Modeling of Immunosensors under Nonequilibrium Conditions

I. Mathematic Modeling of Performance Characteristics

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Immunosensors for the detection of small analytes that use analyte–enzyme conjugates as signal generators require special attention if operated under nonequilibrium conditions. If the size of the analyte and the analyte–enzyme conjugate differ substantially, the two antigens do not diffuse at the same rate. This can cause time-dependent shifts in the sensitivity of competitive immunoassays. Therefore, immunosensors operating at short incubation times require precise timing that meets closely the specifications for which the sensors were calibrated. As an example, we have analyzed kinetic binding curves for the quantitative determination of progesterone with an immobilized monoclonal antibody and a conjugate between horseradish peroxidase and progesterone as signal generator. Mathematical paradigms have been developed to simulate the diffusion, antigen–antibody complex formation, and competitive binding processes in this analytical system. Dose–response curves obtained under nonequilibrium conditions can vary substantially from those obtained at equilibrium of antigen–antibody interaction. The degree of this variation depends on the performance characteristics of the major components of the immunosensor. The developed mathematical solutions reflect experimental results and can be used to model optimal conditions for immunosensors operating under nonequilibrium conditions. In this paper (Part I), we report on the mathematical modeling of the interaction between analyte, analyte–enzyme conjugate, and an immobilized antibody. In Part II (W. Schramm and S.-H. Paek (1991) Anal. Biochem. 196), we present experimental results and compare them with the theoretical models.

In the area of sensor technology, one of the performance characteristics is the “response time.” For many sensors, a fast response time is desirable. Obviously, the definition of “fast” is relative, depending on the particular problem to be solved by a measurement and on the technique used for analyte detection and signal generation by a sensor. Recent developments in sensor technology (1,2) use biomolecules for the detection and quantitative determination of analytes in solutions. In physiological systems, the natural function of biomolecules is to control regulatory mechanisms that extend frequently over time periods of minutes or even hours. However, their often inherent slow response times can cause limitations if used in biosensors.

One class of biomolecules used in sensors designed to detect complex organic molecules is the immunoglobulin (immunosensors (3,4)). With the immunoassay technique that forms the basis for immunosensors, incubation times of several hours, even days, are not uncommon. The antigen–antibody complex formation (a) is a reversible process and (b) obeys an equilibrium reaction that follows the law of mass action. The number of antigen molecules bound to immunoglobulins increases asymptotically over time to reach a state at which the number of molecules associating with the antibodies and those molecules dissociating from the antibodies are equal. Many quantitative determinations with immunoglobulins are performed at this equilibrium state.

However, immunoglobulins can be used in the non
equilibrium state to reduce response times in immuno-
sensors. In this communication (Part I), we outline
some of the problems to be considered for the engineer-
ing of immuno sensors operating under nonequilibrium
conditions, and we also present solutions for calculating
the performance of the sensors under these conditions.
The theoretical determination of kinetic variables using
mathematical models permits the appropriate selection
of immunoglobulins and the synthesis of analyte–en-
zyme conjugates with optimal binding characteristics.
In a subsequent paper (Part II) (5), we analyze how the
models predict experimental results.

**ANALYTE-ENZYME CONJUGATE AS SIGNAL GENERATOR**

For the majority of analytical systems that use immu-
noglobulins as bioreceptors, binding of an antigen to an
antibody cannot be directly monitored because the re-
sulting complex does not usually provide a suitable phys-
ical signal. Therefore, for many applications, "labels" are
used for signal generation. For many years, radioiso-
topes have been the most popular labels but these are
often replaced today by other molecules such as lumines-
cent and fluorescent markers. One of the most com-
monly used labels is enzymes. Enzymes can generate
either a photometrically or an electrochemically detect-
able signal. We will investigate the kinetic reactions
with enzymes as signal generators and we have selected
the competitive immunoassay as an example. Similar
considerations might apply for other types of antigen–
antibody binding reactions used for the construction of
biosensors. We have used a solid-phase immunoassay as
a model which is probably representative for most im-
munosensors. In a solid-phase assay, one of the com-
ponents participating in the analytical reaction is immobi-
lized on a solid matrix, e.g., on an electrode or an optic
fiber. For the investigated example, the immunoglobu-
lin is immobilized.

