

**Improved Rate
Constant
Estimates Using
the BIAcore**

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Standard Mass Action Law

The equation normally used to estimate rate constants using the BIAcore is the standard mass action law:

$$\frac{d\tilde{B}}{d\tilde{t}} = \tilde{k}_a C_T (R_T - \tilde{B}) - \tilde{k}_d \tilde{B}. \quad (1)$$

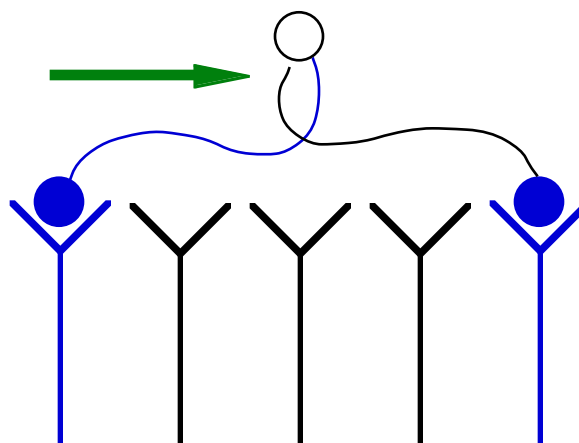
- \tilde{B} is the number of bound receptors, averaged along the surface of the channel, as measured by the BIAcore.
- C_T is the concentration of analyte *at the channel inlet*.
- R_T is the number of receptor sites, so $R_T - \tilde{B}$ is the average number of empty receptors.
- \tilde{k}_a is the *association rate constant*.
- \tilde{k}_d is the *dissociation rate constant*.
- \tilde{t} is time.

Key Assumption

- Equation (1) comes from standard chemical reaction theory, where the reactants are *well-mixed*.
- ✗ But the reactants *aren't* well mixed in the BIAcore; the receptor is attached to a wall!
- ✗ In addition, as reactant proceeds, there must necessarily be less analyte at the exit of the channel than at the entrance.
- ✓ We must consider transport effects.

Transport Effects

- In presence of imposed flow, analyte can bind to a *different* site downstream.



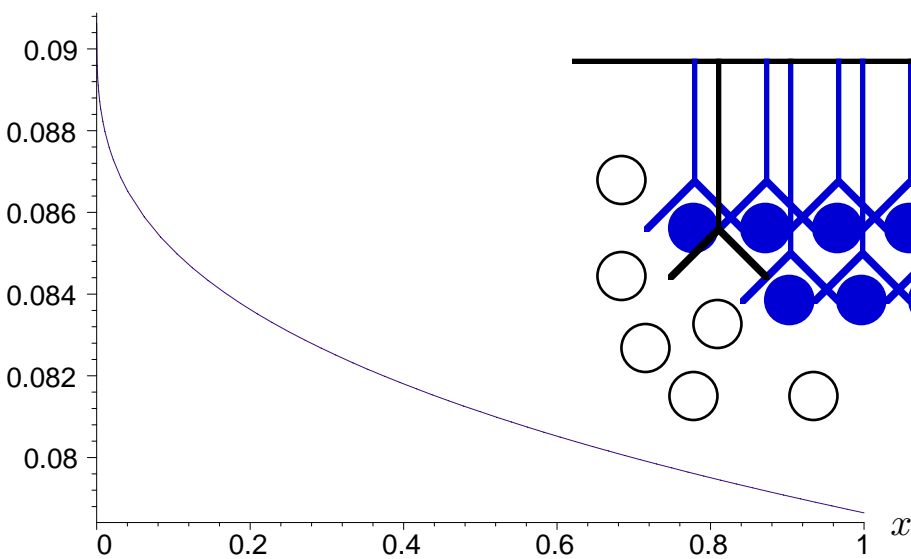
- ✗ reactants no longer well-mixed (more analyte at entry of channel)

- ✗ Therefore, the terms in red in (1) are *not* uniform in x (distance along the channel) at all.

- ✗ BIAcore measures only *averages* of B .

