0273-2289/97/6701-02—0001$13.25

Antibody–Antigen Binding Kinetics

A Model for Multivalency Antibodies for Large Antigen Systems

AJIT SADANA[1,2] AND TUAN VO-DINH*,[1]

[1]*Advanced Monitoring Development Group, Life Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN*
[2]*Chemical Engineering Department, University of Mississippi, University, MS*

Received August 7, 1996; Accepted November 11, 1996

ABSTRACT

This work presents a theoretical analysis of the influence of multivalency of antigen on external mass transfer-limited binding kinetics to divalent antibody for biosensor applications to polycyclic-aromatic systems. Both cases are considered wherein the antigen is in solution and the antibody is either covalently or noncovalently attached to a cylindrical fiber-optic biosensor, and the antibody is in solution and the antigen is attached to the surface. Both single-step and dual-step binding processes are considered. The rate of attachment of antigen to antibody (or vice versa) is linear for the valencies (or reaction orders) analyzed in the time frame (100 min) considered. The rate of attainment of saturation levels of antigen or antibody in solution close to the surface is very rapid (within 20 min). An increase in the valency of the antigen in solution has the effect of decreasing the order of reaction (for valency, $v \geq 1$). An increase in the number of steps increases the order of reaction, as expected. An increase in the valency of the antigen in solution decreases the saturation level of the antigen close to the surface and the rate of antigen attachment to the antibody on the surface for all Damkohler numbers. A decrease in the diffusional limitations decreases the effect of valency (or reaction order) on saturation levels of c_s/c_0. Nondimensional plots presented in the analysis help extend the analysis to different antigen–antibody systems. An increase in the valency of the antibody in solution has the effect of increasing the order of reaction (for $v > 2$). The

*Author to whom all correspondence and reprint requests should be addressed.

effects in this case are reverse to those described earlier. For valency greater than 2, the reaction order is dependent on the antigen valency, whether it is in solution or immobilized on the surface. The general analysis presented here should be applicable to most surface reactions that involve ligand–receptor binding wherein multiple-binding sites are involved on either the receptor or the ligand.

Index Entries: Antigen–antibody binding; immunosensor; binding kinetics; biosensor; polycyclic-aromatic compounds (PACs).

INTRODUCTION

Recently, there has been increasing interest in the use of immunological techniques for chemical detection in environmental and biomedical applications *(1)*. This is owing to the excellent specificity of antibody–antigen reactions, the advances in sensitive optical-sensing technologies, and new developments in monoclonal antibody-production technology. Over the last 10 yr, our laboratory has been interested in the development of antibody-based sensors using fiberoptic probes and laser excitation *(2–7)*. Detection techniques involved direct measurement of the antigens *(2,4,5)* or competitive-binding measurement of labeled antigens *(6)*. In this system, the antibody was targeted to one specific type of antigen (e.g., benzo(a)pyrene *[2]* or benzopyrene-tetraol *[3,4]*. The design of antibody-targeted agents for a large class of chemical species such as the polycyclic-aromatic compounds (PACs) could be an important development for biosensors. Such biosensors could be used to screen samples for their overall content of PACs rather than for specific PACs.

In this chapter, we investigate the reaction mechanisms that would be involved in a situation in which an antibody is targeted to a group of antigens having multiple-antigenic sites. This model is relevant to the situation in which the antibody is designed to have a paratope targeted to only a monocyclic aromatic—or part of a monocyclic ring. Such an antibody would be capable of recognizing not only one PAC, but a family of PACs. Figure 1 schematically depicts such an antibody targeted to a family of PACs. The concept of multivalency for antibodies requires certain conditions. In general, antibodies are larger than antigens. Therefore, certain size and steric conditions must be fulfilled to allow more than one antibody to be attached to an antigen. This could occur for antigens with sufficiently large size or with antibodies specifically designed to have a small size or sterically favorable paratope geometry. Of course, the combining site on the antibody should not be so large that it completely "encloses" the PAC (antigen). Note that the steric hindrance may be particularly significant if the binding pockets are generally deep. Also, one has to keep in mind that

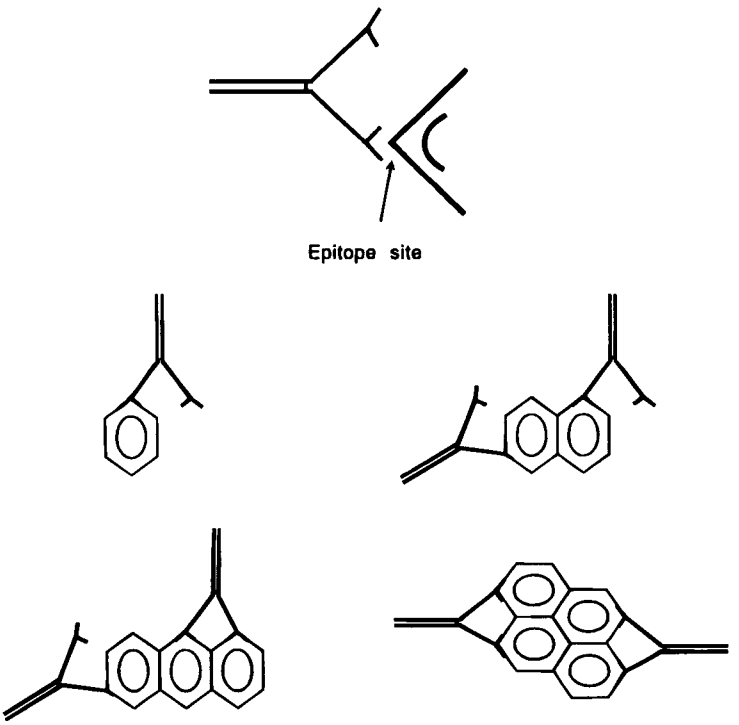

Fig. 1. Schematic diagram of antibodies having paratopes targeted to the antigen series of polycyclic aromatic compounds.

it may be difficult or challenging to engineer or design an antibody to have a PAC-combining site smaller than a PAC that would have a useful binding affinity. Another approach is to design systems consisting of parts of the antibody by cleaving and combining the appropriate paratopes.

There is an ever-increasing need for sensitive biosensor systems capable of detecting and distinguishing a wide range of substances. Biosensors use bioreceptor molecules as sensing mechanisms. Their performance (sensitivity, selectivity, and reproducibility) can be improved by a better understanding of their mode of operation. The success rate of the detection scheme will be significantly enhanced if one can grasp better physical insights into the different steps that are involved in the molecular-recognition (biosensing) process. One such detection scheme is the solid-phase immunoassay technique that has already gained importance in biosensors. The binding process of antigens to antibodies is the basis of immunosensors.

External-diffusional limitations also plays a role in these types of assays and the influence of diffusion in such systems has been mentioned *(8)* and analyzed to some extent using a conventional analysis *(9–15)*, and fractals *(16–18)*. Previous studies on antigen–antibody binding for biosensor applications, including a recent analysis of a chemical-sensor applica-

tion *(19)* have been restricted to monovalent and divalent antigens or antibodies. The binding of multivalent antigens or antibodies to each other could have importance in the design of biosensor probes.

