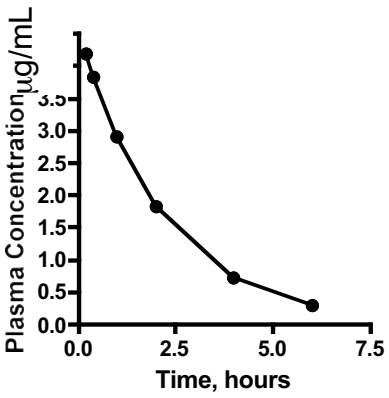
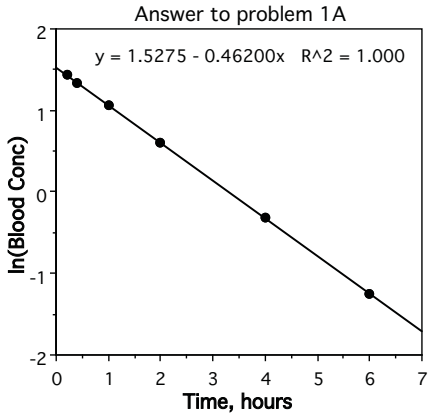
20.201 '13 Pharmacokinetics Homework ANSWER KEY
9 Oct 2013

Please prepare your answers in electronic format and submit the answers before or on October 11. While this is not a graded problem set, you will get credit for completing the problem set and submitting it. An answer key will be posted on October 12, so no credit will be given for late submissions!

1) A drug was injected intravenously in 200 g rats at a dose of 185 mg/kg and blood samples were obtained at several times. The plasma concentrations are as follows:

Time (Hours)	Plasma Concentration (mg/ml)
0.2	4.2
0.4	3.83
1	2.9
2	1.83
4	0.726
6	0.288





a) What type of kinetics is observed? Zero order, first order, second order? Why?

A plot of the log of the plasma concentration versus time is linear, so it is likely that the elimination is governed by first-order kinetics.

b) Is there a plasma half-life for the drug? If so, what is it?

Yes, since the kinetics are first-order. The slope of the log plot is -0.462 so the rate constant is 0.462 hr^{-1} and the half-life = 0.693/0.462 = 1.5 hours.

c) What is the rate constant for loss of the drug from plasma? -slope = 0.462 hr^{-1}

d) Calculate the volume of distribution of the drug.

The Y-intercept of the log plot is 1.528, so the plasma concentration at t = 0 is 4.6 μg/mL. With a dose of 37 μg, the V_d = 37 μg/4.6 μg/mL = 8.0 mL. This is much smaller than the total body

water of a rat (60% * 0.2 kg = 120 mL), so the drug is probably limited to the blood compartment.

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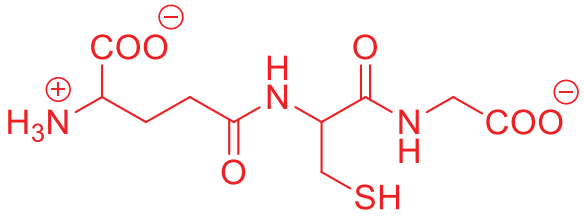
2) In addition to its role in phase II metabolism, glutathione provides one line of protection in the cell against electrophiles and oxidants. Answer the following questions:

a) Draw the structure of glutathione at pH 7.4. What is its net charge?

Net charge at pH 7.4 = -1



γ-glutamylcysteinylglycine

b) If the second order rate constant for the reaction of glutathione with cisplatin at 37 °C is 1 x 10^{-2} $M^{-1}s^{-1}$, what is the pseudo-first order rate constant of the reaction at 10 mM glutathione and what is the half-life of cisplatin under these conditions?

PLEASE NOTE: SECOND-ORDER KINETICS WILL NOT BE COVERED ON THE QUIZ! THIS IS A "HOLD-OVER" FROM PREVIOUS YEARS AND AN INTERESTING THOUGHT PROBLEM ABOUT THE REACTIVITY OF AN ANTICANCER DRUG (CISPLATIN) WITH AN ENDOGENOUS NUCLEOPHILE. THE RATE CONSTANTS ARE TOTALLY FICTITIOUS.

$$d[\text{cisplatin}]/dt = d[\text{GSH}]/dt = -k[\text{GSH}][\text{cisplatin}]$$

At [GSH] = 10 mM:

$$d[\text{cisplatin}]/dt = d[\text{GSH}]/dt = -k(10 \times 10^{-3}\ M))[\text{cisplatin}]$$