Typical Profile

bound state



bound state vs. x (distance along channel)*

* $Da=0.05$

When Can We Neglect Transport Effects?

- ✓ if the characteristic velocity V of the ligand (or equivalently, the flow rate) is *large enough* to sweep analyte to the end of the channel where it has been depleted
- ✓ if the diffusion coefficient \tilde{D} of the analyte is *large enough* that the analyte can diffuse quickly through the unstirred layer to the reacting surface
- ✓ if the association rate constant is *small enough* that the reaction does not significantly deplete the analyte at the entrance to the channel.
- ✓ if the number of receptor sites is *small enough* that the reaction does not significantly deplete the analyte at the entrance to the channel.
- ✗ However, to increase the reliability of the BIAcore readings, one usually *increases* R_T .
- What do the words in red mean? How do we measure “large enough”?

The Damköhler Number

We introduce the concept of the *Damköhler number* Da :

$$\text{Da} = \frac{\tilde{k}_a R_T L^{1/3} h^{1/3}}{V^{1/3} \tilde{D}^{2/3}} = \frac{\text{reaction rate}}{\text{diffusion rate in unstirred layer}}$$

- L is the length of the BIAcore channel.
- h is the height of the BIAcore channel.
- ✓ Note that if any of the phenomena described in red occurs, $\text{Da} \ll 1$.
- ✓ Therefore, if $\text{Da} \ll 1$, the reaction rate is much slower than the diffusion rate, so transport effects do not play a role.
- ✗ However, the larger Da is, the larger the effects of transport. In particular, if $\text{Da} \ll 1$, then the bound state evolves in a way quite different from that predicted by (1). (See Figure 1.)
- Da can range from 10^{-5} for a well-designed experiment to 10^5 for a poorly designed one.
- How did we determine that Da is the relevant parameter?

Including Transport to Obtain Da

- ✓ flow in most of channel does not affect reaction
- ✓ need to study transport only in unstirred L ev eque boundary layer
- The relevant *dimensionless* set of equations is listed below. For a more complete explanation of how they are derived, see appendix 1 (on yellow boards) or [1].

$$\eta \frac{\partial C}{\partial x} = \frac{\partial^2 C}{\partial \eta^2} \quad (2a)$$

$$\frac{\partial C}{\partial \eta}(x, 0, t) = Da \frac{\partial B}{\partial t} \quad (2b)$$

$$\frac{\partial B}{\partial t} = (1 - B)C(x, 0, t) - KB \quad (2c)$$

- ✓ All the quantities in red in (1) are no longer assumed to be uniform in x , but are now allowed to depend on x , and hence are listed in green.
- B is the fraction of receptor sites that are bound, so $1 - B$ is the fraction of empty receptors.
- C is the ratio of analyte at a point to the analyte at the channel inlet.
- K is the dimensionless affinity constant.
- t is time measured on the reaction time scale.
- η is a stretched variable near the reacting surface.
- Equation (2a) describes the transport of analyte in the unstirred layer. Note that the velocity is now described by a linear flow, and that the transport equation for C is in steady state.
- Equation (2b) describes the transport of analyte to the reacting surface.
- Equation (2c) describes the reaction at the surface.

Advantages and Disadvantages of (2)

- ✗ reaction assumed to occur at the surface $\eta = 0$. (Further research: include effects of dextran layer.)
- ✓ Note that if $\text{Da} = 0$, then there is practically no change to the analyte concentration from the reaction. Then C is indeed uniform, and (1) is the correct equation.
- ✗ But this never occurs! What to do if $\text{Da} \neq 0$?