The analysis of multivalent antigen–antibody binding is still in an initial stage. In this chapter, we present a theoretical analysis of the external-diffusion limited-binding kinetics of multivalent antigens or antibodies in solution to multivalent antibodies or antigens immobilized on the biosensor surface. The effect of multivalency on reaction order is presented. The model is generalized to include the binding of n-valent antigens or antibodies to each other.

THEORY

The binding of antigen in solution to antibody immobilized on a surface will be presented. This will be followed by an analysis of the binding of antibody in solution to antigen immobilized on a surface. Some assumptions are utilized in the derivation. They include: Although nonspecific binding will be mentioned and explained briefly in a qualitative sense, the binding models will not express this explicitly. As expected, nonspecific binding will have a deleterious effect on specific binding. An analysis of the influence of nonspecific binding on specific antigen–antibody binding kinetics for biosensors for first-order, one and a half-order, and second-order kinetics is available *(20)*. Lateral interactions owing to immobilized species being too close to each other are also ignored.

Antigen in Solution/Antibody on the Surface (Monovalent Antigen/Divalent Antibody)

First-Order Reaction Kinetics

Figure 2A illustrates the steps that are involved in the binding of the antigen in solution to the antibody that is either covalently or noncovalently attached to a surface. The rate of binding of a single antigen by an antibody is given by (Fig. 2A):

$$d\Gamma_1 / dt = k_1 c_s(\Gamma_0 - \Gamma_1) - k_{-1}\Gamma_1 \tag{1}$$

Here, Γ_0 is the total concentration of the antibody sites on the surface, Γ_1 is the surface concentration of antibodies that are bound by antigens at any time, c_s is the concentration of the antigen close to the surface, k_1 is the forward reaction-rate constant, and k_{-1} is the reverse reaction-rate constant. The simplified binding reaction scheme is:

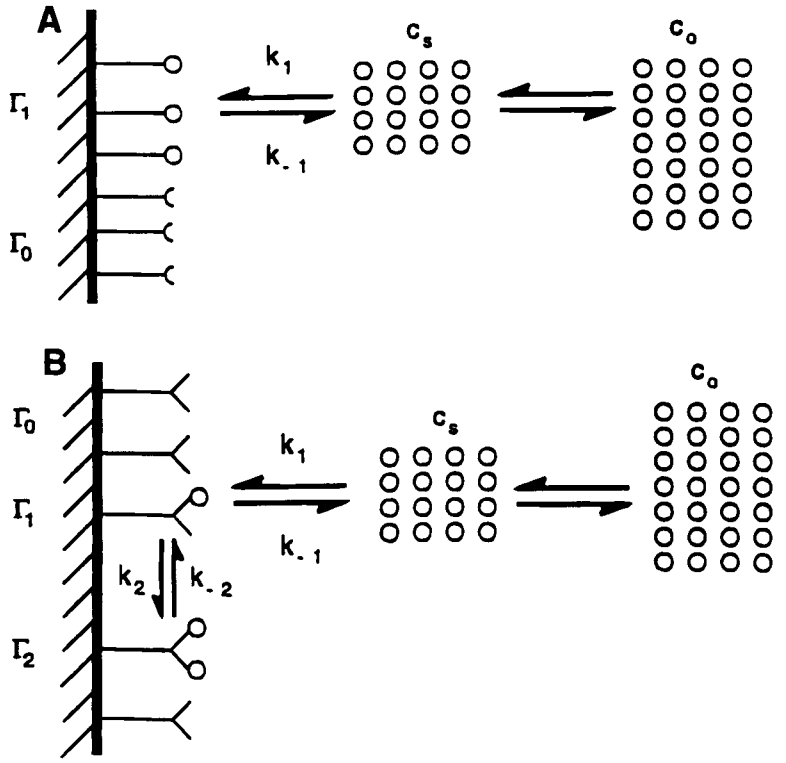

Fig. 2. **(A)** Elementary steps involved in the single-step binding of monovalent antigen in solution to monovalent antibody noncovalently or covalently attached to the surface. **(B)** Elementary steps involved in the dual-step binding of monovalent antigen in solution to divalent antibody noncovalently or covalently attached to the surface.

$$Ab + Ag \underset{k_r}{\overset{k_f}{\leftrightarrow}} Ab \cdot Ag \tag{2}$$

Here Ab is the antibody binding site and Ag is the antigen.

Because we are interested in initial-binding kinetics, $\Gamma_1 \ll \Gamma_0$. Also, $k_1 c_s \Gamma_0 \gg k_{-1}\Gamma_1$ at sufficiently short times *(9)*. Another assumption utilized is that the reaction has not reached dynamic equilibrium during the course of the experiment. The aforementioned two conditions simplify Eq. 1 to:

$$d\Gamma_1/dt = k_1 c_s \Gamma_0 = d\Gamma_{Ag}/dt = k_f[Ag]\Gamma_0 \tag{3}$$

Here, Γ_{Ag} is the surface concentration of the bound reactant or antigen. The first-order dependence on antigen concentration close to the sur-

face is expected if one antigen molecule in solution binds to a single (antibody) binding site on the surface. Another way of looking at the first-order process is that it is dependent on the concentration of a single reactant (in this case the antigen), because the antibody is in considerable molar excess. For first-order reaction kinetics, note that k_f and k_r are equal to k_1 and k_{-1}, respectively.

It is appropriate to indicate at this point that the solvent significantly influences the binding affinity and specificity. This fact is recognized, especially because PACs are only soluble in aqueous solutions to a very small extent. More often than not, practical immunoassays and immunosensors would require some cosolvent to keep the PAC soluble. This would significantly affect the binding affinity and specificity.

Second-Order Reaction Kinetics

The dual-step binding that follows is basically an extension of the single-step binding analysis that has just been presented. The elementary steps involved in the reaction schemes are given in Fig. 2B.

The rate of binding of the single antigen to the bound antibody is given by:

$$d\Gamma_1/dt = k_1 c_s(2\Gamma_0 - \Gamma_1 - 2\Gamma_2) - k_2\Gamma_1 c_s + 2k_{-2}\Gamma_2 - k_{-1}\Gamma_1 \qquad (4)$$

Γ_2 is the surface concentration of the antibody which binds two antigens. k_1 is the binding-rate coefficient of a single arm of the antibody to (a single arm of) the antigen. This is consistent with Eqs. 1 and 4, and applies throughout the chapter. In the aforementioned case, the antibodies have two antigen-binding sites. Then, antibodies without any antigen bound have two binding sites available, antibodies with one antigen bound have one site available, and antibodies with two antigens bound have no sites available. The stoichiometric coefficient 2 is added in front of the Γ_0 term to reflect the possibility that the binding of a single antigen may occur at either of the two arms of the antibody. Further details of the derivation are available *(13,14)*.

The rate at which the antibody binds two antigens is given by:

$$d\Gamma_2/dt = k_2 c_s\Gamma_1 - 2k_{-2}\Gamma_2 \qquad (5A)$$

The stoichiometric coefficient 2 is added to reflect the possibility that either of the two antigens may dissociate.

One may also consider the following one-step binding reaction scheme:

$$Ab + 2Ag \underset{k_{-3}}{\overset{k_3}{\leftrightarrows}} Ab \cdot Ag \cdot Ag \qquad (5B)$$

However, the probability of a reaction involving three molecules at once is negligible, at least in comparison to the above two reactions given in Eqs. 4 and 5A.