With k = 1 x 10^{-2} $M^{-1}s^{-1}$:

$$d[\text{cisplatin}]/dt = d[\text{GSH}]/dt = -(1 \times 10^{-2}\ M^{-1}s^{-1})(10 \times 10^{-3}\ M)[\text{cisplatin}]$$

Therefore, $k^* = (1 \times 10^{-2}\ M^{-1}s^{-1}) \times (10 \times 10^{-3}\ M) = 1 \times 10^{-4}\ s^{-1}$

$t_{1/2} = 0.692/(1 \times 10^{-4}\ s^{-1}) = 6.92 \times 10^{3}$ s

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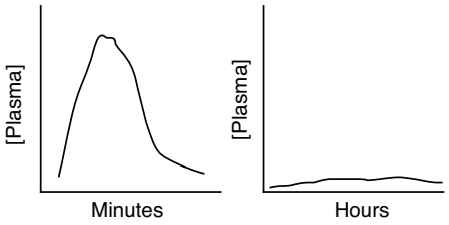
4) Glyceryl trinitrate, erroneously called nitroglycerin, is metabolized by reaction with glutathione-organic nitrate reductase, high levels of which are found in the liver.

a) Shown below are hypothetical profiles for the plasma pharmacokinetics of nitroglycerin following sublingual (under the tongue) and oral (swallowed) doses. Match each profile with the correct route of administration and rationalize your choices.

Oral: the graph on the right side; the oral dose is absorbed from the gut and enters the portal venous blood that flows directly into the liver where the drug is extensively metabolized.
Sublingual: the left graph below; sublingual administration allows the drug to enter the general (systemic circulation) without first passing through the liver.

b) What is the relationship of the liver to the circulatory system that results in these pharmacokinetic profiles?

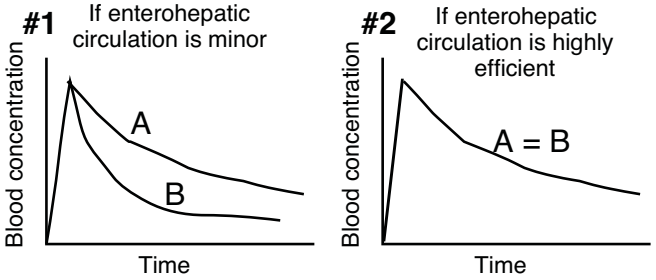
All blood flow form the gut enters the liver in the portal circulation before it enters the general circulation.



5) Two chemicals (A and B) behave identically in the body except that, while both chemicals are removed from the body by excretion in the urine, chemical B is also excreted in the bile and is subject to enterohepatic circulation.

Sorry! Parts a and c are identical questions! I hope this did not escape your attention!

a) Compare the pharmacokinetics of the two chemicals by sketching plots of the log(blood concentration) *versus* time for their clearance from blood. Explain the basis for the differences, if any. Assume i.v. injection with instantaneous distribution.



A very tricky problem! There are two possible answers to this problem depending on the degree to which compound B is subjected to enterohepatic circulation. B has two routes of excretion: urine and bile. If enterohepatic circulation is inefficient (*i.e.*, B excreted in the bile is not subject to significant reabsorption from the gut into the blood), then B will be excreted in the feces to an appreciable extent. In this case, plot #1 would be correct since two routes of excretion will result in a faster loss of the drug from the blood compared to a single route. However, if absorption of B from the gut is highly efficient (*i.e.*, none is excreted in the feces), then its major route of excretion will be in the urine, as with A. In this case, plot #2 is correct.

b) If the blood half-life of the chemical A is 10 hours, what is the first-order rate constant for its clearance from the blood?

$k = 0.692/t_{1/2} = 0.692/10 = 0.0692\ hr^{-1}$

c) Sketch plots of ln(blood concentration) *versus* time for chemical A if: (i) it is excreted in the urine; and (ii) it is excreted in both urine and bile.

See part a above

d) Assume that chemical B is metabolized in the liver. the log(blood concentration) *versus* time for chemical B in (i) presence and (ii) absence of liver failure.

Liver failure slows the metabolism of the drug and thus slows its clearance from the blood.

6) Kinetics of transport and metabolic processes.

a) Define the difference between zero-order and first-order pharmacokinetics using two examples: (1) the metabolism of a chemical; and (2) the transport of a chemical across a cell membrane

Zero order kinetics occurs when an enzyme or transport protein becomes saturated with substrate molecules. Enzymes and transport proteins generally operate with first order kinetics (i.e., the rate of the "reaction" depends on the concentration of the substrate). However, when the substrate concentration is high enough, the rate of the enzyme or transport protein reaches a maximum and higher substrate concentrations do not increase the rate of the "reaction."

b) What is the predominant mechanism of transportation of foreign chemicals across cell membranes? What "order" is this process in terms of transport kinetics? Justify your answer.