Solving Equation (2)

- Using transform techniques [1], one may solve equations (2) for B :

$$\frac{\partial B}{\partial t} = [1 - \text{Da}C_1(x)](1 - B) - KB, \quad (3a)$$

$$C_1(x) = \frac{1}{3^{1/3}\Gamma(2/3)} \int_0^x \frac{\partial B}{\partial t}(x - \xi, t) \frac{d\xi}{\xi^{2/3}} \quad (3b)$$

- ✓ Note that if $\text{Da} = 0$, then (3a) (written in dimensional form) becomes (1), since there are no transport effects.
- $\text{Da}C_1(x)$ is the deviation of C from its inlet value 1
- $C_1(x)$ incorporates the nonuniformity of B into its evolution equation.
- ✗ Equation yields form for B only; we must still perform averaging to obtain \bar{B} , the average over the scanning range which the BIAcore returns.
- ✗ Equation now too complicated for general-purpose software, though equation may be solved numerically [2] (see Figure 1).
- If we design our experiment such that Da is small, can we simplify things?

Making Da Small

To force $\text{Da} \ll 1$, we set

$$V \gg \frac{\tilde{k}_a^3 R_T^3 hL}{\tilde{D}^2}.$$

- ✗ bound depends on the unknown \tilde{k}_a
- ✓ should be able to obtain useful order-of-magnitude estimates for \tilde{k}_a from other sources

Then since transport effects are small, we should have that

$$\bar{B} = B_0 + \text{Da}\bar{B}_1 + \dots, \quad (4)$$

where B_0 is the dimensionless form of the solution to (1), which is uniform in space. In other words, the true average of the bound state should be very near the result from equation (1).

Effective Rate Constants

- new averaged equation (for derivation, see Appendix 2 [on yellow boards] or [3], [4])

$$\frac{d\bar{B}}{dt} = [(1 - \bar{B}) - K\bar{B}](1 - p), \quad p = \frac{\text{Da}(1 - \bar{B})\bar{F}}{1 + \text{Da}(1 - \bar{B})\bar{F}}, \quad (5a)$$

or, in dimensional form,

$$\frac{d\tilde{B}}{d\tilde{t}} = [\tilde{k}_a C_T (R_T - \tilde{B}) - \tilde{k}_d \tilde{B}] (1 - p), \quad p = \frac{\text{Da}(R_T - \tilde{B})\bar{F}}{R_T + \text{Da}(R_T - \tilde{B})\bar{F}}. \quad (5b)$$

- p represents the probability that a dissociated analyte molecule will rebind further down the channel.
- \bar{F} is a constant that depends only on flow and geometry, so (5) holds for a wide array of systems [4].

Advantages of (5)

- ✓ absence of transport ($Da = 0$) yields $p = 0$ and hence equation (1)
- ✓ as transport effects increase ($Da \rightarrow \infty$), $p \rightarrow 1$ (maximum probability of rebinding)
- ✓ utilizes averaged data provided by the BIAcore
- ✗ using standard model (1) underestimates rate constant by $1 - p$
- ✓ Nonlinear curve fitting is now more difficult (since Da has \tilde{k}_a in it), but still within easy reach of most programs. Easier than solving (3).
- ✓ Figure 1 shows the results from (3) and (5) lying right on top of one another. Figure 2 shows the error *vs.* time.
- ✓ Error is proportional to Da^2 as $Da \rightarrow 0$.

Moderate Da

- ✗ The *formal* derivation of (5) depends on Da being small.
 - Is the small error in Figures 1 and 2 a fluke?
 - How far wrong is (5) when Da is not small? Answer: not much!
 - Figure 3 shows the largest error between the solutions of (3) and (5) during a typical association experiment.
- ✓ Note that even for large Da , the error is quite small.
- ✓ Error for small Da is proportional to Da^2 , as predicted.
- ✓ Error for large Da asymptotes to a constant as $p \rightarrow 1$. (Transport effects saturate as $Da \rightarrow \infty$.)

Conclusions

- ✗ The standard model (1) does not include the effects of transport in the BIAcore.
- ✗ Therefore, it underestimates rate constant by $1 - p$, where p is the probability of rebinding.
- Including the effects of transport leads to equation (3), which can be solved numerically [2].
- ✗ However, equation (3) is much more difficult to solve.
- ✓ Error between (3) and (5) is $O(\text{Da}^2)$ as $\text{Da} \rightarrow 0$, and small even when Da is moderate or large.
- ✓ Therefore, equation (5) should replace (1) in order to obtain accurate estimates for a wide range of Da .

