9/22

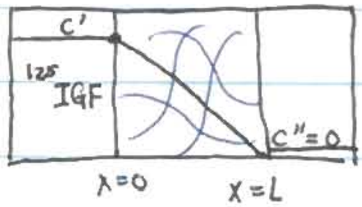
Today: (I) Summarize $\boxed{\text{Separation of Variables Soln}}$

binding site.

(II) Include Binding: Ex: 1st order, Reversible, Biomolecular R in Equilibrium

(III) Re-Solve Full Transient: Diffusion – Reaction

$$\frac{\partial c}{\partial t} = D_{IGF}\frac{\partial^2 c}{\partial x^2} + \cancel{R}^{\,0}$$



Find $c(x,t)$: $c(x,t=0)=0$ I.C.

$c(x=0,t)=c'$, $c(x=L,t)=c''=0$ } B.C.

$$c(x,t) = \hat{c}(x,t) + \underbrace{c'\left(1-\frac{x}{L}\right)}_{\text{St. State}}$$

Find $\hat{c}(x,t)$: $\hat{c}(x,t=0) = -c'\left(1-\frac{x}{L}\right)$

$\hat{c}(x=0,t)=0$, $\hat{c}(x=L,t)=0$ } B.C. (homogeneous)

$$\boxed{\frac{\partial \hat{c}(x,t)}{\partial t} = D\frac{\partial^2 \hat{c}}{\partial x^2}} \; : \text{Try } \hat{c}(x,t) = X(x)T(t)$$

$$\frac{1}{DT(t)}\cdot\frac{\partial T(t)}{\partial t} = \frac{\partial^2 X}{\partial x^2}\cdot\frac{1}{X} = -k^2 \; ; \; T(t) = Ae^{-k^2Dt}$$

$$X(x) = \cancel{C_1}^{\,0}\cos kx + C_2 \sin kx, \quad k = \left(\frac{n\pi}{L}\right)$$

$$\hat{c}(x,t) = \sum_{n=1}^{\infty} A_n' \sin\left(\frac{n\pi x}{L}\right)e^{-t/\tau_n}$$

Finally: Find A_n

to satisfy I.C. $\left(\sum_{n=1}^{\infty} A_n \sin\frac{n\pi x}{L}\right) = -c'\left(1-\frac{x}{L}\right)$

⇒ Fourier

$$\int_0^L\left(\sum_{n=1}^{\infty} A_n' \sin\frac{n\pi x}{L}\right)\sin\frac{m\pi x}{L}\,dx = \int_0^L -c'\left(1-\frac{x}{L}\right)\sin\frac{m\pi x}{L}\,dx \quad (= n=1)$$

Find A_1: $(n=1)$

... $\int$ term by term $\quad c(x,t) = c'\left(1-\frac{x}{L}\right) - \sum_{n=1}^{\infty} A_n \sin\frac{n\pi x}{L} e^{-t/\tau_n}$

$\tau_n = \frac{L^2}{n^2\pi^2 D}$; slowest decay time: $n=1$, $\boxed{\tau_{diff} = \frac{L^2}{\pi^2 D}}$

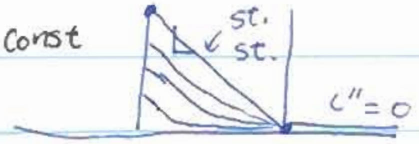
from boundary conditions.

Note #1

$\frac{1}{\tau}\frac{\partial c}{\partial t} = D_{IGF}\frac{\partial^2 c}{\partial x^2}\frac{1}{L^2}$ $\quad$ "char. time": $\tau = \frac{L^2}{D}$

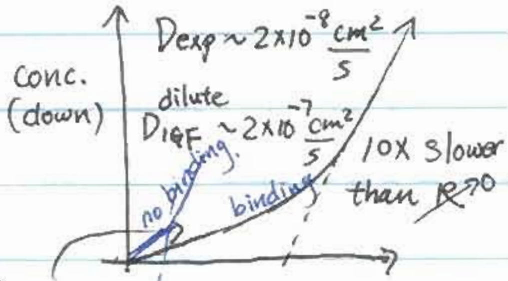
$$\boxed{\frac{1}{\tau} = \frac{D}{L^2}}$$

Note ② $N = \text{const}$



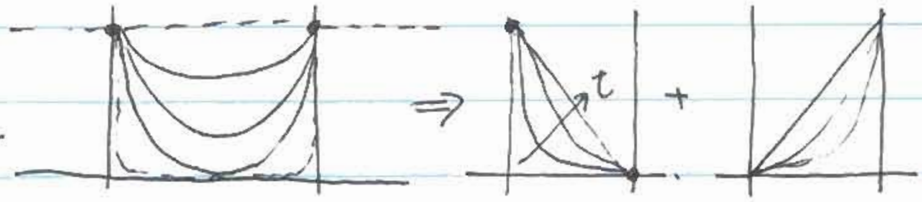
$\tau_{diff} = \frac{4L^2}{\pi^2 D}$ (slower)

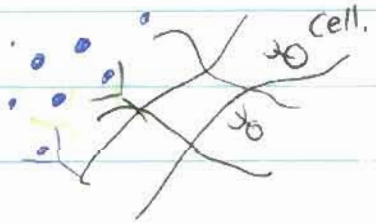
Add Equil. Binding



finding D $\quad$ ~~tim~~ lag time

"τ_{lag}" $= \frac{L^2}{6D}$





$\boxed{\text{Equil. binding}}$

$C_F = [IGF]_{free}$

$C_B = [IGF]_{bound}$

n = conc. of binding sites $n = 50\,nM$

(IGFBP6)

$$IGF\text{-}site \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} IGF + site$$

$$n = \underbrace{[IGF\text{-}site]}_{C_B} + [site]$$

bound rate eqn: $\frac{\partial C_B}{\partial t} = k_{on} C_F (n - C_B) - k_{off} C_B = -R$.

known (k_{on})

Equil: $= 0 \Rightarrow \frac{k_{off}}{k_{on}} = \frac{C_F(n-C_B)}{C_B}$

$\equiv K_{diss}$

known.

