significant role in the specific and the non-specific binding of the antigen. The diffusion-limited reaction can be determined by considering the equation:

$$\frac{\partial c}{\partial t} = D\nabla^2 c = D\frac{\partial^2 c}{\partial x^2} \tag{1a}$$

Here t is the reaction time, x is the distance away from the fiber optic surface, and D is the diffusion coefficient. For all practical purposes, the single dimension diffusion on a flat plate analysis is appropriate considering the dimensions of the molecule, the diffusion coefficient of the reactant in solution, and the radius of the fiber-optic surface [9–11]. Cylindrical geometry (or coordinates) may also be utilized for understanding biosensor applications. In that case the diffusion limitation of the reaction scheme can be determined for purely radial diffusion by considering the following equation in one dimension:

$$\frac{\partial c}{\partial t} = D\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial c}{\partial r}\right) \tag{1b}$$

In other coordinate systems (for example, spherical) Shoup and Szabo [19] indicate that the diffusive motion can play an important role in determining the rates of biomolecular reactions such as ligand binding to cell macromolecules or cell-bound receptors.

Eq. 1a may be rewritten in dimensionless form as:

$$\frac{\partial y}{\partial \theta} = \frac{\partial^2 y}{\partial z^2} \tag{1c}$$

Here $y = c/c_0$, $z = x/L$, where L is a characteristic length dimension, for example, the diameter of a fiber-optic biosensor, and $\theta = t/(L^2/D)$.

The appropriate initial condition for Eq. 1a in dimensionless form is:

$$y(z,0) = 1 \quad \text{for } z > 0, \theta = 0$$
$$y(0,0) = 0 \quad \text{for } z = 0, \theta = 0 \tag{2a}$$

The above initial condition is equivalent to the rapid immersion of a sensor into a solution with antigens.

A boundary condition in dimensionless form for Eq. 1a is:

$$y(\infty,\theta) = 1 \quad \text{for } \theta > 0, z = \infty \tag{2b}$$

This boundary condition was not mentioned in similar previous analysis by other groups, for example, Stenberg et al. [7], as well as by ours [8–11,13,14]. We now feel that this more correctly represents the actual situation. This condition indicates that the concentration of the analyte in solution is unaffected 'far away' from the biosensor surface. This is a typical boundary condition used for diffusional-limited problems in mass (or temperature is measured in that case) transfer.

Another boundary condition for Eq. 1a is:

$$D\frac{\partial c}{\partial x} = \frac{\mathrm{d}\Gamma^{\mathrm{s}}_{\mathrm{Ag}}}{\mathrm{d}t} + \frac{\mathrm{d}\Omega^{\mathrm{n}}_{\mathrm{Ag}}}{\mathrm{d}t} \quad \text{for } t > 0, x = 0 \tag{2c}$$

Eq. 2c arises because of mass conservation, wherein the flow of antigens to the surface must be equal to the rate of antigen reacting with the antibody on the surface (specific binding), and the binding of the antigen to the surface itself (non-specific binding). Here $\mathrm{d}\Gamma^{\mathrm{s}}_{\mathrm{Ag}}/\mathrm{d}t$ and $\mathrm{d}\Omega^{\mathrm{n}}_{\mathrm{Ag}}/\mathrm{d}t$ represent the rates of specific and non-specific binding, respectively. For different reaction orders the right hand side is different. First- and second-order reactions are selected for study since the analysis for these reaction orders where only specific binding is present for biosensor applications is available [9–11]. The details for obtaining this boundary condition are presented below for first- and second-order reactions. The analysis is then easily extended for one and one-half and general nth order reactions (fractal-like kinetics).

2.1. First-order reaction

The rate of binding of a single antigen by an antibody is given by:

$$\frac{\mathrm{d}\Gamma^{\mathrm{s}}_1}{\mathrm{d}t} = k^{\mathrm{s}}_{\mathrm{f}} c_{\mathrm{s}}(\Gamma^{\mathrm{s}}_0 - \Gamma^{\mathrm{s}}_1) - k^{\mathrm{s}}_{\mathrm{r}}\Gamma^{\mathrm{s}}_1 \tag{3a}$$

where Γ^{s}_0 is the total concentration of the antibody sites on the surface, Γ^{s}_1 is the surface concentration of antibodies that are bound to a single antigen at any time t, c_{s} is the concentration of the antigen close to the surface, $k^{\mathrm{s}}_{\mathrm{f}}$ is the forward reaction rate constant, and $k^{\mathrm{s}}_{\mathrm{r}}$ is the reverse reaction rate constant. In this case, even though the antibody molecule has two binding sites, for all practical purposes we believe that an antigen molecule reacts with an antibody as if it had only one binding site.

