Mathematical Modeling for Immunocolloid Labeling

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ABSTRACT

For many biological applications, it is becoming increasingly important to extend imaging capabilities to simultaneously identify multiple molecular species in order to gain insight into the structural and functional elements of cells. A key part of this drive is the ability to rationally optimize various labeling techniques, which in turn requires an understanding of the basic physical processes operating during the labeling process, and how these processes interact. The kinetics of the immunocolloid labeling process are typically modeled using a Langmuir kinetic equation [1]. However, very different behavior which can not be explained by a Langmuir-type model has been noted in certain experiments [2], [3]. We develop a mathematical model for the labeling process which exhibits these two behaviors in different parameter limits, gives some insight into how to optimize the labeling process, and can easily be extended to more complicated situations.

Keywords: immunocolloid labeling, immunogold labeling, mathematical model, reaction-diffusion model, surface-volume reaction

1 INTRODUCTION

Immunocolloid labeling is a technique for imaging molecular scale features in many biological applications. The method starts by conjugating colloidal heavy metal particles to ligand molecules which bind to the target molecules of interest. Gold is frequently used as the heavy metal, hence the process is often referred to as immunogold labeling. The specimen to be labeled is bathed in a suspension of the composite metal-ligand label particles, the label particles diffuse to and bind to the target molecules, and once the labeling process is complete, the labeling suspension is washed away and the specimen is prepared and viewed under an electron microscope. While the target molecules may be difficult to detect directly, the label particles are easily detected.

This process widely used, but in order to maximize its effectiveness and advance the development of multiple labeling techniques, in which several different target molecules are simultaneously marked with label particles which can be distinguished under the electron microscope by size, shape, or composition, a theoretical framework needs to be developed that will give some insight into how to choose the experimental parameters such as concentration of labeling particles, the time for which the specimen is exposed to the labeling suspension, and so on.

To this end, we have developed a mathematical model based on the two most basic physical mechanisms needed to describe the labeling process: diffusion of the label particles through the suspension to the immobile target sites, and the chemical reaction between the label particles and target molecules. In its simplest form, the model depends on only one nondimensional parameter, the Damköhler number Da, which can be thought of as the ratio of a time scale for the reaction to a time scale for diffusive transport of the label particles. In the limit of small Da, this model reproduces the results of the Langmuir-type model typically used to describe the labeling process [1], which assumes that the process is limited by the rate of the binding reaction.

On the other hand, it is well known that in certain situations the process is limited by diffusion of the label particles [1]. This can happen when the target molecules are densely packed on a surface, leading to depletion of the label particles close to the surface, as is the situation in certain experiments described in [2], [3]. This corresponds to the large Da limit of the model described herein.

The model has several nice features. In its simplest form, as presented here, it depends on only one nondimensional parameter, the Damköhler number Da. It is clear how all the experimentally accessible parameters enter into Da and hence easy to understand what happens when multiple experimental parameters are varied. Asymptotic solutions can be developed in the two limits $0 \ll \mathrm{Da} \ll 1$ and $\mathrm{Da} \gg 1$ which in fact turn out to be good approximations to the true solutions over almost the entire range of Da. The model is easily solved numerically, especially in the middle range when $\mathrm{Da} \approx 1$ and neither of the asymptotic solutions does a good job. Finally, the model provides a good starting point. It can easily be extended to allow more complicated chemistry at the boundary, interactions between the label particles, complicated boundary geometry, multiple labeling, and so on.