$C_F n - C_F C_B = K_{diss}\, C_B$

$\rightarrow C_B (K_{diss} + C_F) = n C_F$

$$C_B = \frac{n_1 C_F}{K_{diss} + C_F}$$ "Langmuir Isotherm"

① $$\frac{\partial C_F}{\partial t} + \frac{\partial C_B}{\partial t} = D_{IGF} \frac{\partial^2 C_F}{\partial x^2} \cancel{+ R}$$

↑free IGF ↑bound IGF diffusion

Suppose: reaction is very fast!!!

② rate: $$\frac{\partial C_B}{\partial t} = k_{on}\, C_F (n - C_B) - k_{off} C_B \simeq 0$$

Then ⇒ $$\boxed{\frac{\partial C_F}{\partial t} + \frac{\partial}{\partial t}\left(\frac{n C_F}{K_{diss} + C_F}\right) = D_{IGF} \frac{\partial^2 C_F}{\partial x^2}}$$

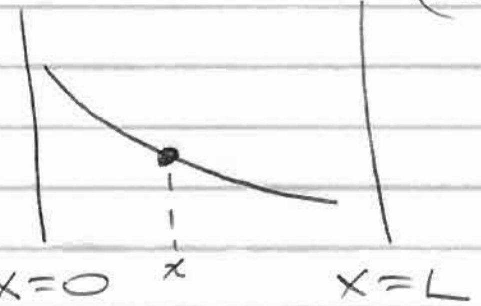
$$\frac{\partial}{\partial t} C_F = D_{eff} \frac{\partial^2 C_F}{\partial x^2}$$

For $T_{diff} \sim \frac{L^2}{\pi^2 D_{diff/reac.}} \gg T_{reac.} \sim \frac{1}{k_{diff.}}$ $\sim 50s.$

$D_{eff} = \frac{D_{IGF}}{}$

BE.430 Lect. #5 (9/22/04): <u>Last "Blackboard"</u>

(AJG)



(1) $\dfrac{\partial c_F}{\partial t} + \dfrac{\partial c_B}{\partial t} = D_{IGF}\dfrac{\partial^2 c_F}{\partial x^2}$

(2) $\dfrac{\partial c_B}{\partial t} = k_{on}(n - C_B) - k_{off}C_B$

We assume that reaction is much faster than the overall diffusion process $\Rightarrow$ $\therefore$ at each position x, Eq.(2) is in "quasi-equilibrium"

$\Rightarrow \left[k_{on}(n - C_B) \simeq k_{off}C_B\right] \Rightarrow \boxed{C_B = \left(\dfrac{nC_F}{K_d + C_F}\right)}$ (3) (Langmuir Isotherm)

Thus, while $C_B(x,t)$ & $C_F(x,t)$ change slowly as the diffusion process evolves, C_B is always related to C_F by Eq.(3) at every x and t (relatively "instantaneous" reaction)

$\Rightarrow$ (1) becomes $\dfrac{\partial c_F}{\partial t} + \dfrac{\partial}{\partial t}\left(\dfrac{nC_F}{K_d + C_F}\right) = D\dfrac{\partial^2 c_F}{\partial x^2}$ (4)

By chain rule: $\dfrac{\partial}{\partial t}\left(\dfrac{nC_F}{K_d + C_F}\right) = \dfrac{\partial}{\partial t}\text{"}(*)\text{"} = \left(\dfrac{\partial *}{\partial C_F}\right)\left(\dfrac{\partial C_F}{\partial t}\right) = \left(\dfrac{nK_d}{[K_d + C_F]^2}\right)\dfrac{\partial C_F}{\partial t}$

$\therefore$ (4) $\Rightarrow \boxed{\dfrac{\partial c_F}{\partial t}\left(1 + \dfrac{nK_d}{(K_d + C_F)^2}\right) = D_{IGF}\dfrac{\partial^2 c_F}{\partial x^2}}$ (5)

- This is still nonlinear $\Rightarrow$ computer in general.
- <u>BUT</u>, in our example, the inital addition of radiolabeled ^{125}I-IGF to the upstream bath involves addition of a tiny amount of IGF (→ next page)

(9/22/04)

For our system: $K_d \sim 5$ nM (known)
$n \sim 50$ nM (known)

→ initial addition involves $[{}^{125}IGF] < 0.5$ nM

∴ We can linearize (5) for case $C_F \ll K_d$

$$\Rightarrow \frac{\partial c_F}{\partial t}\left(1 + \frac{n}{K_d}\right) = D_{IGF}\frac{\partial^2 c_F}{\partial x^2}$$

$$\boxed{\frac{\partial c_F}{\partial t} = D_{eff}\frac{\partial^2 c_F}{\partial x^2}} \quad (6)$$

where $D_{eff} = \dfrac{D_{IGF}}{\left(1 + \frac{n}{K_d}\right)} \simeq \left(\dfrac{D_{IGF}}{11}\right)$

So $D_{eff} \ll D_{IGF}$ ⇒ diffusion-limited reaction greatly slows overall diffusive transport.

But initial addition still characterized by the form of a "diffusion equation"
→ Non linear in general.

⇒ CAUTION 🙂: Just because the solution to a diffusion equation of the form (6) can be fit to DATA does not mean that binding is absent!