For initial binding kinetics, after some simplification, one obtains [9,10]:

$$\frac{d\Gamma^{\mathrm{s}}_1}{\mathrm{d}t} = k^{\mathrm{s}}_{\mathrm{f}}\Gamma^{\mathrm{s}}_0 c_{\mathrm{s}} \tag{3b}$$

Integration of the above equation yields the relative concentration of the antigen bound by specific

binding. This is proportional to the optical signal of the sensor at any time, t. Therefore:

$$\frac{\Gamma^s_{Ag}}{c_0} = k^s_f \Gamma^s_0 \int_0^t y\,dt \tag{3c}$$

The rate of non-specific binding of the antigen is given by:

$$\frac{d\Omega^n_1}{dt} = k^n_f c_s(\Omega^n_0 - \Omega^n_1) - k^n_r \Omega^n_1 \tag{4a}$$

Here Ω^n_0 is the total concentration of the non-specific binding sites on the fiber-optic surface, Ω^n_1 is the concentration of the antigen bound by non-specific binding, and k^n_f and k^n_r are the forward and reverse binding rate constants for non-specific binding, respectively.

In the initial kinetic regime, $\Omega^n_1 \ll \Omega^n_0$ and $k^n_f c_s \Omega^n_0 \gg k^n_r \Omega^n_1$. Then, from Eq. 4a one obtains the following for non-specific binding:

$$\frac{d\Omega^n_1}{dt} = k^s_f \Omega^n_0 c_s \tag{4b}$$

The simplified reaction scheme for the binding of the antigen by specific as well as by non-specific binding is given by:

$$\mathrm{Ab} + \mathrm{Ag} \rightleftharpoons \mathrm{Ab \cdot Ag}$$

$$\mathrm{NS} + \mathrm{Ag} \rightleftharpoons \mathrm{NS \cdot Ag} \tag{5}$$

Here Ab represents the antibody binding site, NS represents the non-specific binding site, and Ag represents the antigen.

On substituting for the rates of specific (Eq. 3b) and non-specific binding (Eq. 4b) into the boundary condition (Eq. 2c) yields in dimensionless form:

$$\frac{\partial y}{\partial z} = \mathrm{Da}(1 + \alpha)\, y \tag{6}$$

Here $\alpha = (k^n_f \Omega^n_0)/(k^s_f \Gamma^s_0)$ and Da is the Damkohler number. α is the ratio of the maximum binding rate due to non-specific and specific binding. Also, $\mathrm{Da} = k^s_f \Gamma^s_0 L/D$. The Damkohler number is the ratio between the maximum reaction rate and the maximum rate of external mass transport.

2.2. Second-order reaction

The modelling of the specific binding of an antigen in solution to an antibody immobilized on the surface is done by a two-step process. The elementary steps involved in the reaction scheme are shown in Fig. 1b [11].

The rate of binding of a single antigen by an antibody is given by:

$$\frac{d\Gamma^s_1}{dt} = k^s_1 c_s(\Gamma^s_0 - \Gamma^s_1 - \Gamma^s_2) - k^s_{-1}\Gamma^s_1 + k^s_{-2}\Gamma^s_2 - k^s_2 c_s \Gamma^s_1 \tag{7a}$$

where Γ^s_0 is the total concentration of the antibody sites on the surface, Γ^s_1 is the surface concentration of antibodies that are bound to a single antigen at any time, t, and Γ^s_2 is the surface concentration of the antibody that binds two antigens. The rate at which the antibody specifically binds two antigens is given by:

$$\frac{d\Gamma^s_2}{dt} = k^s_2 c_s \Gamma^s_1 - k^s_{-2}\Gamma^s_2 \tag{7b}$$

For initial binding kinetics, after some simplification one obtains [9]:

$$\frac{d\Gamma^s_1}{dt} = k^s_f c_s^2 \Gamma^s_0 \tag{7c}$$

The second-order dependence on antigen concentration is not surprising since two molecules of the antigen can bind to two binding sites on the same antibody molecule. On integrating the above equation one obtains the concentration of the antigen bound due to specific binding. This bound concentration is given by:

$$\frac{\Gamma^s_1}{c_0} = \frac{\Gamma^s_{Ag}}{c_0} = k^s_f \Gamma^s_0 c_0 \int_0^t y^2\,dt \tag{7d}$$