If an enzyme-labeled antigen is used for signal genera-
tion in immuno sensors, the size of the label can sub-
stantially differ from that of the native antigen (ana-
yte). This has consequences for the kinetics of the
different binding reactions. A competitive immunoas-
say as shown in Fig. 1 implies that the analyte competes
with the analyte–enzyme conjugate for binding sites on
the antibody. This happens only if the two species are
reasonably equally recognized by the antibody. Pro-
vided that is the case, binding over time for the native
antigen is different than for the enzyme-labeled antigen
if their molecular masses vary substantially because the
smaller molecule diffuses faster to the immobilized an-
tibody than does the larger conjugate. Therefore, we
have a sequential immunoassay where one of the two
competing components (i.e., the analyte) reaches the
antibody first to initiate the binding reaction that culmi-
nates in the equilibrium state where the on-rate and the
off-rate for the analyte–antibody complex formation
are equal. The other antigen (the larger conjugate)
reaches equilibrium later. It is the combination of these
two superimposed, temporarily delayed equilibrium re-
actions that determines the performance characteris-
tics of the immunosensor.

This is of little consequence if the incubation time is
long enough to permit both antigen species to reach
binding equilibrium. However, if the immunosensor is
operated under nonequilibrium conditions to reduce the
response time, the different kinetics of the two binding
reactions have to be taken into consideration for calibra-
tion purposes. We have made an attempt to model the
response time of the described type of immunosensor
under nonequilibrium conditions.

**THEORETICAL DETERMINATION OF KINETIC VARIABLES**

Many performance characteristics of immuno sensors
can be predicted by theoretical models. In this section,
we introduce the mathematical model for the theoretical
determination of kinetic variables and present two
approaches to calculate the binding of antigen to anti-
body over time. However, to execute the calculations
according to the model, a minimum of independent vari-
ables need to be experimentally determined (see Part
II). This is comparatively simple for variables derived
from labeled antigens since these are directly generat-
ing a measurable signal. The native antigen, however,
cannot be measured directly in many designs of immu-
nosensors and some kinetic parameters need to be indi-
rectly determined.

**Mathematical Model**

For the development of the mathematical model we
have selected the geometry of a microwell (Fig. 2). An
antibody is chemically bound to a surface area contacted by 200 µl of medium. These same conditions were used for the validation of the theoretical predictions by experimental studies. The symbols and dimensions used for the model are described in Table 1.

Diffusion of molecules and binding of antigens to the immobilized antibody at the liquid–solid interface are the two processes that are mathematically described.

**Differential rate equation for diffusion.** If an immunoSENSOR with an immobilized antibody on the surface is exposed to the solution that contains the analyte to be measured, a concentration gradient of the analyte (antigen) ensues. The analyte is removed from the bulk solution by binding to the antibody. As a dynamic equilibrium process, the antigen–antibody binding rate is determined by the on- and off-rate, i.e., the association of the antigen with the antibody and the dissociation from the antigen–antibody complex. The question arises: Is the approach to equilibrium limited by the ability of the antigen molecules to reach the antibody on the solid surface? In other words, is the establishment of equilibrium diffusion limited? In practical terms, the investigator wants to know if a certain design of an immunoSENSOR can be operated with or without agitation. Diffusion of reagents on solid–liquid interfaces of microwells toward the formation of antigen–antibody complexes without agitation was previously described (6). We are theoretically investigating the diffusion processes under agitation. In Part II of this study, we describe the experimental results for diffusion processes under agitation.

Diffusion is the transport of a constituent from a region of higher concentration to that of a lower concentration. This process consists of three components which are described in the context of an immunoSENSOR with an immobilized antibody on a solid surface and a binding reaction at the liquid–solid interface (for review see (7)):

1. Convective diffusion, the movement of molecules (or particles) from the bulk solution to the surface. This is a three-dimensional process (8,9).
2. Lateral diffusion, the movement of molecules on the surface of the solid phase toward an immobilized immunoglobulin. This is a two-dimensional process (10,11).
3. Rotational diffusion, to direct the antigen spatially toward the idiotypic site of the immunoglobulin so that

