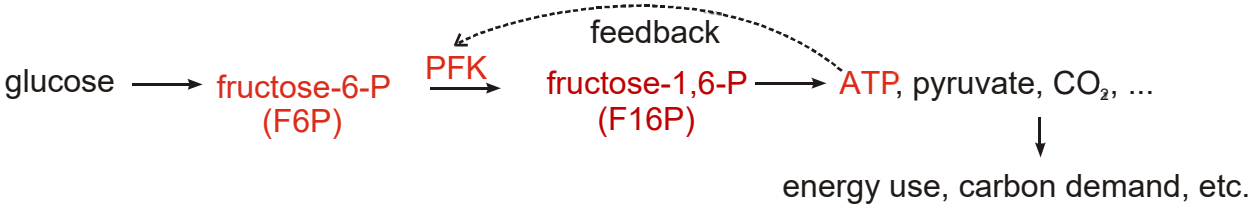
Quantitative Physiology I / Molecular and Cellular Systems; BMEN E4001x
HW2: Kinetics and Diffusion
Due Oct 08, 2025, 11:00PM (US Eastern Time)

1) Enzyme inhibition (10 points)

A key point of regulation of the glycolytic reaction is in conversion of fructose-6-P to fructose-1,6-P by the enzyme phosphofructokinase (PFK). ATP is a downstream product of this reaction, but also acts to inhibit PFK, slowing down generation of ATP in a negative feedback loop. We explore here this feedback loop.

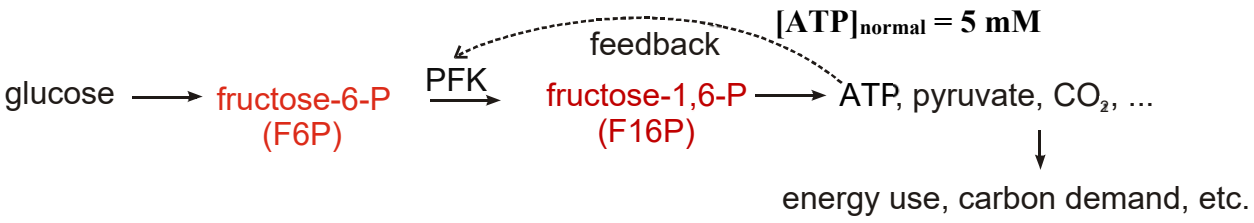


Assume:

- This question only focuses on an inhibitory site on PFK for ATP. While there is an ATP-binding catalytic site that is essential for this reaction to take place, ignore this interaction here. The components that are important to this question are marked in red.
- ATP acts as an allosteric, uncompetitive inhibitor of PFK.
  (note: the actual type of inhibition is a bit different, and the assumption of uncompetitive inhibition is used here to explore the models discussed in class)
- $\mathbf{V_{max}}$ for the reaction (generating F16P from F6P at saturating amounts of substrate and in the absence of inhibitor) is **5 μM/s**. Assume PFK is present at a concentration of 1 μM.
- The standard concentrations of F6P and ATP prior to perturbation of the system are:
  $\mathbf{[F6P]_{normal} = 0.2\ mM;}$ $\mathbf{[ATP]_{normal} = 5\ mM}$
- Binding constants of F6P and ATP to PFK are:
  $\mathbf{K_{M,F6P} = 0.2\ mM;}$ $\mathbf{K_{I,ATP} = 5\ mM}$
- The concentration of ATP inside the cell is going to be a balance between ATP production and downstream usage. Following a perturbation, the concentration of ATP will approach some steady-state value that is dependent on the other parameters of the system. This could be a very complicated equation.
  As a simplifying assumption here, the steady-state concentration of ATP is proportional to the reaction rate. That is:
  $\mathbf{[ATP]_{steady\ state} = (3000*seconds) * reaction\ rate.}$

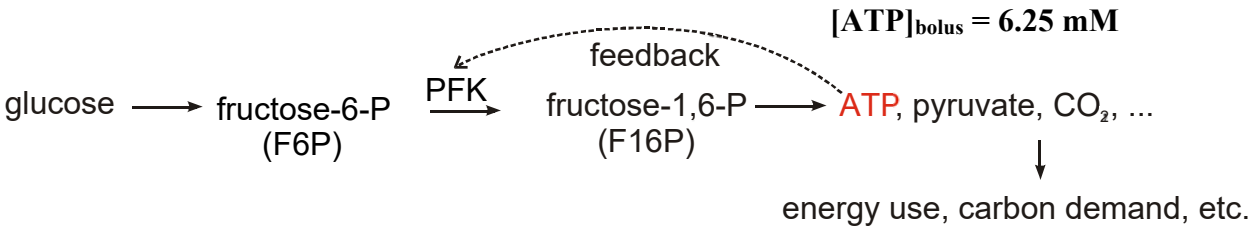
Explore the ATP-PFK feedback loop:

1.1)



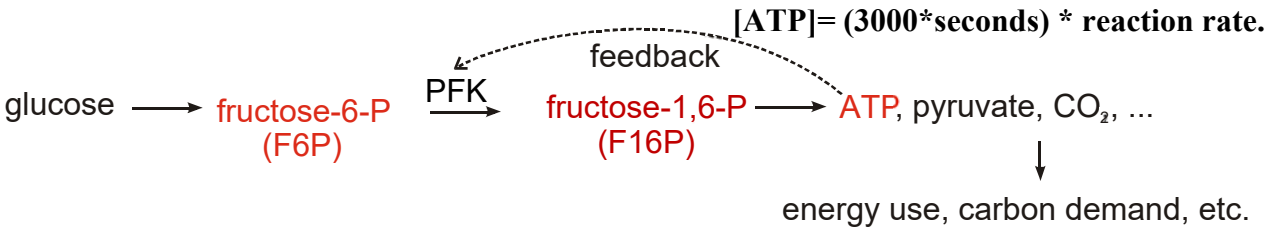
Unregulated reaction. Look at how the system responds to a change in F6P level, separate from feedback. If the concentration of F6P suddenly increases by 25% to 0.25 mM, what is the initial change (in percent) of reaction rate, before the concentration of ATP has time to adjust? That is, assume the concentration of ATP remains at 5 mM.

1.2)



Feedback mechanism. As a separate perturbation, assume a bolus of ATP is suddenly introduced into the cell, instantaneously raising the level of ATP by 25% to 6.25 mM. Compared to the standard conditions (F6P at 0.20 mM, ATP at 5 mM), what is the change (in percent) of reaction rate?

1.3)



Regulated reaction. Now, put the two processes together. Starting with the perturbed system of part 1.1 (in which F6P increases to 0.25 mM), allow the increase in ATP production and resultant increase in steady state ATP to now provide negative feedback. Compare the steady-state reaction rate of this feedback system with the unregulated reaction rate of part 1.1; how much does feedback offset the effects of an increase in F6P? *Note: Feel free to use a numerical approach, such as a graphic calculator, MATLAB, or the Solver tool in Excel to find your solution.*

For your reflection (not graded):

- Can this feedback mechanism completely offset the effect of increased [F6P] on [ATP]?
- How is this system similar or different than other feedback system you have studied, such as operational amplifiers?

Solution

The core equation for this problem is the uncompetitive inhibition relation:

$$V(F6P, ATP) = (5\mu M/s)\frac{1}{1 + [ATP]/K_i}\frac{[F6P]}{\left(\frac{K_M}{1 + [ATP]/K_i}\right) + [F6P]}$$

This is going to be explored in the context of a negative feedback loop, looking at using an abundance of ATP to lessen the impact of a perturbation in the reaction. As such, the goal is to compare reaction rate and ATP levels under the influence of perturbations and feedback against a standard condition.

Standard Conditions, for [ATP]=5 mM, [F6P]=0.2 mM, the reaction rate 01.667 μM/s

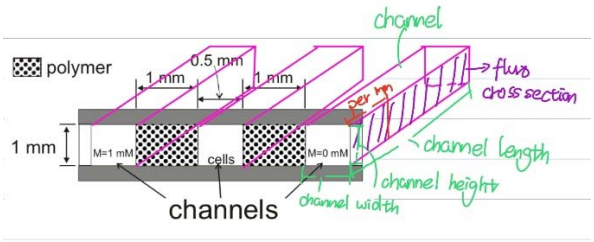
1.1) Reaction rate after increasing F6P by 25% = 1.786 μM/s. This is a 7.1 % increase compared to standard conditions. Note that the percent change in rate is less than the percent change in substrate concentration. This is because the substrate concentration is similar to $K_{m,F6p}$. This increase in reaction rate would cause ATP concentration to rise. The effect of this rise will be examined in the following sections.