The rate of non-specific binding of the antigen to the surface is given by:

$$\frac{d\Omega^n_1}{dt} = k^n_f c_s(\Omega^n_0 - \Omega^n_1) - k^n_r \Omega^n_1 \tag{8}$$

Here Ω^n_0 is the total concentration of the non-specific binding sites on the fiber-optic surface, and Ω^n_1 is the concentration of the filled non-specific binding sites on the surface. In the initial regime, $\Omega^n_1 \ll \Omega^n_0$, and $k^n_f c_s \Omega^n_0 \gg k^n_r \Omega^n_1$. Then, the total rate of antigen bound by specific and non-specific binding is given by:

$$\frac{d\Gamma'_{Ag}}{dt} = k^s_f \Gamma^s_0 c_s^2 + k^n_f \Omega^n_0 c_s \tag{9}$$

On substituting for the total rate of antigen bound into Eq. 2c, one obtains the boundary condition for the second-order reaction at $x = 0$ in dimensionless form as:

$$\frac{\partial y}{\partial z} = \mathrm{Da}\left(y^2 + \alpha' y\right) \tag{10}$$

The Damkohler number, Da now is equal to $k_f^s \Gamma_0^s L c_0^{n-1}/D$, and $\alpha' = \alpha c_0^{n-1}$. Here $n-1$ is the order of reaction.

2.3. Other order reaction kinetics

For the present, no reaction mechanisms are proposed for the one and a half-order reaction kinetics. Nevertheless, it is useful to display curves of Γ_{Ag}^s with respect to time for these reaction orders. Fractal-like kinetics, as indicated earlier, display non-integer orders of reaction [15].

One and a half-order reaction. For the one and a half-order reaction, the rate of antigen bound by specific binding is given by:

$$\frac{\mathrm{d}\Gamma_{Ag}^s}{\mathrm{d}t} = k_f^s \Gamma_0^s c_s^{\frac{3}{2}} \tag{11a}$$

On integrating the above equation one obtains the relative concentration of the antigen bound by specific binding. This is given by:

$$\frac{\Gamma_{Ag}^s}{c_0} = k_f^s \Gamma_0^s c_0^{\frac{1}{2}} \int_0^t y^{\frac{3}{2}} \mathrm{d}t \tag{11b}$$

The boundary condition in dimensionless form is:

$$\frac{\partial y}{\partial z} = \mathrm{Da}\left(y^{\frac{3}{2}} + \alpha' y\right) \tag{12}$$

In this case the Damkohler number is given by $k_f^s \Gamma_0^s L c_0^{1/2}/D$. Similar expressions can also be derived for the one half-order case on substituting $1/2$ in place of $3/2$ where the reaction order exponent is used.

The solution for the diffusion equation (Eq. 1a) for the different reaction orders may be obtained on utilizing different but appropriate boundary conditions at $x = 0$, and the same initial condition at $t = 0$ (Eq. 2a), and the same boundary condition at $x = \infty$. Note that since the boundary condition is nonlinear except for the first-order reaction, and the initial condition exhibits a discontinuity, the solution to the