We are interested in initial-binding kinetics. Therefore, $\Gamma_1 \ll \Gamma_o$ and $\Gamma_2 \ll \Gamma_o$ *(9)*. Also, $k_1\Gamma_o c_s \gg k_2\Gamma_1 c_s$, or in effect $k_1\Gamma_0 \gg k_2\Gamma_1$ and $k_{-2}\Gamma_2$ and $k_{-1}\Gamma_1$ is very small. Then, Eqs. 4 and 5A reduce respectively to:

$$d\Gamma_1/dt = 2k_1c_s\Gamma_0 \tag{6}$$

and

$$d\Gamma_2/dt = k_2c_s\Gamma_1 \tag{7}$$

The reaction scheme shown in Fig. 2B may be combined as:

$$Ab + Ag \underset{k_r}{\overset{k_f;Ag}{\leftrightarrow}} Ag:Ab:Ag \tag{8A}$$

where Ab is the antibody molecule and Ag is the antigen molecule.

Let us now relate k_f and k_r to the elementary steps involved in the reaction scheme shown in Fig. 2B. Consider:

$$Ab + Ag \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} Ab:Ag \underset{k_{-2}}{\overset{k_2;Ag}{\leftrightarrow}} Ag:Ab:Ag \tag{8B}$$

One may obtain the steady-state concentration of [Ab : Ag] and [Ag : Ab : Ag] by utilizing the pseudo steady-state approximation. Substitution for [Ab : Ag] and [Ag : Ab : Ag] in the rate expression for [Ag : Ab : Ag] obtained from eqs. 8A, B, and comparison of these rate expressions yields:

$$k_f = 2k_1k_2/(k_2[Ag] + k_{-1}) \qquad k_r = k_{-1}k_{-2}/(k_2[Ag] + k_{-1}) \tag{9}$$

Note that these equations are similar to the Stenberg et al. *(9)* model except for the [Ag] term in the denominator.

Case 1

If $k_{-1} \ll k_2[Ag]$ in Eq. 9 (a highly probable case), then $k_f = 2k_1/[Ag]$. Also, assume that $k_{-1} \cong k_{-2}$ *(9)*; then $k_r \cong 0$, which indicates negligible dissociation and is consistent with previous findings *(9)*.

CASE 2

If there is rapid dissociation (not very likely), then k_2 [Ag] $\ll k_{-1}$, and $k_f = 2K_1k_2$ (where $K_1 = k_1/k_{-1}$) and $k_r = k_{-2}$.

Let us analyze the more probable case (Case 1). Then, from Eq. 6:

$$d\Gamma_1 / dt = k_f c_s^2 \Gamma_0 = k_f [Ag]^2 \Gamma_0 = d\Gamma_{Ag} / dt \quad (10)$$

where Γ_{Ag} is the concentration of the bound reactant. Note that in the study by Stenberg et al. *(9)* the dependence was first-order, because one molecule of the antibody in solution can bind to one binding site of the antigen molecule on the surface. It should be asserted at this point that IgG antibodies have two binding sites, and thus the second-order dependence given by Eq. 10 may be more appropriate than the first-order dependence given by Eq. 3. In this (single-step) case, because the antibody is in excess, it would be an error to invoke second-order kinetics in the rate expression by including terms for the concentration of both antigen and antibody. Note that the concentration term for antibody does "appear", but only as Γ_0 (invariant). If the system were such that neither the antibody nor the antigen were in excess, then the derivation of the rate expression could lead to second-order kinetics. Then, this expression would include terms for the concentration of both the antigen and the antibody. Considering the antibodies as monovalent systems that lead to the first-order dependencies is an overly simplistic treatment of the true situation. Nevertheless, the first-order dependence was briefly presented here because it is available in the literature *(9)*, and because it provides the basis for the second-order and other (higher)-order dependence.

Higher-Order Reaction Kinetics

There is ample evidence in the literature indicating antigens need not necessarily be monovalent in nature; that divalent and higher-valency antigens could occur in nature or could be designed. We next analyze the binding kinetics of divalent and higher-valency antigens to divalent antibodies.

Third-Order Kinetics (Divalent Antigen/Divalent Antibody)

Figure 3A illustrates the steps that are involved in the binding of the divalent antigen in solution to the divalent antibody that is either covalently or noncovalently attached to a surface. An analysis similar to the one performed for the monovalent antigen in solution and divalent antibody immobilized on the surface may be performed for the divalent antigen in solution and the divalent antibody immobilized on the surface. Dual-step binding may be utilized to describe the binding of a single divalent antigen to a bound antibody. The elementary steps involved in the reaction scheme are given in Fig. 3A. The rate of binding of a divalent antigen by an antibody is given by:

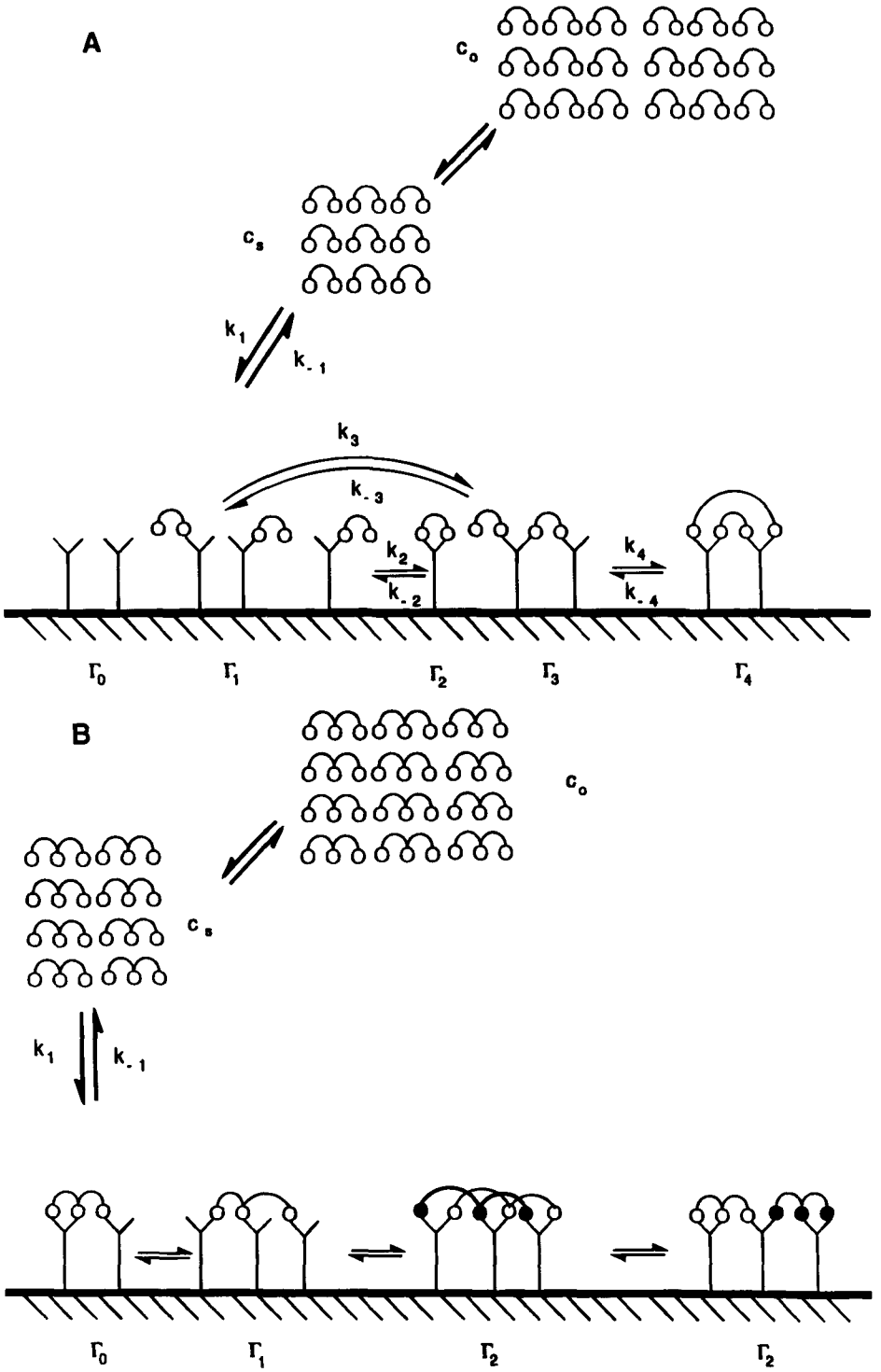

Fig. 3. **(A)** Elementary steps involved in the binding of divalent antigen in solution to divalent antibody noncovalently or covalently attached to the surface. **(B)** Elementary steps involved in the binding of trivalent antigen in solution to divalent antibody noncovalently or covalently attached to the surface.

$$d\Gamma_1/dt = k_1c_s(4\Gamma_0 - \Gamma_1 - 2\Gamma_2 - 3\Gamma_3 - 4\Gamma_4) - k_2\Gamma_1 - k_3\Gamma_1^2 + 2k_{-3}\Gamma_3 + k_{-2}\Gamma_2 \quad (11A)$$

Γ_2 is now the concentration of the single bound divalent antibody that binds to the single bivalent antigen. Γ_3 is the surface concentration of two antibodies that are bound by two different antigens. Three out of the four possible binding sites on the two-antibody complex on the surface are occupied by two divalent-antigen molecules. Γ_4 is the surface concentration of two antibodies that are bound by two different antigen molecules. In this case, all of the four possible binding sites on the two-antibody complex on the surface are occupied by two divalent-antigen molecules. Rate expressions for $d\Gamma_2/dt$, $d\Gamma_3/dt$, and $d\Gamma_4/dt$ could be derived. However, as indicated for the derivations for the first- and second-order kinetics, they are not utilized because we are interested in initial-binding kinetics. For initial-binding kinetics, and based on assumptions done previously for first- and second-order kinetics, one obtains:

$$d\Gamma_1/dt = 4k_1c_s\Gamma_0 \quad (11B)$$

The number 4 reflects the possibility that either of the two arms of the antigen in solution can bind to either of the two arms of the antibody.

The reaction scheme shown in Fig. 3A may be written showing the elementary steps as:

$$\begin{array}{ccccc} & & \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} & & \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} \\ Ab + Ag & & & Ab:Ag & & Ab:Ag' \\ & Ab:Ag, k_3 \Downarrow\Uparrow k_{-3} & & & & \\ & & & & \overset{k_4}{\underset{k_{-4}}{\rightleftharpoons}} & \\ & Ab:Ag:Ab:Ag & & & & Ab:Ag:Ab:Ag' \end{array} \quad (12)$$

The reaction scheme shown in Fig. 3A may be combined as:

$$Ab + Ag \overset{Ab;Ag;k_f}{\underset{k_r}{\leftrightarrow}} Ab:Ag:Ab:Ag' \quad (13)$$

One may obtain the steady-state concentrations of [Ab : Ag] and [Ab : Ag : Ab : Ag'] on utilizing the steady-state approximation. Substitution for [Ab : Ag] and [Ab : Ag : Ab : Ag'] in the rate expression for [Ab : Ag : Ab :

Ag'] obtained from Eqs. 12 and 13, and followed by a comparison of these rate-expressions yields expressions for k_f and k_r.

Substitution of the expression for k_1 obtained in terms of k_f in Eq. 11B yields:

$$d\Gamma_1/dt = k_f\Gamma_0[Ag] \tag{14}$$

Figure 3B shows the binding of the trivalent antigen in solution to the divalent antibody immobilized to the surface. A similar analysis for the dual-step binding of the trivalent antigen to a divalent antibody yields:

$$d\Gamma_1/dt = k_f\Gamma_0[Ag]^{2/3} \tag{15}$$

Equations 3, 10, and 15 may easily be generalized to obtain an expression for the binding of a v-valent antigen in solution to a bivalent antibody on the surface by a dual-step mechanism. This expression is:

$$d\Gamma_1/dt = k_f\Gamma_0[Ag]^{s/v} \tag{16}$$

Here, v is the valency of the antigen, s is the number of steps (single or dual) involved in the binding of the antigen in solution to the antibody on the surface, and Γ_0 is the initial concentration of the antibody on the surface. Note that for a dual-step reaction ($s = 2$) and for the binding of a monovalent ($v = 1$) antigen in solution to a divalent antibody immobilized on a surface, Eq. 16 reduces to Eq. 10 as it should. For a single-step reaction ($s = 1$), and for the binding of a monovalent antigen in solution to a monovalent antibody on a surface, Eq. 16 reduces to Eq. 3 as it should. An increase of the valency of the antigen in solution has the effect of decreasing the order of reaction (for $v \geq 1$). An increase in the number of steps increases the order of reaction, as expected. More often than not, noninteger reaction orders should be anticipated unless $s = v$. This is a theoretical analysis, and no examples are analyzed for the binding of either PACs or any other well known antibody–antigen system. The application of the theory to real examples will be done at a later date.

It is also of interest to analyze the reverse case, wherein the antibody is in solution and the antigen is covalently or noncovalently immobilized to the surface.

Antibody in Solution/Antigen on the Surface

First-Order Reaction Kinetics

Figure 4A describes the single-step mechanism that is involved in the binding of the monovalent antibody in solution to monovalent antigen that