<p>| TABLE 1 |
| Symbol and Their Descriptions Used for the Theoretical Determination of Kinetic Variables by Mathematical Models |</p>
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>Thickness of penetration layer</td>
<td>mm</td>
</tr>
<tr>
<td>[Ab]₀, [Ab]ₜ</td>
<td>Density of binding sites of antibody on the solid surface (subscript f stands for unoccupied and t for total binding sites)</td>
<td>mol mm⁻²</td>
</tr>
<tr>
<td>[Ag]₀, [Ag]ₜ, [Ag]ₜ</td>
<td>Concentration of antigen (subscript b in bulk solution, s on solid surface, and t for total)</td>
<td>mol mm⁻³</td>
</tr>
<tr>
<td>[Ab:Ag]</td>
<td>Density of antigen–antibody binding complex</td>
<td>mol mm⁻²</td>
</tr>
<tr>
<td>S</td>
<td>Amount of antigen bound to antibody</td>
<td>mol/well</td>
</tr>
<tr>
<td>C</td>
<td>Defined by N₈ₜkₕ{(1 + 2X)/(rλ)}</td>
<td>mm² s⁻¹</td>
</tr>
<tr>
<td>D</td>
<td>Effective diffusion coefficient of antigen</td>
<td>mm</td>
</tr>
<tr>
<td>h</td>
<td>Height of liquid head in microwell</td>
<td>mm</td>
</tr>
<tr>
<td>Jₘᵢ, Jₜₑ</td>
<td>Influx and efflux across a defined boundary</td>
<td>mol mm⁻² s⁻¹</td>
</tr>
<tr>
<td>kₑ</td>
<td>Convective mass transfer coefficient (D/a)</td>
<td>mm s⁻¹</td>
</tr>
<tr>
<td>kₜₑ</td>
<td>Off-rate constant of antigen from antigen–antibody complex</td>
<td>s⁻¹</td>
</tr>
<tr>
<td>kₜₑ</td>
<td>On-rate constant of antigen for antigen–antibody complex formation</td>
<td>mm³ mol⁻¹ s⁻¹</td>
</tr>
<tr>
<td>[N₆ₜ][Nₗₜ]</td>
<td>Amount of antigen in bulk solution (b) and penetration layer (p)</td>
<td>mol</td>
</tr>
<tr>
<td>N₆ₑ</td>
<td>Dimensionless Damköhler number (kₜₑ[Ab]₀/kₑ)</td>
<td></td>
</tr>
<tr>
<td>r</td>
<td>Inner radius of microwell</td>
<td>mm</td>
</tr>
<tr>
<td>S</td>
<td>Surface area of microwell that is covered with liquid</td>
<td>mm²</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
<td>s</td>
</tr>
<tr>
<td>T</td>
<td>Total amount of antigen</td>
<td>mol/well</td>
</tr>
<tr>
<td>T'</td>
<td>Total amount of binding sites</td>
<td>mol/well</td>
</tr>
<tr>
<td>V</td>
<td>Volume of liquid in microwell</td>
<td>mm³</td>
</tr>
<tr>
<td>λ</td>
<td>Ratio of h/r in microwell</td>
<td></td>
</tr>
</tbody>
</table>
binding can occur. This is also a two-dimensional process (12).

These three diffusion processes lead to the establishment of an encounter complex which is the precondition for the antigen-antibody complex. The relative rate of formation of these two complexes indicates if the sensor is diffusion controlled or reaction controlled.

It is difficult to measure the diffusion processes separately, but the overall (effective) diffusion coefficient can be experimentally determined. Alternatively, the effective diffusion coefficient can be calculated if the molecular dimensions of the antigen(s) participating in the antigen-antibody reaction are known. However, these calculations are complicated by the effect that repulsive forces (e.g., charge interactions) and nonsymmetric configurations of molecules in solution and on the surface can have on the diffusion of molecules.

For this model, we have not taken into consideration the surface charge of the antigen and the surface area. The electrostatic forces between the surface and antigen is inversely proportional to the distance. Therefore, the three-dimensional diffusion is not substantially affected by charges. The antigen present near the solid surface may interact with the charged surface, the strength of repulsion or attraction depending on the charge density. These forces can affect the lateral diffusion rate (13).

Three-dimensional diffusion can be accelerated by agitation the incubation mixture so that molecules reach the surface faster. However, even with most vigorous agitation, this type of diffusion can never be completely abolished. Near the surface, a layer of water and medium components forms that is organized by electrostatic forces resulting from the interaction of the surface and the components of the medium, including the diffusive substance (i.e., antigen). This is called the penetration layer or hydrodynamic layer (a in Fig. 2 and Table 1; (8)). The diffusive substance may only marginally affect the thickness of the penetration layer if it is vastly outnumbered by other medium components (e.g., gelatin used in incubation buffers).