2 MATHEMATICAL MODEL

As indicated above, our basic model consists of a diffusion equation for the concentration C of label particles in the suspension, coupled to an equation modeling the binding of the label particles to the target molecules on the boundary. The model assumes that there is a uniform surface concentration B at the boundary, which is appropriate for the experiments discussed in [2], [3], and hence ignores variations along the boundary. We have:

$$0 < x < \infty : \qquad C_t = C_{xx}, \tag{1}$$

where C is the nondimensionalized concentration, scaled by the dimensional initial concentration C_0, and x is the nondimensionalized distance from the boundary to which the target molecules are confined. We also need to keep track of the target molecules on the boundary, or equivalently, the surface concentration of bound label particles. This is done through the following two boundary conditions:

$$x = 0 : \qquad B_t = C_x, \qquad \mathrm{Da}^{-1} B_t = C(1 - B). \tag{2}$$

The first of these states that the rate of change of the surface concentration B of bound label particles is equal to the net flux of label particles to the surface from the suspension. The second states that the labeling process follows mass action kinetics, with the reaction rate proportional to the product of the label particles in the suspension adjacent to the boundary and the surface concentration of free (unlabeled) target molecules. For simplicity, we ignore the dissociation reaction. The surface concentration is nondimensionalized with reference to B_0, the surface concentration of target molecules, or equivalently, the maximum surface concentration of bound label particles. We assume that the length scales associated with the problem are very small compared to the size of the experimental setup, and impose a no flux condition at infinity:

$$x = \infty : \qquad C_x = 0. \tag{3}$$

Finally, we have the initial conditions:

$$t = 0 : \qquad C = 1, \qquad B = 0. \tag{4}$$

This is the form the equations take if we use diffusive length and time scales. The diffusive length scale is B_0/C_0, which can be thought of as the thickness of a layer of the initial suspension which contains exactly enough label particles to bind to all target molecules. The diffusive time scale is $B_0^2/(C_0^2 D)$, where D is the diffusion constant for the label particles in suspension. Since the particles are colloidal and subject to Brownian motion, D can be approximated by the Stokes-Einstein formula,

$$D = \frac{\kappa T}{6\pi\mu R}, \tag{5}$$

where κ is Boltzmann's constant, T is the temperature, μ is the dynamic viscosity of the fluid in which the particles are suspended, and R is the radius of the particle.

The only nondimensional parameter is the Damkhöler number,

$$\mathrm{Da} = \frac{k_A B_0^2}{D C_0} = \frac{B_0^2/(C_0^2 D)}{1/(k_A C_0)} = \frac{\text{diffusion time scale}}{\text{reaction time scale}} \tag{6}$$

The experimental parameter which may most easily be adjusted over a wide range is the initial concentration of labeling particles, C_0. Assuming that the labeling process is limited by the reaction, i.e. looking at the parameter regime $0 < \mathrm{Da} \ll 1$, really means that with everything else held fixed, we have chosen C_0 so that the process consumes only a small portion of the available supply of label particles. This may be difficult to arrange if the target molecules are densely packed, i.e. if B_0 is large. This complementary situation with $\mathrm{Da} \gg 1$ leads to a depletion of the supply of label particles adjacent to the boundary, so that the process is limited by the diffusive transport of labels to the boundary.

3 LARGE Da LIMIT

If we formally set $\mathrm{Da} = \infty$ in (2), we get a simplified solution with two possible solutions. The second condition in (2) reduces to two possibilities: either $B = 1$, or $C(x = 0, t) = 0$. The first of these leads to the very simple solution $B = 1$, $C = 1$, which corresponds to the long time limit in which the labeling process is complete. The second possibility also leads to a simplified problem, in which we can first solve for C, from (1), (3), (4) and $C(x = 0, t) = 0$. This problem has the solution

$$C = \mathrm{erf}\left(\frac{x}{2\sqrt{t}}\right), \tag{7}$$

where erf is the error function. We can then compute $C_x(x = 0, t) = 1/\sqrt{\pi t}$, and use the first equation in (2) to find that

$$B = \frac{2\sqrt{t}}{\sqrt{\pi}}. \tag{8}$$

This solution can only be valid for $0 \le t \le \pi/4$ at best, since we have scaled the problem so that $0 \le B \le 1$. In fact, an asymptotic analysis of the problem shows that in the limit $\mathrm{Da} \to \infty$, the solution to the problem converges to the limiting solution

$$B = \begin{cases} 2\sqrt{t/\pi} & 0 \le t < \pi/4 \\ 1 & t \ge \pi/4 \end{cases}. \tag{9}$$

Corrections to this solution for large but finite Da can be obtained systematically, but are not reproduced here because they are somewhat complicated.