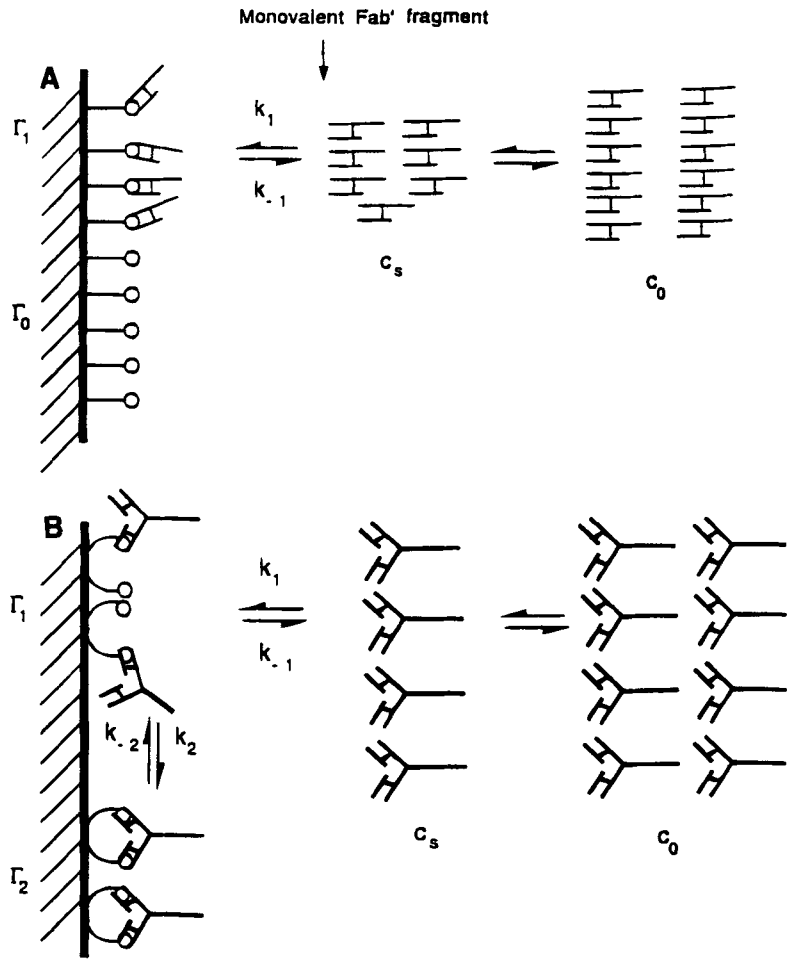

Fig. 4. **(A)** Elementary steps involved in the single-step binding of monovalent antibody in solution to monovalent antigen noncovalently or covalently attached to the surface. **(B)** Elementary steps involved in the dual-step binding of divalent antibody in solution to divalent antigen noncovalently or covalently attached to the surface.

is noncovalently or covalently immobilized to the surface. Because the derivation procedure is similar to the one presented for the case when the antigen is in solution, and the antibody is immobilized on the surface, it is not presented here to avoid repetition. Also, the assumptions utilized are similar. Therefore, just the final reaction-rate kinetic forms are presented.

For first-order kinetics (Fig. 4A), the reaction rate form is:

$$d\Gamma_1/dt = k_1 c_s \Gamma_0 = d\Gamma_{Ab}/dt = k_f \Gamma_{Ag,0}[Ab] \tag{17}$$

In this case, c_s is the concentration of the antibody in solution close to the biosensor surface, and Γ_0 is the initial concentration of the antigen on the surface.

Figure 4B shows the dual-step mechanism involved in the binding of the divalent antibody in solution to the divalent antigen that is noncovalently or covalently immobilized to the surface. The reaction rate expression for this case is:

$$d\Gamma_1/dt = 4k_1c_s\Gamma_0 = d\Gamma_{Ab}/dt = k_f[Ab]\Gamma_{Ag,0} \quad (18)$$

In this case too, one obtains a first-order reaction-rate expression similar to Eq. 17. The only difference here is the number 4, which reflects the possibility that either of the two arms of the antibody in solution can bind to either of the two arms of the divalent antigen immobilized on the surface.

Second-Order Kinetics

Figure 5A shows the dual-step mechanism involved in the binding of the divalent antibody in solution to the monovalent antigen that is covalently or noncovalently immobilized to the surface. Following the aforementioned procedure yields the following expression for the reaction-rate kinetics:

$$d\Gamma_1/dt = k_f\Gamma_{Ag,0}\Gamma_{Ag}[Ab] = 2k_1c_s\Gamma_{Ag,0}\Gamma_{Ag} \quad (19)$$

The number 2 reflects the possibility that either of the two arms of the antibody in solution can bind to the monovalent antigen immobilized on the surface.

Other-Order Kinetics

Figure 5B shows the dual-step mechanism involved in the binding of the divalent antibody in solution to the trivalent antigen that is noncovalently or covalently immobilized to the surface. The reaction-rate expression for this case is:

$$d\Gamma_1/dt = k_f[Ab]^{3/2}\Gamma_{Ag,0} = 6k_1c_s^{3/2}\Gamma_{Ag,0} \quad (20)$$

The number 6 reflects the possibility that either of the two arms of the antibody in solution can bind to any of the three arms (or antigenic sites) of the trivalent antigen immobilized on the surface. One may obtain a general reaction-rate expression for a reaction involving a v-valent ($v > 2$) antigen covalently or noncovalently immobilized to the surface, and a divalent antibody in solution. The general expression is:

$$d\Gamma_1/dt = k_f[Ab]^{v/2}\Gamma_{Ag,0} \qquad v > 2 \quad (21)$$

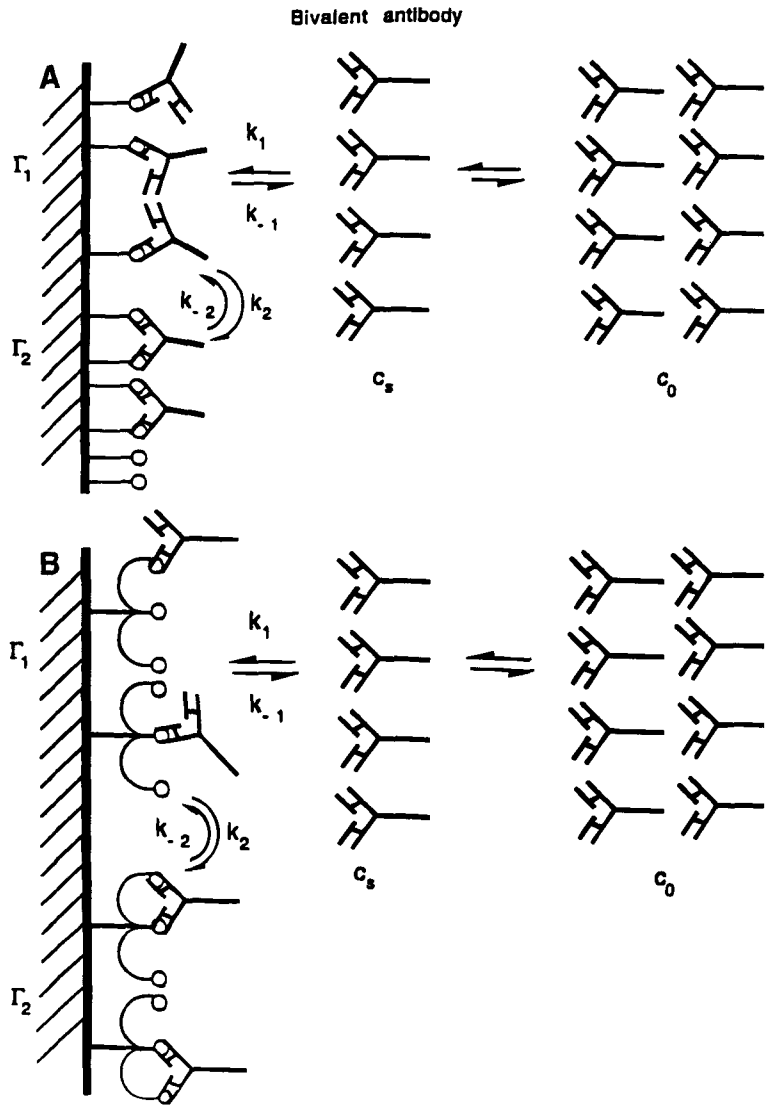

Fig. 5. **(A)** Elementary steps involved in the dual-step binding of divalent antibody in solution to monovalent antigen noncovalently or covalently attached to the surface. **(B)** Elementary steps involved in the dual-step binding of divalent antibody in solution to trivalent antigen noncovalently or covalently attached to the surface.