The formation of the encounter complex (Fig. 3) finishes the process of diffusion and initiates the formation of the antigen-antibody complex, for which the kinetics are determined by the reaction rate \( k_{on} \) and \( k_{off} \).

Equations for two of the three other diffusion rates are difficult to formulate (lateral and rotational). Therefore, we developed an equation only for three-dimensional mass transfer [9]. This expression requires the diffusion coefficient, \( D \), for the solution (\( k = \frac{D}{\alpha} \)). However, we entered into [9] the effective diffusion coefficient (i.e., the sum of the three diffusion processes). Three-dimensional mass transfer seems to contribute the largest component in \( D \) so that potential deviations in the following calculations are negligible. This was finally confirmed by the good correlation of the theoretically developed and the experimentally determined data for kinetic binding curves (Part II).

In Eqs. [1] to [9] we explain the formation rate of the encounter complex (Fig. 3). Although the microwell has the geometry of a cylinder, we will treat all surfaces as one plane. The thickness of the penetration layer for diffusion (8 to 9 x 10^{-2} mm) is much smaller than the radius of the microwell (3.3 mm). Since we are working under stirring conditions, molecules in the bulk solution are uniformly distributed and diffusion takes place only in the thin penetration layer that is at first approximation independent of the well geometry. The efflux of antigen from the bulk solution equals the influx into the penetration layer, while the efflux from the penetration layer reflects binding to the antibody, i.e., withdrawal of the antigen from the system. Two material balance equations for the penetration layer [5] and the bulk solution [8] are developed and then combined to obtain the diffusion rate that reflects the formation of the encounter complex over time [9].

For the determination of the concentration of antigen in the penetration layer, the influx of antigen from the bulk solution is proportional to the diffusion coefficient \( D \) and the concentration gradient \( (\left[ Ag \right]_b - \left[ Ag \right]_s) / a \) (Nernst equation (8)).

\[
J_m = \frac{D}{\alpha}(\left[ Ag \right]_b - \left[ Ag \right]_s) = k_c(\left[ Ag \right]_b - \left[ Ag \right]_s). \quad [1]
\]

The efflux of free antigen (withdrawal from the diffusion process) by binding to the immobilized antibody
follows the binding rate (based on a monovalent binding of antigen to antibody).

\[ J_{\text{out}} = k_{\text{on}}[\text{Ab}][\text{Ag}] - k_{\text{off}}[\text{Ab}:\text{Ag}] \quad [2] \]

To calculate the accumulation of free antigen molecules in the penetration layer, we estimate an average concentration derived from that of free antigen on the solid surface and that found in the bulk solution (assuming a linear gradient in the penetration layer).

\[ d[N_{\text{Ag},b}]/dt = d[\text{Ag}](V - aS)/9) / dt \quad [3] \]

The establishment of a linear gradient in the penetration layer in a model similar to that discussed here has been described in detail (6,8,14). Under stirring conditions, the antigen molecules are distributed in a linear concentration gradient within the order of seconds after addition of antigen to the wells. This compares to times of >30 min to hours for reaching the state of equilibrium between immobilized antibody and antigen.

By applying the conservation law of mass (accumulation = influx - efflux) we obtain

\[ d[N_{\text{Ag},b}]/dt = (J_{\text{in}} - J_{\text{out}})S \quad [4] \]

Inserting Eqs. [1], [2], and [3] into [4], the sum of the change of concentration of the antigen in the bulk solution and on the solid surface is

\[ d[\text{Ag}]_{b}/dt + d[\text{Ag}]_{s}/dt = (2/a)\{k_{c}([\text{Ag}]_{b} - [\text{Ag}]_{s}) - (k_{on}[\text{Ab}][\text{Ag}]_{s} - k_{off}[\text{Ab}:\text{Ag}])\}. \quad [5] \]

For the bulk solution, the net flux across the boundary between bulk solution and penetration layer [6] and accumulation within the bulk solution [7] are

\[ J_{\text{in}} - J_{\text{out}} = -k_{c}([\text{Ag}]_{b} - [\text{Ag}]_{s}) \quad [6] \]

\[ d[N_{\text{Ag},b}]/dt = d([\text{Ag}](V - aS))/dt. \quad [7] \]