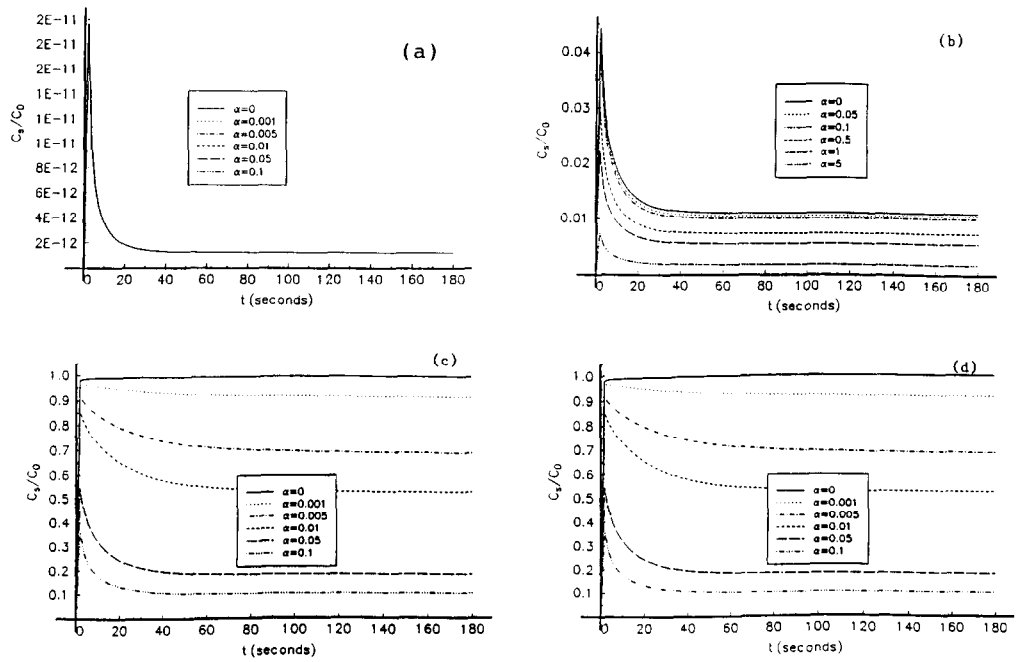

Fig. 2. Influence of α on the normalized antigen concentration in solution, c_s/c_0: (a) one-half-, (b) first-, (c) one and a half- and (d) second-order reactions.

diffusion equation is obtained by a numerical method. After obtaining the numerical solution of the diffusion equation one can proceed to obtain the concentration of the antigen bound to the antibody on the biosensor surface by numerically integrating the appropriate equations for the different reaction orders.

Different numerical techniques were considered in the solution of the diffusion equation (Eq. 1a). The explicit finite difference method was considered unsuitable due to severe restrictions placed by the stability conditions on the interval size. The Crank–Nicholson implicit finite difference method was also considered to be unsuitable since in this method very slowly decaying finite oscillations can occur in the neighbourhood of discontinuities in the initial values or between the initial and boundary values. In our model, the initial condition in the neighborhood of $z = 0$ is discontinuous.

The technique in which the partial differential equation is reduced to a system of ordinary differential equations was found to be suitable for solving the diffusion equation (Eq. 1a). Appropriate expressions for the different reaction orders can easily be obtained [20]. Once the solution of the diffusion equation is obtained, then the concentration of the antigen bound to the antibody due to specific binding can be obtained using the Hermite cubic quadrature. Chen [20] has utilized a subroutine called SDRIV2 for solving the initial value problem. This subroutine is particularly suitable for solving a variety of initial value problems.

2.4. Influence of non-specific binding

2.4.1. Antigen concentration in solution

Fig. 2a–d show the influence of α on the concentration of antigen in solution near the surface, c_s/c_0 for the different reaction orders. For the one-half order reaction there is just a single curve for the amount of antigen in solution near the surface, c_s/c_0 (Fig. 2a). Apparently, at this low reaction order the α value does not have any noticeable influence on the amount of antigen in solution near the surface. Fig. 2b–d show the influence of the α value on c_s/c_0 for first-, one and a half-, and second-order reaction orders. Note that as expected an increase in