Here, v is the antigen valency in solution. For valency, v greater than 2 an increase in the valency of the antigen in solution increases the order of reaction. Also, one obtains noninteger orders of reaction for odd-valent antigens immobilized on the biosensor surface.

Diffusional Limitations

External diffusional limitations play a significant role in the binding of the antigen in solution to the antibody on the surface, or vice versa. The diffusional limitations of the antigen–antibody binding kinetics for biosensor applications has been previously described in detail *(15)*. Only the salient features are briefly presented here. The diffusional limitations of the reaction scheme can be determined for purely radial diffusion by considering the following equation:

$$\delta c / \delta t = D(1/r)\delta / \delta r(r\delta c / \delta r) \tag{22A}$$

where D is the diffusion coefficient. In cylindrical geometry (or coordinates) the aforementioned analysis is useful in understanding biosensor applications.

For a plane surface, Eq. 22A may be written in dimensionless form as:

$$\delta y / \delta \theta = \delta^2 y / \delta z^2 \tag{22B}$$

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 analysis assumes that the surface is uniformly reactive (versus discrete sites).

The boundary condition for Eq. 22B is:

$$\delta y / \delta z|_{z=0} = Da\, u^n \tag{23}$$

where $u = c(0,t)/c_0$, and Da is the Damkohler number and is equal to $c_0^{n-1}Lk_1\Gamma_0/D$. Here n is the reaction order (and is equal to $v/2$ when the antibody is in solution and the antigen is on the surface. Also, n is equal to s/v when the antigen is in solution and the antibody is on the surface). Equation 23 basically is a mass-balance equation, which states that the reactant that diffuses to the surface reacts at the surface. The formula $z = 0$ represents the origin of the coordinate system and is physically the surface of, for example, the fiber to which the antibody is attached. The Damkohler number is the ratio between the maximum-reaction rate and the maximum rate of external diffusional-mass transport. When the Damkohler number is large, then diffusional limitations are present. When the Damkohler number is less than one, then diffusional limitations are, in general, absent.

The appropriate initial condition is:

$$\begin{aligned} c(x,0) &= c_0 \quad \text{for } x > 0, \ t = 0 \\ c(0,0) &= 0 \quad \text{for } x = 0, \ t = 0 \end{aligned} \tag{24}$$

Equation 22B may be solved numerically with the initial condition (Eq. 24) and the boundary condition [Eq. (23)] to obtain solutions for $c(0,t)$ and $\Gamma_{Ag}(t)$. The boundary condition is nonlinear and the solution to Eq. 22B is obtained by a numerical method developed by Patankar *(21)*. This is a finite difference method with specific points in the domain called grid points. This is a standard method and is used frequently in heat-transfer solutions. The advantage of the finite difference method is that it is easy to formulate to the discretization equations. The solution of the discretization equation can be obtained by the standard Gaussian-elimination method. Because of the particularly simple form of the equation, Patankar *(21)* turns it into a convenient algorithm called the Thomas algorithm or tridiagonal-matrix algorithm (TDMA). The method computes the concentration on a configuration with given boundary condition and two initial conditions.

RESULTS

Sadana and Sii *(13)* showed the influence of reaction order and Damkohler number on the saturation level of c_s/c_0 and on Γ_{Ag}/c_0. Their curves may easily be adapted (or used) to show the influence of valency and Damkohler number on the saturation level of c_s/c_0 and on the amount of bound reactant normalized by the initial concentration of antigen in solution, Γ_{Ag}/c_0. This is so because the reaction order n, is, in general, equal to either the number of steps, s divided by the valency, v of the antigen in solution (antigen in solution/antibody on the surface), or one-half of the valency, v of the antigen immobilized on the surface (antibody in solution/antigen on the surface).

Antigen in Solution/Antibody on the Surface

Equation 16 indicates that for a constant number of steps, s, the order of the reaction decreases as the valency of the antigen in solution increases. Figure 6 shows that a decrease in the reaction order (or in our case an increase in the valency of the antigen in solution) decreases the saturation level of c_s/c_0. In other words, higher-valency antigens in solution will attain lower-saturation levels of c_s/c_0 for all Damkohler numbers. Also, note that as the Damkohler number increases, the disparity between the saturation levels increases. In other words, a decrease in the diffusional limitations has an effect of decreasing the effect of reaction order (or in our case valency) on saturation levels of c_s/c_0. Thus, increasing mass-transfer limitations are particularly deleterious for high-valency antigens in solution as far as the saturation levels of c_s/c_0 are concerned.

Figure 7 shows the influence of reaction order and Damkohler number on Γ_{Ag}/c_0. Figure 7 shows that for the same Damkohler number an increase in the reaction order, increases the rate and level of binding. Thus, lower-