Equations [6] and [7] are combined

\[ d[\text{Ag}]_{b}/dt = -k_{c}S/(V - aS)(([\text{Ag}]_{b} - [\text{Ag}]_{s}) \quad [8] \]

Inserting Eq. [8] into [5], the final differential equation for the diffusion rate under stirring conditions is

\[ d[\text{Ag}]_{s}/dt = k_{c}(2/a) + S/(V - aS))/([\text{Ag}]_{b} - [\text{Ag}]_{s}) - (2/a)(k_{on}[\text{Ab}][\text{Ag}]_{s} - k_{off}[\text{Ab}:\text{Ag}]). \quad [9] \]

The concentration of antigen that reaches the surface by diffusion, [\text{Ag}]_{s}, represents that of the encounter complex which is the precursor for antigen–antibody complex formation.

**Equation for antigen–antibody binding reaction.** The rate of formation of antigen–antibody complex, [\text{Ab}:\text{Ag}], (reaction rate) is a function of the concentrations of the unoccupied binding sites, [\text{Ab}]; the antigen at solid surface, [\text{Ag}]_{s}; and the antigen–antibody binding complex, [\text{Ab}:\text{Ag}]

\[ d[\text{Ab}:\text{Ag}] / dt = k_{on}[\text{Ab}][\text{Ag}] - k_{off}[\text{Ab}:\text{Ag}]. \quad [10] \]

**Equations for material balance (law of mass action).** To solve Eqs. [9] and [10] for the four unknown variables ([\text{Ag}]_{b}, [\text{Ag}]_{s}, [\text{Ab}], and [\text{Ab}:\text{Ag}]), two additional equations are required. These are provided by the material balance equations for the antigen and the antibody binding sites.

The total molar amount of antigen is the sum of the molar amount in the bulk solution, in the penetration layer, and the amount bound to antibody:

\[ V[\text{Ag}]_{b} = (V - aS)[\text{Ag}]_{b} + aS(\frac{[\text{Ag}]_{s} + [\text{Ag}]_{b}}{2}) + S[\text{Ab}:\text{Ag}]. \quad [11] \]

The molar amount of antibody on the solid surface is

\[ [\text{Ab}]_{s} = [\text{Ab}]_{s} + [\text{Ab}:\text{Ag}]. \quad [12] \]

The unknown variables ([\text{Ag}]_{b}, [\text{Ag}]_{s}, [\text{Ab}], and [\text{Ab}:\text{Ag}]) can be calculated from Eqs. [9] to [12] by either the analytical or the numerical method (see below). With these equations, the concentrations of antigen on the solid surface and bound antigen can be calculated, provided that the independent kinetic variables are experimentally determined.

**Determination of Time-Variable Concentrations of Bound Antigen**

Equations [9] to [12] can be reduced to two differential equations by substituting [\text{Ag}]_{b} and [\text{Ab}]_{s} in [9] and [10] with [11] and [12]. The two final differential equations are nonlinear and, therefore, difficult to mathematically resolve for two dependent variables (concentrations of antigen on the surface [\text{Ag}]_{s} and the concentration of the binding complex [\text{Ab}:\text{Ag}]). However, if we apply two approximations (by assuming pseudo-steady-state antigen concentrations on the surface, [\text{Ag}]_{s}, and by neglecting the volume of the penetration layer), the equations become mathematically solvable.

The nonlinear equations can also be solved using a numerical method which requires different approximations (see below). We have explored both approaches for
the kinetic study of antigen–antibody binding at liquid–solid interfaces.

**Analytical solution.** By definition, a pseudo steady state exists when the diffusion rate of antigen from the bulk solution to the surface is equal to the reaction rate of antigen–antibody binding; no encounter complex or free antigen would have the chance to accumulate in the penetration layer. As we describe in Part II of these investigations, the assumption of a steady-state antigen concentration in the penetration layer is adequate if stirring conditions are used to facilitate the mass transport from the bulk solution to the solid surface (8).