1.2) Raising ATP to 6.25 mM lowers the reaction rate to 1.538 μM/s, a 7.7 % decrease compared to the standard conditions. This demonstrates the basic mechanism that increasing ATP decreases ATP production. Conceptually, the decrease in reaction rate (which is ATP production) will cause a decrease in ATP concentration in the cell over time.

1.3) Following an increase in F6P, ATP production will rise, increasing the concentration in the cell. This increase offsets the effect of rising F6P concentration to some degree. Importantly, downstream ATP use must change to balance changes in production; otherwise, the concentration of ATP would continue to change. So, allow the downstream demand for ATP change as a function of ATP concentration. This can be a very complex relationship. For the purposes of this problem, assume that downstream use is proportional to the concentration of ATP. Since downstream use is equal to reaction rate at steady state, we get the stated simplification of [ATP]= (3000*seconds) * reaction rate (watch concentration units).
Solve this linear relationship and the overall rate equation simultaneously, [ATP]=5.260 μM, so reaction rate = 1.753 μM/s, a 5.18 % increase as compared to standard conditions, only 73 % of the increase seen in part 1.1.

2) Diffusion-based transport (10 points)

Cells are able to detect chemical gradients. One system for studying this effect consists of three parallel, microscale channels, separated by regions of a polymer material. The cross section of this system, looking along the channels, is shown above.



added annotations by Y. Wang

The two outer channels are connected to external pumps that continually replenish these regions, thus maintaining the concentration of a bioactive chemical in these channels. Cells are placed on the bottom of the center channel. The two polymer blocks serve to stop the bulk flow of fluid between the channels. For this problem, assume:

- The concentration of the compound being studied, denoted as M, is held at 1 mM in the left channel and at 0 mM in the right channel, as indicated in the figure above.
- The three channels are filled with media that can be treated as water.
- The diffusion coefficient of M in the middle channel, D_m, is 0.5 μm^2/msec. The partition coefficient of M in this region (media) is 1.0.
- The diffusion coefficient of M in the polymer regions, D_p, is 0.4 μm^2/msec. The partition coefficient of M in the polymer regions is 0.8.
- The two polymer regions and the middle channel are considered non-stirred.
- Diffusion from left to right can be considered a 1-D, Cartesian problem. Consider the left edge of the left-most region of polymer as the origin, x = 0.

A) For this system, determine:

2.1 The concentration of M as a function of position, x, across the microfluidic channel. This can be a graph or a set of relations covering the range of x. Remember to take partition coefficient of each region into consideration.

2.2 The change in concentration per distance in the center channel. That is, what is the concentration gradient that the cells see?

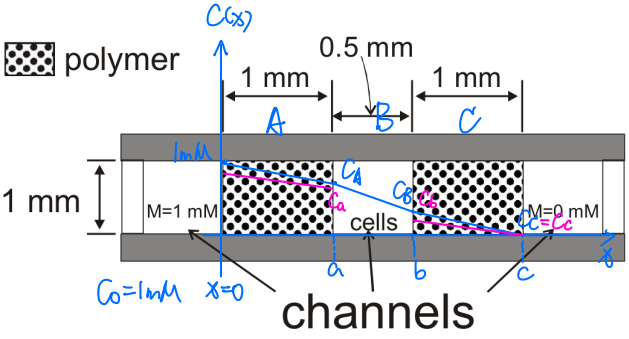
2.3 The flux of M across this system (left to right) per mm of channel length (measured perpendicular to the cross section, into the page).

2.4 The time it takes for a molecule of M to diffuse across the middle channel. Use $\sqrt{\langle x(t)^2 \rangle}$ as a measure of how far the average particle diffuses in time t.

B) You observe cell migration in response to this gradient, but want to carry this experiment out again with a gradient that is twice as steep as before. The concentration of M at the left side of the middle channel should be the same as in section A of this question. Using a new 3D printing system, you can adjust the widths of the two polymer regions (1 mm each in part A).

2.5 What should the widths of these regions be to meet the new design criteria? The left and right polymer regions could even be different widths.

For your own consideration: graph the concentration of M as a function of position for this modified system.