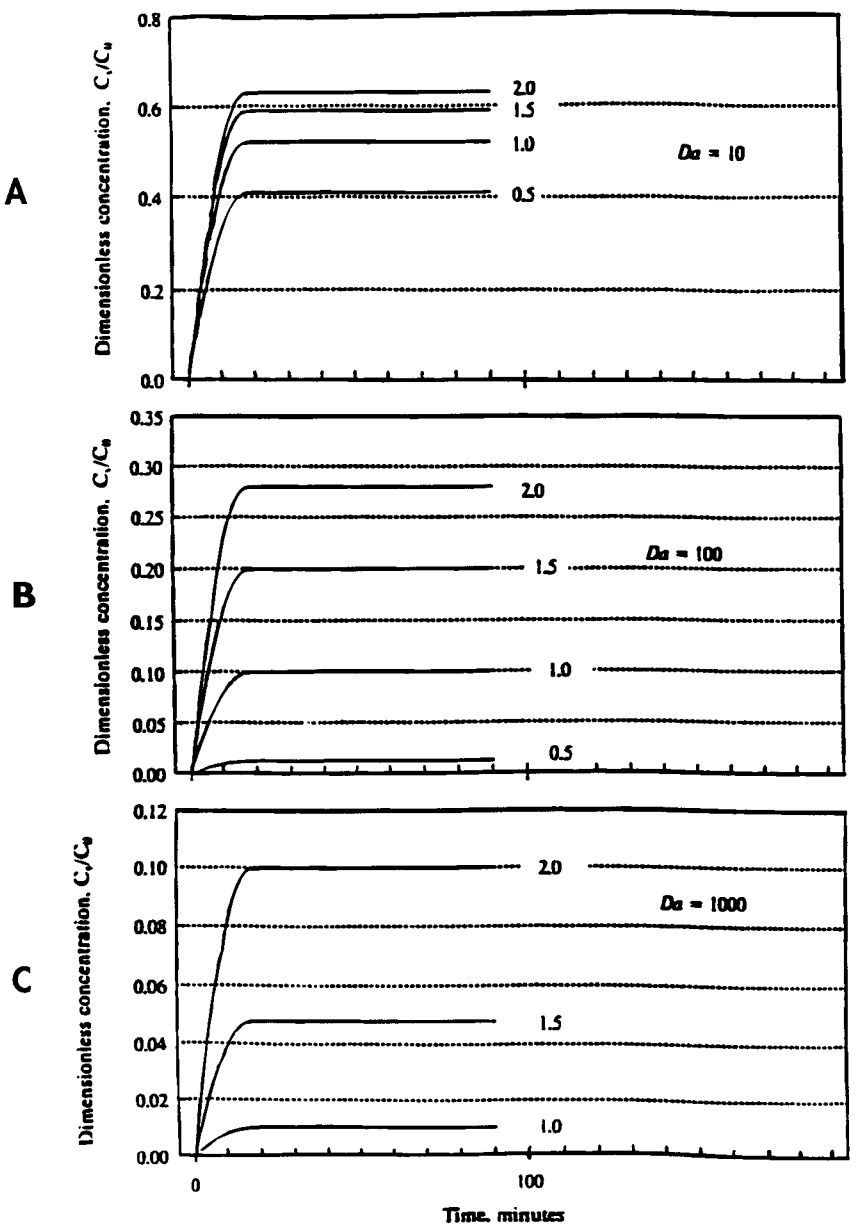

Fig. 6. Influence of reaction order (and/or valency) on dimensionless concentration c_s/c_0 as a function of time *(13)*: **(A)** Da = 10, **(B)** Da = 100, **(C)** Da = 1000.

valency antigens in solution bind faster than and attain higher Γ_{Ag}/c_0 levels than the higher-valency antigens. An increase in Da increases the differences between the rate of attachment of the antigen to the antibody for the different reaction orders. In other words, and as is to be intuitively expected, a decrease in the external-diffusional limitations minimizes the effect of

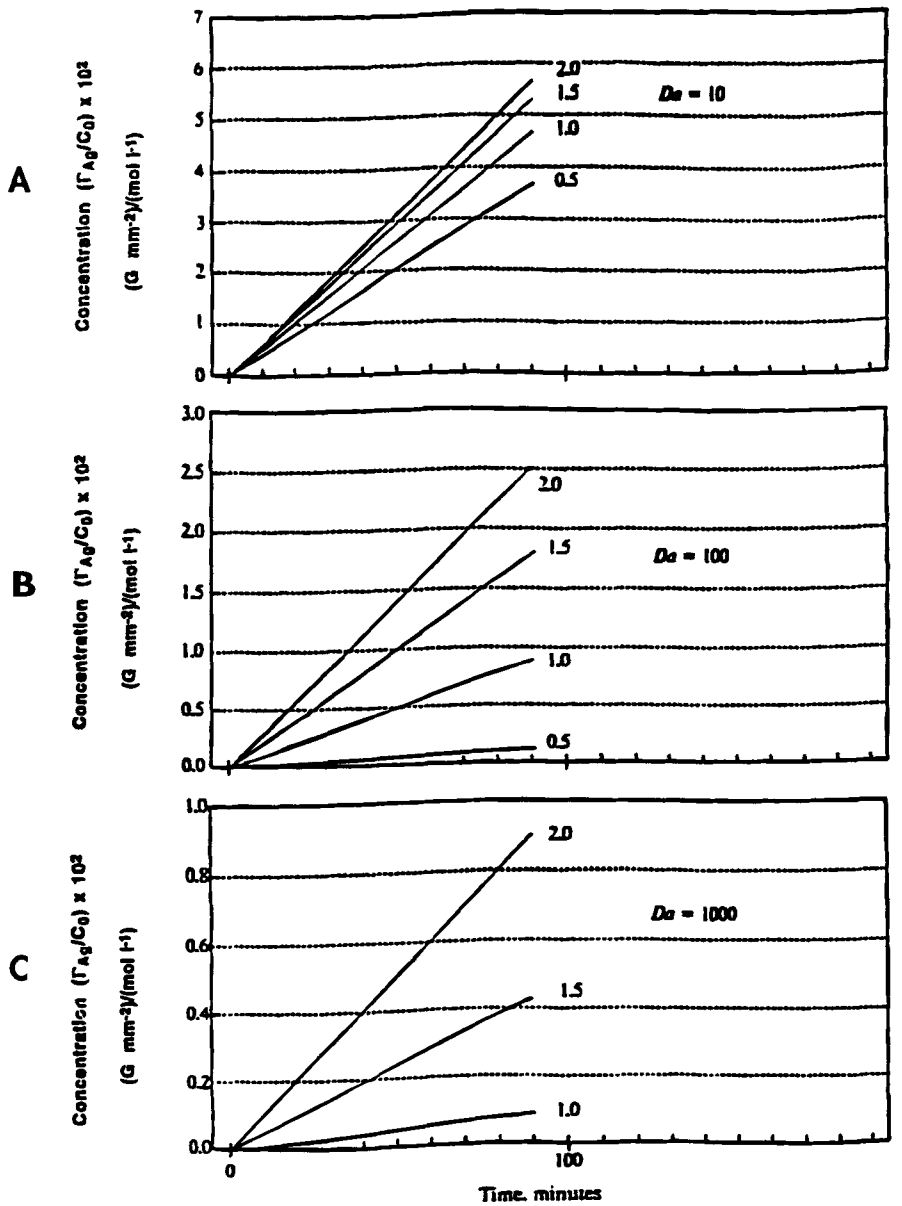

Fig. 7. Influence of reaction order (and/or valency) on Γ_{Ag}/c_0 as a function of time *(13)*: **(A)** Da = 10, **(B)** DA = 100, **(C)** Da = 1000.

reaction order (or the antigen valency) on the rate of attachment of the antigen to the antibody. Also, as expected, for the same antigen valency (or reaction order), lower rates of binding and levels are attained as one increases the Damkohler number or the mass-transfer limitations.

Antibody in Solution/Antigen on the Surface

Equation 21 indicates that as the valency of the antigen ($v > 2$) immobilized on the surface increases, the order of the reaction increases. In this case, because the effect of an increase in valency is the reverse of that observed for the previous case (wherein the antigen is in solution and the antibody is immobilized on the surface) the analysis is not repeated here to conserve space.

Experiments with fiber-optic biosensors have indicated some degree of nonspecific binding or adsorption that changes (with respect to the specific adsorption) with changes in the initial concentration of the antigen in solution. Nonspecific binding or adsorption should be considered in the development of an appropriate model. Non-first-order concentration dependence of the reactant in the reaction-rate expression may be owing to different reasons. Hence, the importance of the present analysis, wherein the effect of reaction order (and/or valency of the antigen or antibody as the case may be) is considered on the amount of antigen attached to the antibody on the surface (or vice versa). Sadana and Chen *(20)* indicated that nonspecific binding begins to play an increasing role with respect to specific binding as the reaction order is increased. In other words, the amount of antigen bound specifically to the surface is rather sensitive to the order of reaction, and this sensitivity increases as the reaction order is increased. When the antigen is in solution and the antibody is immobilized on the surface, an increase in the valency of the antigen in solution leads to lower-reaction orders, and hence, as one might expect, to lower contributions from nonspecific binding in the overall reaction. Furthermore, Sadana and Chen *(20)* indicated that when nonspecific binding is present, there is an optimum value of the forward binding-rate coefficient that leads to the maximum amount of antigen that can be specifically bound to an antibody immobilized on the surface. It would be of interest to analyze and to note if nonspecific binding alleviates or exacerbates the influence of varying antigen valency in external diffusion-limited antigen–antibody binding reactions.