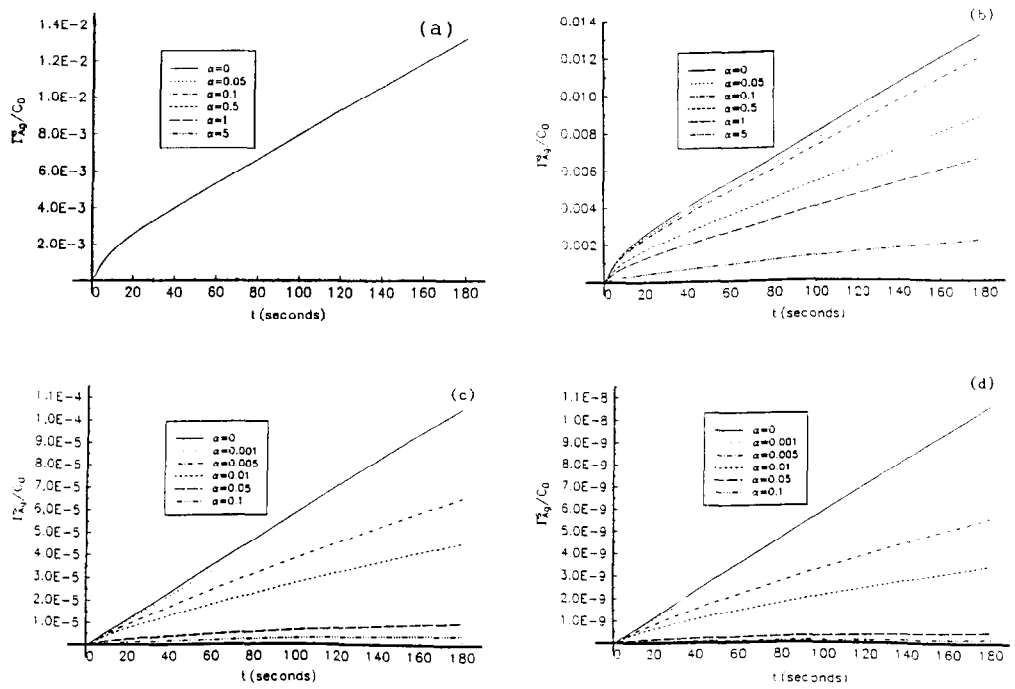

Fig. 3. Influence of α on the amount of antigen bound to the antibody immobilized on the biosensor surface, Γ_{Ag}^{∞}/c_0: (a) one-half-, (b) first-, (c) one and a half- and (d) second-order reactions.

α leads to a decrease in the c_s/c_0 with time. Note that the higher order reactions are more sensitive to changes in the α value.

Fig. 3a–d show the influence of α on the amount of antigen bound specifically to the antibody immobilized on the biosensor surface, Γ^s_{Ag}/c_0. In accordance with Fig. 2a there is just a single binding curve for the specific binding of the antigen to the immobilized antibody (see Fig. 3a). Fig. 3b–d show the influence of the α value on Γ^s_{Ag}/c_0 for first-, one and a half-, and second-order reactions. Note that, and as expected, an increase in the α value leads to a decrease in the amount of antigen bound specifically to the antigen on the surface. When $\alpha = 0.01$ and for a reaction time of 3 min, Γ^s_{Ag}/c_0 decreases by about 46 and 40 percent relative to the $\alpha = 0$ for the second-, and one and a half-order reactions, respectively. The decrease in Γ^s_{Ag}/c_0 is negligible for first-order reactions for this α value. When $\alpha = 0.1$, and for a reaction time of 3 minutes Γ^s_{Ag}/c_0 decreases by about a factor of 250, 20, and by 9 percent relative to the value for $\alpha = 0$ for the second-, one and a half-, and first-order reactions, respectively. Apparently, an increase in the reaction order considerably increases the sensitivity of Γ^s_{Ag}/c_0 to the α value. Note that for the second- and the one and a half-order reactions even a small value of α such as 0.001 leads to a noticeable decrease in the signal transmitted when compared to the $\alpha = 0$ case. Therefore, in biosensor design we need to consider the influence of non-specific binding, especially for the reaction orders which are one and higher.

2.4.2. *Antibody concentration on the surface*

Fig. 4a–b shows the influence of α on the normalized concentration of the antigen in solution, c_s/c_0 for different antibody concentrations on the biosensor surface for a first-order reaction. Note that when non-specific binding is not present an increase in the antibody concentration on the biosensor surface leads to a decrease in the c_s/c_0 value as expected. When α is greater than zero, then there is a significant drop in the c_s/c_0 value in solution, as expected. For example, for a 3-minute reaction time and for a Γ^s_0 value of $5 \cdot 10^{-13}$, c_s/c_0 decreases by 25 percent from 0.12 to 0.09 as the α increases from 0 to 0.5.

Fig. 5a,b shows that for a first-order reaction as

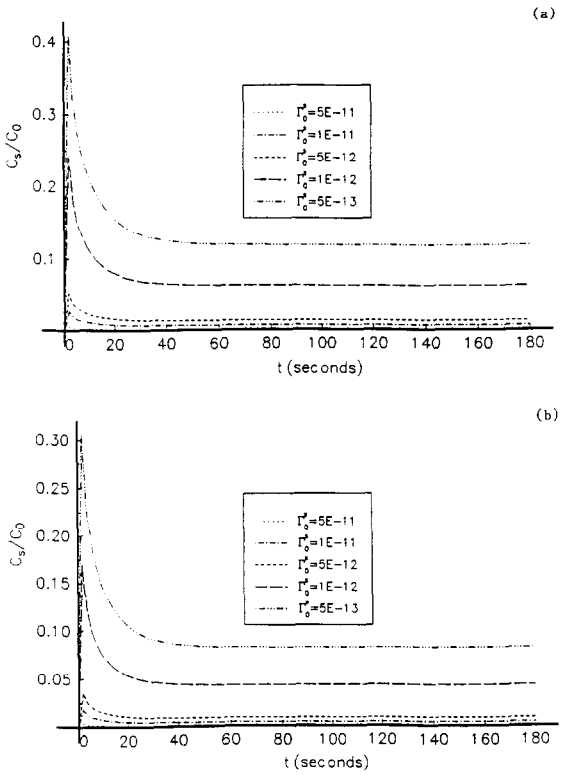

Fig. 4. Influence of antibody concentration immobilized on the surface, Γ^s_0 on the normalized concentration in solution, c_s/c_0 for a first-order reaction for different α values: (a) 0, (b) 0.5.

the concentration of the antibody immobilized on the surface, Γ^s_0 increases the amount of antigen bound specifically to the antibody on the surface, Γ^s_{Ag} increases for both $\alpha = 0$ and for 0.5. As expected for the same Γ^s_0 value Γ^s_{Ag}/c_0 decreases for the $\alpha = 0.5$ (non-specific binding) case compared to the $\alpha = 0$ case.