A) 2.1

$$J=\frac{1}{R_A+R_B+R_C}\Delta C=\frac{1}{R_T}\Delta C,\quad R_T=R_A+R_B+R_C$$

$$R=\frac{L}{AD\beta},\quad \beta_A=\beta_C=\beta_p=0.8,\quad \beta_B=\beta_m=1.0,$$

$$L_A=L_C=1mm,\quad L_B=0.5mm,$$

$$D_A=D_C=D_p=0.4\mu m^2/msec,\quad D_B=D_m=0.5\mu m^2/msec$$

$$R_A:R_B:R_C=\frac{L_A}{AD_A\beta_A}:\frac{L_B}{AD_B\beta_B}:\frac{L_C}{AD_C\beta_C}=\frac{L_A}{D_A\beta_A}:\frac{L_B}{D_B\beta_B}:\frac{L_C}{D_C\beta_C}$$

$$=\frac{1mm}{0.4\mu m^2/msec\cdot 0.8}:\frac{0.5mm}{0.5\mu m^2/msec\cdot 1.0}:\frac{1mm}{0.4\mu m^2/msec\cdot 0.8}$$

$$=25:8:25$$

$$\Delta C_A:\Delta C_B:\Delta C_C=25:8:25,\quad \Delta C_A+\Delta C_B+\Delta C_C=\Delta C=1mM-0mM=1mM,$$

$$\Rightarrow \Delta C_A=\frac{25}{58}mM,\quad \Delta C_B=\frac{4}{29}mM,\quad \Delta C_C=\frac{25}{58}mM$$

$$C_A=1mM-\frac{25}{58}mM=\frac{33}{58}mM,\quad C_B=\frac{33}{58}mM-\frac{4}{29}mM=\frac{25}{58}mM,\quad C_C=0mM$$

[$\Delta C_a=\beta_A\Delta C_A=0.8\cdot\frac{25}{58}mM=\frac{10}{29}mM$, $\Delta C_b=\beta_B\Delta C_B=1.0\cdot\frac{4}{29}mM=\frac{4}{29}mM$, $\Delta C_c=\beta_C\cdot\Delta C_C=0.8\cdot\frac{25}{58}mM=\frac{10}{29}mM$

$C_{0,A}=\beta_A\cdot C_0=0.8\cdot 1mM=0.8mM=\frac{4}{5}mM$, $C_a=C_{0,A}-\Delta C_a=\frac{4}{5}mM-\frac{10}{29}mM=\frac{66}{145}mM$

$C_b=\beta_B\cdot C_B=0.8\cdot\frac{25}{58}mM=\frac{10}{29}mM$, $C_c=C_C=0mM$]

x is measured in mm,

when $x<0$ mm, $C(x)=1mM$;

when $0mm<x<1mm$, $C(x)=\beta_A(C_0-\frac{\Delta C_A}{L_A}x)=0.8(1mM-\frac{\frac{25}{58}mM}{1mm}x)=0.8(-\frac{25}{58}x+1)=-\frac{10}{29}x+\frac{4}{5}$ (mM);

[$=C_{0,A}-\frac{\Delta C_a}{L_A}x=\frac{4}{5}mM-\frac{\frac{10}{29}mM}{1mm}x=-\frac{10}{29}x+\frac{4}{5}$ (mM);]

when $1mm<x<1.5mm$, $C(x)=C_A-\frac{\Delta C_B}{L_B}(x-L_A)=\frac{33}{58}mM-\frac{\frac{4}{29}mM}{0.5mm}(x-1mm)=-\frac{8}{29}x+\frac{49}{58}$ (mM);

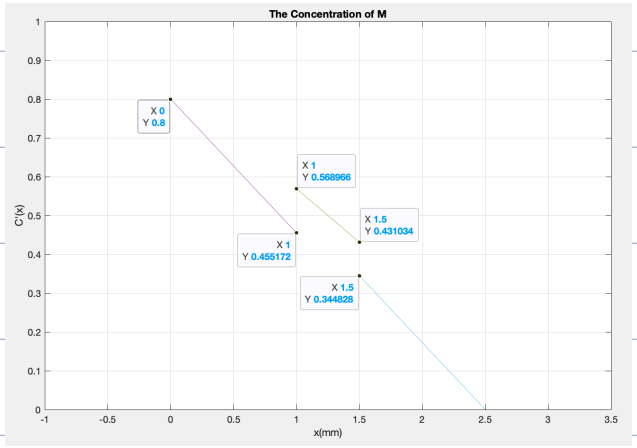
when $1.5mm<x<2.5mm$, $C(x)=\beta_C[C_B-\frac{\Delta C_C}{L_C}(x-L_A-L_B)]=0.8[\frac{25}{58}mM-\frac{\frac{25}{58}mM}{1mm}(x-1mm-0.5mm)]$