References

- [1] Edwards, D. A. “Estimating Rate Constants in a Convection-Diffusion System with a Boundary Reaction.” *IMA Journal on Applied Mathematics*, **63** (1999): 89112.
- [2] Edwards, D. A., and Jackson, S. A. “A Nonlinear Integral Equation Arising in Biochemical Systems,” in production.
- [3] Edwards, D. A., Goldstein, B., and Cohen, D. S. “Transport Effects on Surface-Volume Biological Reactions.” *Journal of Mathematical Biology*, (1999): in press.
- [4] Edwards, D. A. “Biochemical Reactions on Helical Structures.” *SIAM Journal on Applied Mathematics*, in press.

Figure 1. **B** (averaged) vs. **t**, **Da**=0.45, **K**=1

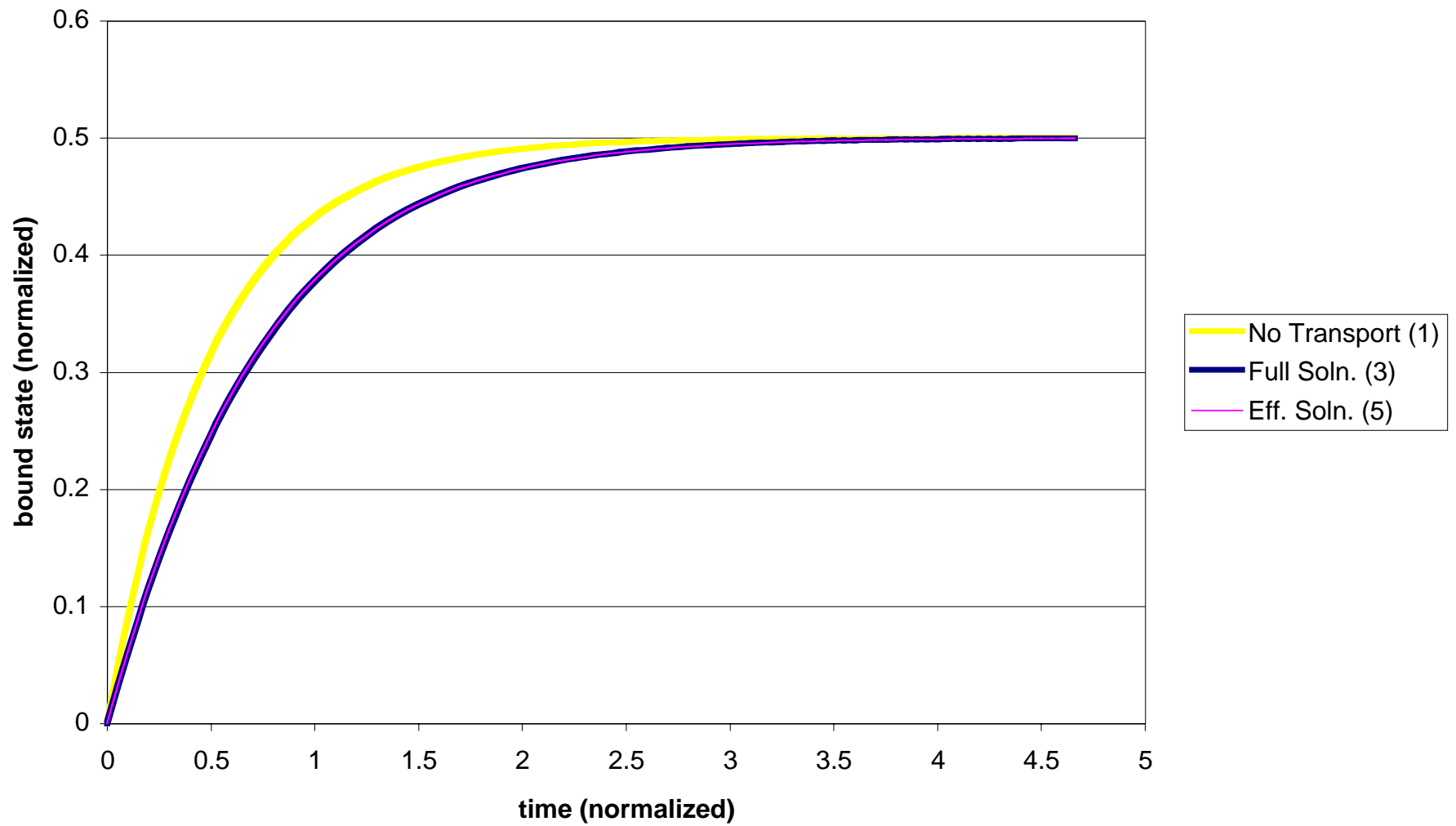


Figure 2. Error between Full and Effective Solutions

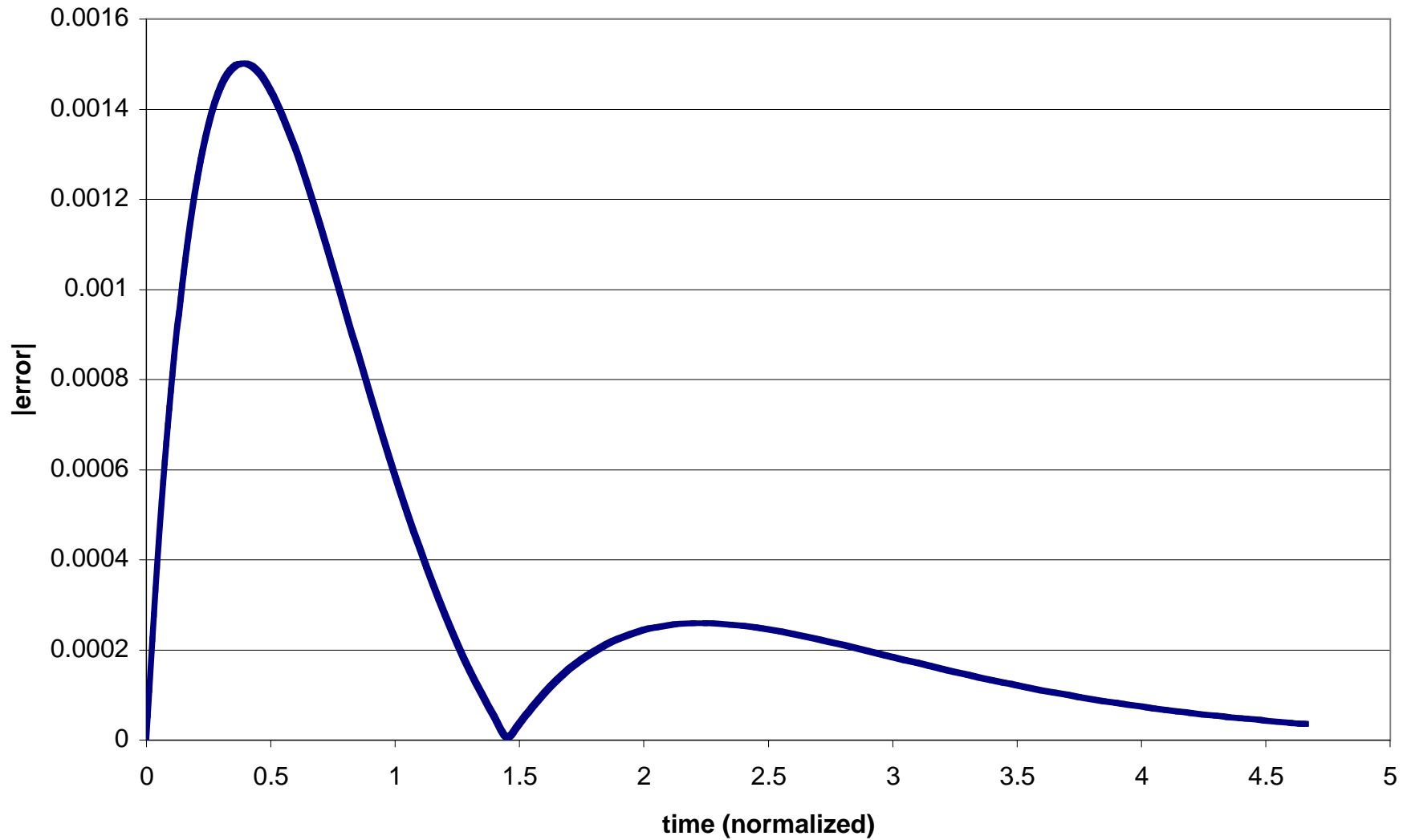
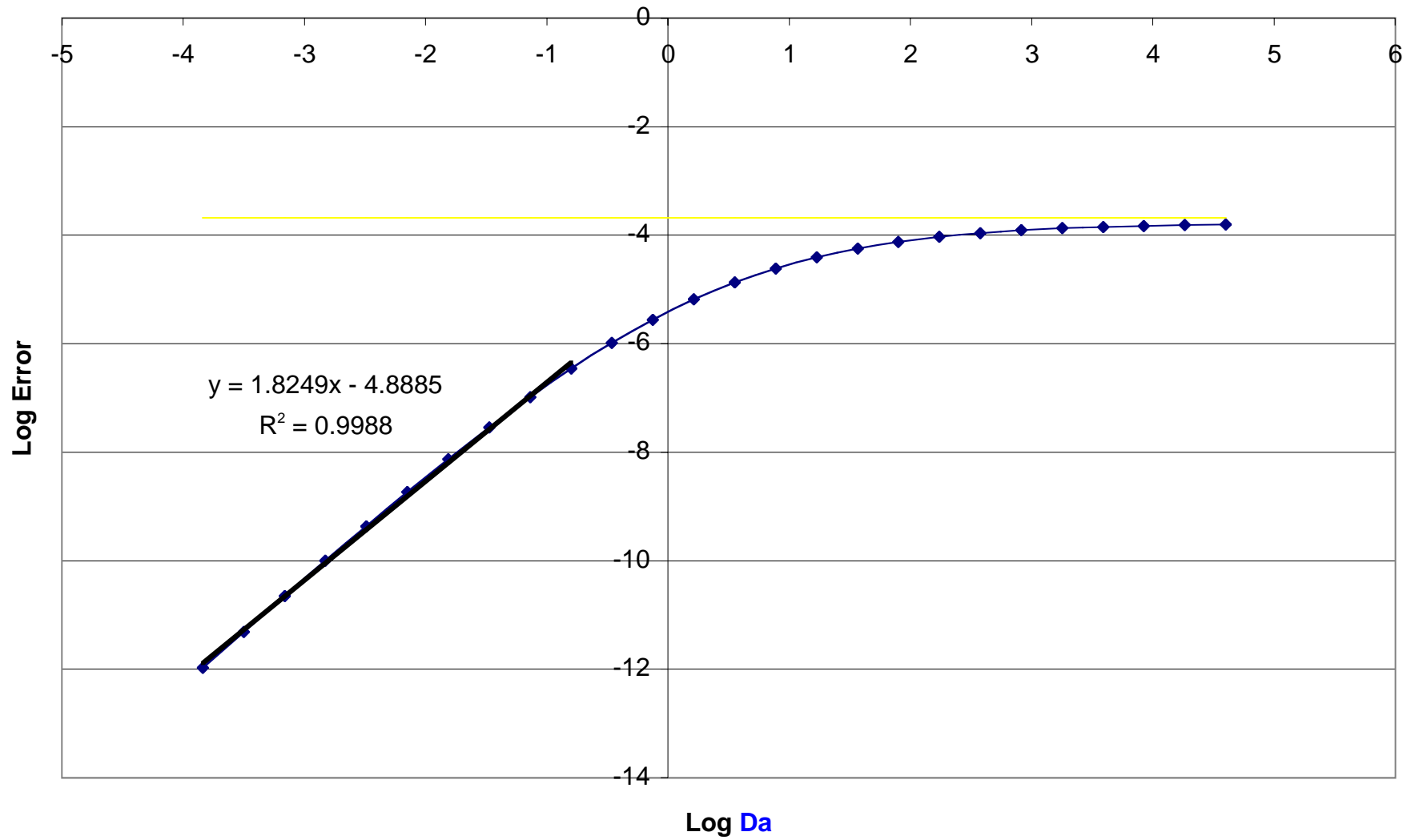