Fig. 6a–c show the influence of α on the normalized concentration of the antigen in solution, c_s/c_0 for different antibody concentrations on the biosensor surface for a one and a half-order reaction. Note that for $\alpha = 0$ an increase in the antibody concentration on the surface leads to a decrease in the c_s/c_0 values as expected. When α is greater than zero then there is a significant drop in the c_s/c_0 value as expected. For example, after a 3-minute reaction time and for an antibody concentration on the surface, Γ^s_0 of $1 \cdot 10^{-11}$ mol/cm^2 the c_s/c_0 values are 0.97, 0.42, and 0.06, for α value of 0, 0.01, and

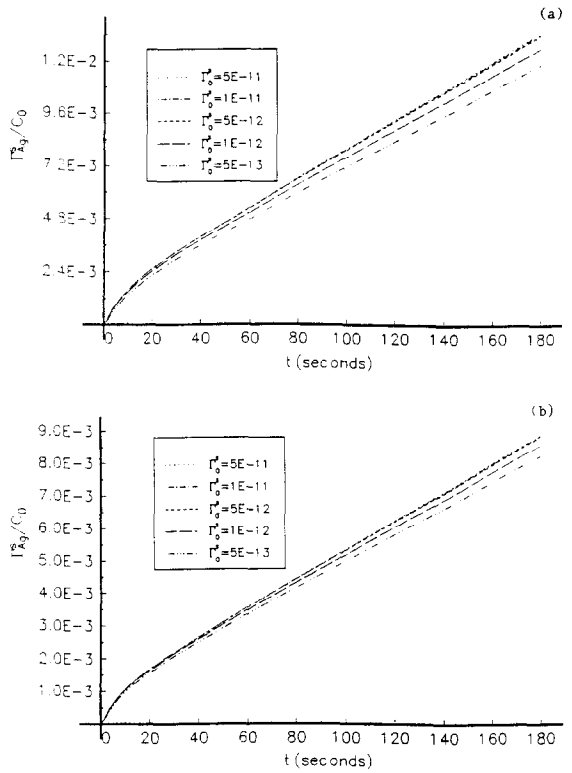

Fig. 5. Influence of antibody concentration on the biosensor surface, Γ_0^s on the amount of specifically bound antigen, Γ_{Ag}^s/c_0 for a first-order reaction for different α values: (a) 0, (b) 0.5.

0.1, respectively. Thus, even small values of α (less than and equal 0.1) very significantly affect the c_s/c_0 values for the one and a half-order reaction.

Fig. 7a–c shows the influence of α on the amount of antigen specifically bound to the antibody immobilized on the biosensor surface for a one and a half-order reaction. As expected, for a zero α value an increase in Γ_0^s increases the amount of antigen specifically bound to the antibody on the surface (Fig. 7a). Note that the introduction of non-specific binding introduces complexities in the Γ_{Ag}^s/c_0 versus time specific binding curve. Initially, as Γ_0^s is increased, Γ_{Ag}^s/c_0 increases for the same time, t. Then, when Γ_0^s is increased beyond a certain value, Γ_{Ag}^s/c_0 no longer increases, but decreases for the same time, t. Similar behavior is observed for the second-order reaction for both the c_s/c_0 versus time and for the Γ_{Ag}^s/c_0 versus time curves when $\alpha > 0$. The Figs. are similar in shape (as in Fig. 7) and are not shown here to conserve space. Note, however, that the second-order reaction is more sensitive than the one and a half-order reaction for changes exhibited by the c_s/c_0 curves, especially for the higher α and lower Γ_0^s values. Note that the specific binding curve, Γ_{Ag}^s/c_0 is consistently higher for the one and a half-order reaction when compared to the second-