$=0.8(-\frac{25}{58}x+\frac{125}{116})=-\frac{10}{29}x+\frac{25}{29}$ (mM);

[$=C_b-\frac{\Delta C_C}{L_C}(x-L_A-L_B)=\frac{10}{29}mM-\frac{\frac{10}{29}mM}{1mm}(x-1mm-0.5mm)=-\frac{10}{29}x+\frac{25}{29}$ (mM);]

when $x\geq 2.5mm$, $C(x)=0mM$

$$C(x) = \begin{cases} 1, & x < 0\,mm \\ -\frac{10}{29}x + \frac{4}{5} = -0.345x + 0.8, & 0mm < x < 1mm \\ -\frac{8}{29}x + \frac{48}{58} = -0.276x + 0.845, & 1mm < x < 1.5mm \\ -\frac{10}{29}x + \frac{25}{29} = -0.345x + 0.862, & 1.5mm < x < 2.5mm \\ 0, & x \geq 2.5mm \end{cases}$$



2.2 $\text{gradient} = \frac{\Delta C_B}{L_B} = \frac{\frac{4}{29}mM}{0.5mm} = \frac{8}{29}mM/mm = 0.276\,mM/mm$

2.3 $J = j \cdot A = \frac{D}{L}\Delta C \cdot \text{channel height} \cdot \text{channel length}$

$= \frac{D_B}{L_B}\Delta C_B \cdot 1mm \cdot 1mm$

$= \frac{0.5\,\mu m^2/msec}{0.5mm} \cdot \frac{4}{29}mM \cdot 1mm \cdot 1mm$

$mM = mmol/L = mmol/(dm^3)$

$= 1.379 \times 10^{-13}\,mol/s$

2.4 $\langle x(t)^2 \rangle = NL^2 = 2Dt$, $D = \frac{L^2}{2\delta t}$, $t = N \cdot \delta t$

$\sqrt{\langle x(t)^2 \rangle} = \sqrt{2Dt} = L \Rightarrow t = \frac{L^2}{2D} = \frac{L_B^2}{2D_B} = \frac{(500\mu m)^2}{2 \times 0.5\mu m^2/msec} = 250\,s = 4.167\,min$

B) 2.5 the gradient is twice as steep as before. $\Delta C_B' = 2\Delta C_B = 2 \cdot \frac{4}{29}mM = \frac{8}{29}mM$

the concentration of M at the left side of the middle channel should be the same as in section A of this question. $C_A' = C_A = \frac{33}{58}mM$, $\Delta C_A' = \Delta C_A = \frac{25}{58}mM$,

$C_B' = C_A' - \Delta C_B' = \frac{17}{58}mM$, $\Delta C_C' = C_B' - C_C = \frac{17}{58}mM - 0mM = \frac{17}{58}mM$.

$$\Delta C_A' : \Delta C_B' : \Delta C_C' = \frac{25}{58}mM : \frac{8}{29}mM : \frac{17}{58}mM = 25:16:17$$

$$\text{so } R_A' : R_B' : R_C' = \frac{L_A'}{A D_A \beta_A} : \frac{L_B'}{A D_B \beta_B} : \frac{L_C'}{A D_C \beta_C} = \frac{L_A'}{D_A \beta_A} : \frac{L_B'}{D_B \beta_B} : \frac{L_C'}{D_C \beta_C}$$

$$= \frac{L_A'}{0.4\mu m^2/msec \cdot 0.8} : \frac{L_B'}{0.5\mu m^2/msec \cdot 1.0} : \frac{L_C'}{0.4\mu m^2/msec \cdot 0.8}$$

$$= \frac{L_A'}{0.32} : \frac{L_B'}{0.5} : \frac{L_C'}{0.32} = 25:16:17$$

$$\Rightarrow \frac{25}{8}L_A' : 2L_B' : \frac{25}{8}L_C' = 25:16:17 \Rightarrow L_A' : L_B' : L_C' = 25:25:17 = 1:1:\frac{17}{25}$$

$$L_A' = L_B' = L_B = \frac{1}{2}mm = 0.5mm, \quad L_C' = \frac{17}{25}L_B' = \frac{17}{25}L_B = \frac{17}{25}\cdot 0.5mm = \frac{17}{50}mm = 0.34mm$$