Figure 1 indicates that the PACs are one possible set of antigens that exhibit multivalencies and different binding sites for antibody binding. It is highly probable that as an antibody binds to an antigen-binding site, it either makes easier or constrains (owing to induced conformational changes on the molecule immobilized on the surface) binding of further antibody molecules to different binding sites on the same antigen molecule. This would lead to temporal binding-rate coefficients, heterogeneities on the surface, and fractal reaction-rate kinetics *(22)*. Furthermore, different PACs may bind with certain antibodies in more than one orientation. Also, a population of antibodies may include some that bind the same PAC in different orientations. This would lead to further heterogeneities, and increasing disorder or inhomogeneities.

Table 1
Reaction Rate Expressions for Varying Valency Antigen/Antibody Systems

Valency of antigen/antibody	Reaction rate expression
Antigen in solution/Antibody on surface	
monovalent/monovalent	$d\Gamma_1/dt = k_f\,[Ag]\,\Gamma_{Ab,0}$ single-step; first order
monovalent/divalent	$d\Gamma_1/dt = k_f\,[Ag]^2\Gamma_{Ab,0}$ dual-step; second order
divalent/divalent	$d\Gamma_1/dt = k_f[Ag]\,\Gamma_{Ab,0}$ dual-step; first order
trivalent/divalent	$d\Gamma_1/dt = k_f[Ag]^{2/3}\Gamma_{Ab,0}$ dual-step; 2/3 order
v-valent/divalent	$d\Gamma_1/dt = k_f[Ag]^{s/v}\Gamma_{Ab,0}$ s-steps; s/v order general expression
Antibody in solution/Antigen on surface	
monovalent/monovalent	$d\Gamma_1/dt = k_f[Ab]\,\Gamma_{Ag,0}$ single-step; first-order
divalent/divalent	$d\Gamma_1/dt = k_f\,[Ab]\,\Gamma_{Ag,0}$ dual-step; first-order
monovalent/divalent	$d\Gamma_1/dt = k_f\,[Ab]\,\Gamma_{Ag}\Gamma_{Ag,0}$ dual-step; second-order
trivalent/divalent	$d\Gamma_1/dt = k_f\,[Ab]^{3/2}\Gamma_{Ag,0}$ dual-step; one and a half order
v-valent/divalent	$d\Gamma_1/dt = k_f\,[Ab]^{v/2}\Gamma_{Ag,0}$ $v > 2$ dual-step, v/2 order general expression

Reaction-rate forms exhibiting noninteger orders of reaction along with temporal binding-rate coefficients are a characteristic of diffusion-influenced reactions occurring on fractal surfaces. Antigen–antibody binding kinetics for biosensor applications has been analyzed within a fractal framework *(17,18)*. The fractal dimension provides an indication of the state of disorder or inhomogeneity on the biosensor surface. On examining the figures presented for antigen-antibody binding and the noninteger orders of reaction obtained, especially for the higher-valency antigens cases, it is reasonable to anticipate that higher-valency antigens would, in general, lead to higher levels of disorder on the surface. It would be of significant interest to relate the antigen valency to the fractal dimension on the surface, and how all of this may be tied down to better understand the stability, sensitivity, reproducibility, and response time of biosensors. The multivalency analysis presented is of a general enough nature, and to a large part, most of the analysis is equally applicable to receptor-ligand binding reactions occurring on the surface.

Finally, it is also instructive to present together the different reaction-rate expressions obtained for the different cases of antigen–antibody binding wherein varying valencies by either the antigen or the antibody is exhibited. Table 1 shows these different reaction-rate expressions.

ACKNOWLEDGMENTS

This work was sponsored by the US Department of Energy, Office of Health and Environmental Research, under Contract DE-AC05-84-OR21400 with Lockheed Martin Energy Systems, and by the ORNL Laboratory Directed Research & Development Program (Project on Biosensors). The research was also supported by the appointment of Ajit Sadana to the ORISE 1995 Summer Faculty Research Program at ORNL. The helpful comments provided by A. E. Karu, University of California, Berkeley are appreciated.

REFERENCES

1. Vo-Dinh, T. and Niessner R. Eds. (1995), *Environmental Monitoring and Hazardous Waste Site Remediations*. SPIE Publishers, Bellingham, Washington.
2. Vo-Dinh, T., Tromberg, B. J., Griffin, G,D., Ambrose, K. R., Sepaniak, M. J., and Gradenhire, E. M. (1987), *Appl. Spectrosc.* **41,** 735.
3. Vo-Dinh, T., Nolan, T., Cheng, Y. F., Sepaniak, M. J., and Alarie, J. P. (1990), *Appl. Spectrosc.* **44,** 128.
4. Vo-Dinh, T., Alarie, J. P., Johnson, R. W., Sepaniak, M. J., and Santella, R. M. (1991), *Clin. Chem.* **37,** 532.
5. Vo-Dinh, T., Sepaniak, M. J., Griffin, G. D., and Alarie, J. P. (1993), *Immunomethods* **3,** 85–92.
6. Tromberg, B. J., Sepaniak, M. J., Alarie, J. P., Vo-Dinh, T., and Santella, R. M. (1988), *Anal. Chem.* **60,** 1901.
7. Alarie, J. P., Sepaniak, M. J., and Vo-Dinh, T. (1990), *Anal. Chim. Acta* **229,** 169–176.
8. Giaver, I. (1976), *J. Immunology*, **116,** 766–771.

9. Stenberg, M., Stiblert, L., and Nygren, H. A. (1986), *J. Theor. Biol.* **120,** 129–136.
10. Nygren, H. and Stenberg, M. (1985), *J. Colloid Interf. Sci.* **107,** 560–566.
11. Stenberg, M. and Nygren, H. A. (1982) *Anal. Biochem.* **127,** 183–192.
12. Place J. F., Sutherland, R. M., and Dahne, C. (1985), *Biosensors* **1,** 321–353.
13. Sadana, A. and Sii, D. (1992a), *J. Colloid Interf. Sci.* **151,** 166–177.
14. Sadana, A. and Sii, D. (1992b), *Biosens. & Bioelectron.* **7,** 559–568.
15. Sadana, A. and Madagula, A. (1993), *Biotechnol. Progr.* **9,** 259–266.
16. Sadana, A. and Madagula, A. (1994), *Biosens. Bioelectron.* **9,** 45–55.
17. Sadana, A. and Beelaram, A. (1994), *Biotechnol. Prog.* **10,** 291–298.
18. Sadana, A. and Beelaram, A. (1995), *Biosens. Bioelectron.* **10,** 310–316.
19. Sadana, A., Alarie, J. P., and Vo-Dinh, T. (1995), *Talanta* **42,** 1567.
20. Sadana, A. and Chen, Z. (1996), *Biophys. Chem.* **57,** 177–187.
21. Patankar, S. V. (1980), *Numerical Heat Transfer and Fluid Flow,* McGraw-Hill, New York.
22. Kopelman, R. (1988), *Science,* **241,** 1620–1626.