Figure 3. Log Maximum Error vs. Log Da



Appendix 1: Deriving Equations (2)

- consider the channel to be two-dimensional
- Reynolds number is small, so use standard Poiseuille flow
- full dimensional equations

$$\frac{\partial \tilde{C}}{\partial \tilde{t}} = \tilde{D} \left(\frac{\partial^2 \tilde{C}}{\partial \tilde{x}^2} + \frac{\partial^2 \tilde{C}}{\partial \tilde{y}^2} \right) - V \frac{\tilde{y}}{h} \left(1 - \frac{\tilde{y}}{h} \right) \frac{\partial \tilde{C}}{\partial \tilde{x}}, \quad (\text{A.1})$$

$$\tilde{D} \frac{\partial \tilde{C}}{\partial \tilde{y}}(\tilde{x}, 0, \tilde{t}) = \frac{\partial \tilde{B}}{\partial \tilde{t}}, \quad (\text{A.2})$$

$$\frac{\partial \tilde{B}}{\partial \tilde{t}} = \tilde{k}_{\text{on}}(R_T - \tilde{B})\tilde{C}(\tilde{x}, 0, \tilde{t}) - \tilde{k}_{\text{off}}\tilde{B}. \quad (\text{A.3})$$

- h is the height of the channel

Convective Scaling

- normalizing by standard length scales and *convective* time scale, (A.1) becomes

$$\frac{\partial C}{\partial t_c} = \text{Pe}^{-1} \left(\epsilon^2 \frac{\partial^2 C}{\partial x^2} + \frac{\partial^2 C}{\partial y^2} \right) - y(1-y) \frac{\partial C}{\partial x}.$$

- ϵ is the aspect ratio of the channel
- Pe is the Peclet number
- ✗ $\text{Pe} \gg 1$, so diffusion is neglected in this scaling (need a slower time scale)

Diffusive Scaling

- Diffusion is important only in a thin unstirred layer (width $\text{Pe}^{-1/3}$) near the reacting surface. Using this length scale and the *diffusion* time scale, (A.2) becomes

$$\frac{\partial B_d}{\partial t_d} = D_d \frac{\partial C_d}{\partial \eta}(x, 0, t_d), \quad D_d = \frac{C_T h / R_T}{\text{Pe}^{1/3}}.$$

- ✗ $D_d \ll 1$, so reaction is neglected in this scaling (*still* need a slower time scale)

Reaction Scaling

- ✓ using forward reaction time scale yields equations (2)

Appendix 2: Deriving Equations (5)

- both B and C are nearly (within $O(\text{Da})$) of their values in (1)
- change to C is driven by the leading-order reaction, so

$$C_1(x) = \frac{dB_0}{dt}F(x) \quad \implies \quad C = 1 - \text{Da} \frac{dB_0}{dt}F(x). \quad (\text{A.4})$$

Substituting (4) and (A.4) into (3a), we obtain

$$\frac{\partial B}{\partial t} = (1 - B) - \text{Da}(1 - B_0) \frac{dB_0}{dt}F(x) - KB + \dots$$

Averaging the above, we obtain

$$\frac{d\bar{B}}{dt} [1 + \text{Da}(1 - \bar{B})\bar{F}] = (1 - \bar{B}) - K\bar{B} + \dots$$

$$\frac{d\bar{B}}{dt} = \frac{(1 - \bar{B}) - K\bar{B}}{1 + \text{Da}(1 - \bar{B})\bar{F}} + \dots$$

- error is $O(\text{Da}^2)$
- ✗ Same trick does not work at next order because next-order correction to B is no longer uniform in x .

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