$$[\Delta C_A' = \beta_A \Delta C_A' = 0.8 \cdot \frac{25}{58}mM = \frac{10}{29}mM, \quad \Delta C_B' = \beta_B \Delta C_B' = 1.0 \cdot \frac{8}{29}mM = \frac{8}{29}mM, \quad \Delta C_C' = \beta_C \cdot \Delta C_C' = 0.8 \cdot \frac{17}{58}mM = \frac{34}{145}mM$$

$$C_{0,A} = \beta_A \cdot C_0 = 0.8 \cdot 1mM = 0.8mM = \frac{4}{5}mM, \quad C_A' = C_{0,A} - \Delta C_A' = \frac{4}{5}mM - \frac{10}{29}mM = \frac{66}{145}mM$$

$$C_B' = \beta_B \cdot C_B' = 0.8 \cdot \frac{17}{58}mM = \frac{34}{145}mM, \quad C_C' = C_C = 0mM]$$

x is measured in mm,

when $x < 0mm$, $C'(x) = 1mM$;

when $0mm < x < 0.5mm$, $C'(x) = \beta_A (C_0 - \frac{\Delta C_A'}{L_A'}x) = 0.8(1mM - \frac{\frac{25}{58}mM}{0.5mm}x) = 0.8(-\frac{25}{29}x + 1) = -\frac{20}{29}x + \frac{4}{5}(mM)$;

$$[= C_{0,A} - \frac{\Delta C_A'}{L_A'}x = \frac{4}{5}mM - \frac{\frac{10}{29}mM}{0.5mm}x = -\frac{20}{29}x + \frac{4}{5}(mM);]$$

when $0.5mm < x < 1.0mm$ ($0.5mm + 0.5mm = 1.0mm$), $C'(x) = C_A' - \frac{\Delta C_B'}{L_B'}(x - L_A') = \frac{33}{58}mM - \frac{\frac{8}{29}mM}{0.5mm}(x - \frac{1}{2}mm) = -\frac{16}{29}x + \frac{49}{58}(mM)$;

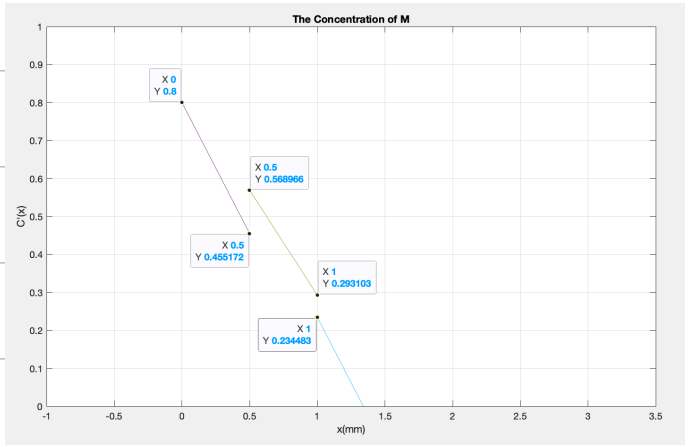
when $1.0mm < x < 1.34mm$ ($0.5mm + 0.5mm + 0.34mm = 1.34mm$), $C'(x) = \beta_C[C_B' - \frac{\Delta C_C'}{L_C'}(x - L_A' - L_B')] = 0.8[\frac{17}{58}mM - \frac{\frac{17}{58}mM}{\frac{17}{50}mm}(x - 0.5mm - 0.5mm)]$

$$= 0.8(-\frac{25}{29}x + \frac{67}{58}) = -\frac{20}{29}x + \frac{134}{145}(mM);$$

$$[= C_B' - \frac{\Delta C_C'}{L_C'}(x - L_A' - L_B') = \frac{34}{145}mM - \frac{\frac{34}{145}mM}{\frac{17}{50}mm}(x - 0.5mm - 0.5mm) = -\frac{20}{29}x + \frac{134}{145}(mM);]$$

when $x \geq 1.34mm$, $C'(x) = 0mM$

$$C'(x) = \begin{cases} 1, & x < 0mm \\ -\frac{20}{29}x + \frac{4}{5} = -0.69x + 0.8, & 0mm < x < 0.5mm \\ -\frac{16}{29}x + \frac{49}{58} = -0.552x + 0.845, & 0.5mm < x < 1.0mm \\ -\frac{20}{29}x + \frac{134}{145} = -0.69x + 0.924, & 1.0mm < x < 1.34mm \\ 0, & x \geq 1.34mm \end{cases}$$



3) Milk (10 points)

Milk is homogenized to simplify processing/distribution and provide a more uniform product to the consumer. Prior to homogenization, fat will separate out into a rich layer at the top of the milk under normal storage conditions for a short time (overnight). In the homogenization process, fat globules, which start off as roughly 5-μm diameter spheres, are broken into smaller pieces, such as 1-μm diameter spheres. This makes the commercial product, which in the grocery appears to not separate.