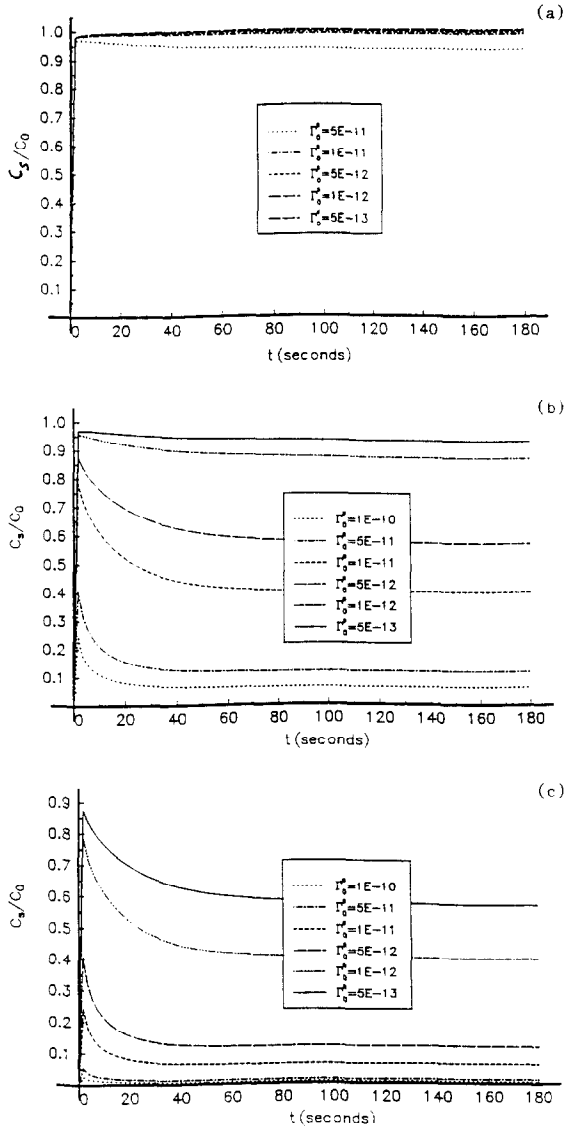

Fig. 6. Influence of antibody concentration immobilized on the surface, Γ_0^s on the normalized antigen concentration in solution, c_s/c_0 for a one and a half-order reaction for different α values: (a) 0, (b) 0.01, (c) 0.1.

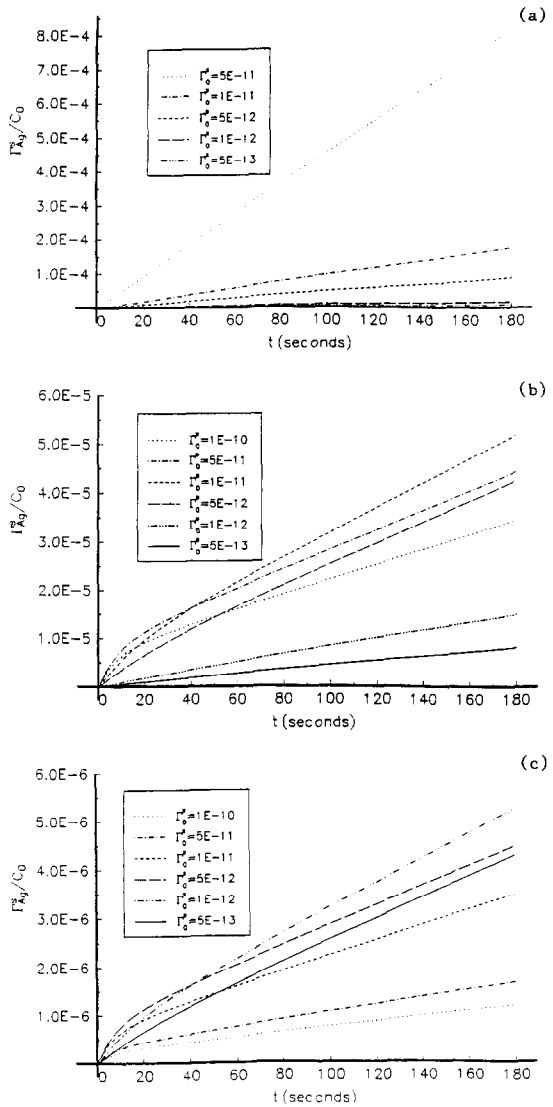

Fig. 7. Influence of antibody concentration on the biosensor surface, Γ_0^s on the amount of specifically bound antigen, Γ_{Ag}^s/c_0 for a one and a half-order reaction for different α values: (a) 0, (b) 0.01, (c) 0.1.

order curve by about four orders of magnitude for $\alpha = 0$, 0.01, and 0.1. However, for the first-order reaction no such complexities as mentioned above are observed even if α is as large as 0.5.