Since the influx (Eq. [1]) is equal to the efflux [2], the following equation replaces [9]:

\[ k_{c}(\text{Ag}_b - \text{Ag}_s) = k_{on}[\text{Ab}][\text{Ag}]_s - k_{off}[\text{Ab:Ag}]. \]  

[13]

We can simplify Eq. [11] by ignoring the volume of the penetration layer (e.g., we have experimentally established that it is \(<7\% of the total volume under stirring conditions):\]

\[ V[\text{Ag}_b] = V[\text{Ag}_s] + S[\text{Ab:Ag}]. \]  

[14]

In Eq. [13], the concentration of antigen at the surface \([\text{Ag}_s]\) can be expressed by \([\text{Ag}_b]\), \([\text{Ab}]_b\), and \([\text{Ab:Ag}]_b\), and then \([\text{Ag}_b]\) is substituted with Eq. [14]:

\[ [\text{Ag}_s] = \left( k_{c}([\text{Ag}_b] - \{(1 + 2\lambda)/(r\lambda)\}[\text{Ab:Ag}]) + k_{off}[\text{Ab:Ag}] / (k_{c} + k_{on}[\text{Ab}]_b) \right). \]  

[15]

\([\text{Ag}_s]\), obtained from [15] is now used in Eq. [10]. To solve [10], \([\text{Ab}]_b\) still needs to be replaced and is obtained from [12]. As a result, Eq. [10] becomes

\[ \frac{d[\text{Ab:Ag}]}{dt} = N_{Da} k_{c} \frac{[\text{Ag}_b]}{(1 + \gamma)} - \left\{ \frac{N_{Da} k_{c} \{(1 + 2\lambda)/(r\lambda)\} + k_{off}}{(1 + \gamma)} \right\} \left( \frac{[\text{Ab:Ag}]}{[\text{Ab}]_b} \right). \]  

[16]

Equation [16] is now integrated in the range between \([\text{Ab:Ag}] = 0\) and \([\text{Ab:Ag}] = [\text{Ab:Ag}]_{\text{int}}\), and in the range \(t = 0\) to \(t = t\). \(B/T\) (bound over total antigen) is then determined.

\[ B/T = \left( \frac{S[\text{Ab:Ag}]_{\text{int}}}{V[\text{Ag}]_{\text{int}}} \right) = \left\{ \frac{1 - \exp\{-[C + k_{off}]t/(1 + N_{Da})\}}{(1 + k_{off}C^{-1})} \right\}. \]  

[17]

where

\[ C = N_{Da} k_{c} \{(1 + 2\lambda)/(r\lambda)\}. \]  

[18]

**Numerical solution.** To circumvent the mathematical approximations in the analytical solution, a numerical method can be used for the theoretical calculations. Numerical methods are algorithms that use only arithmetic and certain logical operations such as algebraic comparisons (15). However, in calculating a function by computer-oriented numerical methods, errors might now be introduced by approximating the solution of a mathematical problem (truncation errors) and in operating a finite number of digits (round-off errors).

For the numerical solution, we use the fourth-order Runge-Kutta method (16) to simultaneously solve the two differential equations [9] and [10] with two supplemental equations [11] and [12]. The purpose of the Runge-Kutta method is to obtain an approximate solution of a system of first-order ordinary differential equations with given initial values.

To calculate the variable of interest, i.e., the ratio of bound over total antigen \((B/T)\) as a function of time, the major steps are:

(a) Scale the variables in Eqs. [9] to [12] \(([\text{Ag}]_b, [\text{Ag}]_s, [\text{Ab:Ag}]_b, [\text{Ab}]_b, [\text{Ab}]_s\) by \([\text{Ag}]_b\).

(b) Scale the time by \((a/k_c)\).

(c) Define the scaled variables.

(d) Rearrange Eqs. [9] and [10] to introduce the dimensionless groups \(\{(k_{on}[\text{Ab}]_b)/k_c\}\) and \(\{(k_{off}V)/(k_{on}[\text{Ab}]_b)[\text{Ab}]_b)\}\).

(e) Execute a computer program (e.g., by FORTRAN) for the solution of the equations by the numerical method; i.e., calculate \(B/T\) at a given time interval by iterations.

The numerical solution for the problem analyzed in this communication is in Part II and is compared with experimental results.

We have calculated dose–response curves and kinetic binding curves for an immobilized antibody to the steroid hormone progesterone and a radiolabeled and an enzyme-labeled progesterone derivative in microwells by the methods introduced in Part I. The results are compared with experimentally obtained data in Part II of this communication.

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