Consider the separation process as sedimentation in a 1 g gravitational field. In this exercise, we will compare the behavior of the fat globules to casein, a family of proteins in milk found as either individual molecules of 1.8-nm diameter or as micellar aggregates of 100-nm diameter. Assume that these protein structures can all be considered spherical objects

Assume that milk is mainly water, so density = 1 g/cm^3. Milk fat has a density of 0.9 g/cm^3, and thus floats. Assume that casein proteins are of density 1.3 g/cm^3; these will sink.

Useful constants:

$k_b = 1.38 \times 10^{-23}$ J/K Earth's gravitational field = g = 9.8 m/sec^2

T = 300K for this problem.

for water: density ρ_W=1 g/cm^3, viscosity = $\eta_W = 1 \times 10^{-2}$ poise;1 poise = 1 dyne*sec/cm^2

For the four entities of 5 μm fat globules, 1 μm fat globules, 100 nm casein aggregates, and 1.8 nm casein proteins:

3.1) **Steady state sedimentation**. First, look at the long-term (equilibrium) separation of each of the entities from mike. That is, calculate a characteristic sedimentation height/depth, z*, in earth's normal gravitational field for the two sizes of fat globules and two forms of protein.

3.2) **Does it separate?** Compared to an average milk carton height of 25 cm, do the numbers in section 3.1 explain what you observe for milk storage? That is, which of these four entities would separate out from milk given enough time? Assume that if |z*| > 25 cm, there is no separation for that entity and if |z*| < 25 cm, that structure separates out from solution.

3.3) **Time matters.** Another factor affecting separation is how long a system would take to reach equilibrium (no net flux). As a measure of this timescale, calculate the drift velocity for the four entities, and calculate how long it would take each to traverse the height of a milk carton (25 cm), at that velocity; don't take diffusion into consideration here. Does this help explain the differences in observed separation behavior of fat globules and casein proteins? Compare an observation period of one month against the time it takes to traverse the 25 cm carton for each entity.

Solution

entity	$\lvert m_{net} \rvert$ (kg)	$\lvert z^* \rvert$ (m)	separate, based on z*?	v (m/s)	time to traverse
5 μm fat globule	6.545E-15	6.455E-8	yes	1.361E-6	1.837E5 s (~2 days)
1 μm fat globule	5.236E-17	8.068E-6	yes	5.444E-8	4.592E6 s (~53 days or 1.5 months)
100 nm casein agg.	1.571E-19	2.689E-3	yes	1.633E-9	1.531E8 s (~5 years)
1.8 nm casein prot.	9.161E-25	4.611E2	no	5.29E-13	4.724E11 s (very long)

3.1 Direct use of the equation from class, $z^* = k_B T/(m_{net}*a)$; where a = acceleration. The absolute value for z* is reported in the table above. The sign of z* is going to depend on how the problem was set up with regards to the direction of acceleration vs. system axes. Most importantly, what the sign of z* means is key; for fat, the z*'s should describe an exponential curve that is increasing with distance from the bottom of the carton, while for casein, it should show exponential decay with distance from the bottom.

3.2 Interpretation of z*. the 5 μm fat, 1 μm fat, and 100 nm casein would all separate, based on z* alone. For the 1 μm fat and 100 nm casein, this is contrary to experience.

3.3 Solve Stokes formula for creeping flow.

That is, use $\mu = \frac{1}{6\pi\eta R} = \frac{v}{F}$ to get: $v = \frac{F}{6\pi\eta R}$. As before, $F = m_{net}*a$.

The time it would take to traverse the carton is listed in the table above. Only the 5 μm fat has time to separate.

Note that using the drift velocity to estimate time to set up a profile is different than using $\sqrt{\langle x(t)^2 \rangle}$. This is more appropriate when there is a drift velocity and a long distance to traverse.