There are two points we want to make here about the limiting solution (9) and its refinements. The first

is that that this simplified solution agrees with the experimental results and the theoretical analysis based on the Brownian transport of label particles reported in [2],[3]. The second is that including the first correction to (9) gives an approximate solution for B which agrees well with the solution obtained by numerically solving the problem for $2 < \mathrm{Da} < \infty$.

4 SMALL Da LIMIT

As noted above, in this limit we expect the solution to evolve on the reactive time scale. Since (1)–(4) were obtained using diffusive length and time scales, we expect that we will need to rescale the problem in this limit. In fact, naively setting $\mathrm{Da} = 0$ in (1)–(4) leads to the solution $C = 1$, $B = 0$, i.e. the solution is pinned at the initial conditions and does not evolve. Setting $\tau = \mathrm{Da}t$, $\xi = \sqrt{\mathrm{Da}}x$, we obtain

$$0 < \xi < \infty : \qquad C_\tau = C_{\xi\xi}, \tag{10}$$

$$\xi = 0 : \qquad C_\xi = \sqrt{\mathrm{Da}} B_\tau, \qquad B_\tau = C(1 - B), \tag{11}$$

$$\xi = \infty : \qquad C_\xi = 0, \tag{12}$$

$$\tau = 0 : \qquad C = 1, \qquad B = 0. \tag{13}$$

If we now set $\mathrm{Da} = 0$, the problems for C and B decouple. Solving for C using (10), $C_\xi(\xi = 0, t) = 0$ from (11), (12) and (13) gives $C = 1$. Therefore, we are left with a linear, constant coefficient, ordinary differential equation, in fact just the Langmuir kinetic equation, for B from (11), with solution $B = 1 - \exp(-\tau)$. If we go back to dimensional variables,

$$B = B_0 \left(1 - \exp(-k_A C_0 t)\right), \tag{14}$$

so that we can use a relatively simple curve fit to determine the association rate constant.

This can be corrected for small but nonzero Da by expanding both B and C as power series in $\sqrt{Da}$ and solving the resulting regular perturbation problem to the order desired. This leading order solution agrees well with the numerical solution of the full problem for $0 < \mathrm{Da} < 1/2$.

5 OUTLOOK AND CONCLUSIONS

We have presented a mathematical model for the immunocolloid labeling process. The model is consistent with existing theoretical results in the two limiting cases in which either the association reaction or the diffusive transport of label particles is the rate limiting step in the process. It allows the rational development of a sequence of approximate solutions valid in each of these limiting cases. In the intermediate range where the two subprocesses occur at commensurate rates, the model can easily be solved numerically.

The model is a first step toward a better understanding of the labeling process. More details of the surface chemistry can easily be included. For instance, dissociation of bound label particles from the targets can be included by a slight modification in the second equation of of (2). The Langmuir model can be modified to allow for crowding of the adsorbate when the target sites are densely packed [4], and the same can be done for this model in general (not just in the reaction limited case), again by a slight modification of the second equation in (2). The multiple labeling process can be approached by coupling together a number of these basic models, with separate variables for the concentrations of each of the label species. In actual applications of immunocolloid labeling, geometrical effects will potentially be an issue, for two reasons: the target molecules will generally not be homogeneously distributed on the boundary as assumed in the model as presented here, and the boundary on which the target molecules are located may itself be complicated. By making the obvious changes to pose the problem on a two or three dimensional spatial domain and allowing B_0 to vary with the location along the boundary, the model can be extended to cover these possibilities as well.

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