Note also that the optimum value of Γ_0^s for which the sensor will exhibit a maximum signal after a certain reaction time is different for different α values. For example, at $\alpha = 0.01$, the maximum signal is obtained after a reaction time of 3 minutes for a Γ_0^s value that is between 10^{-11} and $5 \cdot 10^{-12}$ mol/cm^2. This applies to both one and a half- and second-order reactions. At $\alpha = 0.1$ this optimum value of Γ_0^s changes, and lies between 10^{-12} and $5 \cdot 10^{-12}$ mol/cm^2 both for the one and a half- and second-order reactions.

3. Conclusions

During the external diffusion-limited specific binding of antigen in solution to antibody covalently or noncovalently immobilized on a surface, the presence of non-specific binding does not affect or leads to only minor changes for a half-order reaction. This is because a single curve is applicable for different values of the ratio of non-specific binding to specific binding (α). An increase in the α value leads to a decrease in (a) the normalized concentration of the antigen close to the surface, c_s/c_0, (b) the rate of specific binding, and (c) the amount of antigen specifically bound to the antibody on the surface, Γ_{Ag}^s/c_0 for first-, one and a half-, and second-order reactions. It is of interest to note that an increase in the reaction order increases the sensitivity of the specific binding to the α value.

Mechanistic schemes are presented for first- (single-step) and second-order (dual-step) specific binding of the antigen in solution to the antibody immobilized on the surface. Mechanistic schemes for other order reaction kinetics are not presented though these may arise from intrinsic first- or second-order kinetics when heterogeneity, fractal-like kinetics, steric factors, or other factors are included in the analysis.

An increase in the antigen concentration in solution, c_0, increases the rate of specific binding and the amount of antigen bound to the antibody on the surface for one and a half- and second-order reactions. The specific binding curve for a first-order reaction is unaffected since a single curve represents the rate and amount bound for the different antigen concentrations analyzed. As expected, higher α values lead to lower rates of specific binding and the amount of specifically bound antigen for the one and a half- and second-order reactions.

For a first-order reaction an increase in the antibody concentration on the surface leads to (a) a lower normalized concentration of the antigen close to the surface, c_s/c_0, and (b) higher rates of specific binding and amount of antigen specifically bound to the antibody. Higher α ratios depress both the c_s/c_0 and the Γ^s_{Ag}/c_0 curves for the first-order reaction, as expected.

The introduction of non-specific binding introduces complexities in the specific binding curve for one and a half- and second-order reactions. For a zero α value an increase in the antibody concentration on the surface, Γ^s_0 increases the amount of antigen specifically bound to the antibody on the surface. For α values greater than zero there is an optimum value of Γ^s_0 that maximizes the rate and amount of specific binding of the antigen in solution to the antibody immobilized on the surface for one and a half- and second-order reactions. This Γ^s_0 value is different for different α values; but for the same α value it is the same for both one and a half- and second-order reactions.

The inclusion of non-specific binding in the analysis of specific binding of the antigen to the antibody on the surface provides a more realistic picture of what is happening on the surface, besides leading to temporal binding rate coefficients. Furthermore, quantitative estimates of the amount of antigen specifically bound are available for different degrees of non-specific binding (α). The Figs. that show the numerically calculated binding rates for the different orders when non-specific binding is present represent the most useful part of the analysis for readers interested in constructing biosensors. These curves can be used to interpret a far from ideal binding of antigen to an immobilized antibody surface, or vice versa. In many cases higher α values considerably decrease the rate and amount of specifically bound antigen especially for higher reaction orders. The estimates and trends provided by the modeling should help in controlling these reactions to advantage, besides helping to improve the stability, sensitivity, reproducibility, and reaction time of biosensors. Non-specific binding should in the ideal case be minimized. Nevertheless, it will be present to some degree in most cases if not all, and the modeling presented helps estimate the 'derogatory' influence it has on biosensor performance. Suitable experimental data available would considerably assist in developing better models, which in turn would provide for better and more realistic estimates of the influence of non-specific binding on biosensor performance. Such studies that include both experimental and theoretical approaches are strongly recommended. Finally, the analysis does provide a framework and quick estimates of the effect of non-specific binding on the rates of attachment in antibody–antigen systems for different reaction orders when external diffusional limitations are present. Finally, more-or-less all of the analysis presented above would apply to any interaction between binding molecules and their counterparts.

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