You could answer either diffusion or protein-mediated transport (i.e., active, secondary, facilitated transport), since there is no certainty in the proportion of contribution of each transport mechanism. All are generally first-order processes since the rate depends on the concentration of the transported chemical.

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7) You are the Chief Pharmacokineticist at Acme Pharmaceuticals and you have begun pre-clinical testing of a new drug to treat excessive nose hair. The drug was injected rapidly into the tail vein of a rat and blood samples were withdrawn at various times. Answer the following questions about the pharmacokinetics of the new drug.

a) If the rate constant for elimination of the drug from blood is first-order and has a value of 0.692 min^{-1}, what is the half-life of the drug (*i.e.*, $t_{1/2}$)? Either state the value of the half-life or write the equation used to calculate the half-life.

$t_{1/2} = 0.692/k$ $t_{1/2} = 0.692/(0.692\ min^{-1}) = 1\ min$

b) If the rate constant is zero-order with a value of 10 mg/min, how much of the drug has been eliminated after 1 min? How much of the drug is eliminated between the 5^{th} and 6^{th} minutes after injection? How much drug is eliminated after 10 min?

$$dX/dt = k \bullet [A]^0 = k$$
$$dX = k \bullet dt$$
Integrate: $X_t = X_0 - k \bullet t$

The above equations help to understand the nature of zero order kinetics. The rate of elimination is 10 mg/min, then zero order kinetics dictates that 10 mg of drug are eliminated every minute and that the rate does not vary as a function of the drug concentration. So, after 1 min, 10 mg of drug is eliminated; between the 5th and 6th minute, 10 mg of drug is eliminated; and after 10 min, 100 mg of drug is eliminated (10 mg/min • 10 min).

c) What factors might contribute to the elimination of the drug from the blood?

Metabolism, renal or hepatic excretion, distribution to tissues.

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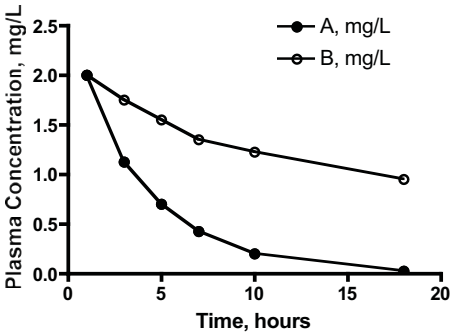
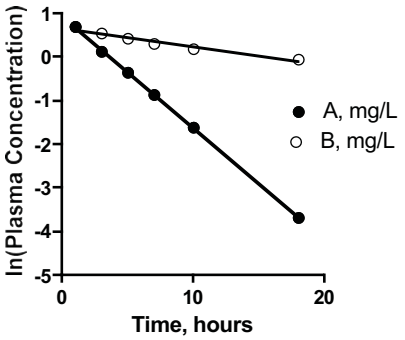
8) The following data were obtained in pharmacokinetic studies of two different drugs given by rapid intravenous injection to Sprague-Dawley rats. Plasma samples were obtained at various times and the concentration of drug determined by HPLC.

a) What type of kinetics *likely* govern the plasma concentration of the drugs?





A plot of plasma concentration versus time is non-linear, while a plot of the natural log of the plasma concentration versus time is linear. Thus, it is likely that first-order govern the elimination of both drugs from the blood. Kudos to those of you who fitted the data to a model for second order kinetics (see table below; plot of 1/[drug] versus time) and found a better fit (i.e., better correlation coefficient). However, this is over-thinking the problem: simple second-order kinetics is highly unlikely and it is more likely that there would be contributions from multicompartment effects or metabolic pathways. Also, keep in mind that I made up the data to illustrate first-order kinetics, so it is completely biologically-irrelevant!

Reaction Order	Differential Rate Law	Integrated Rate Law	Characteristic Kinetic Plot	Slope of Kinetic Plot	Units of Rate Constant
Zero	$-\frac{d[A]}{dt} = k$	$[A] = [A]_0 - kt$	[A] vs t	$-k$	mole L^{-1} sec^{-1}
First	$-\frac{d[A]}{dt} = k[A]$	$[A] = [A]_0 e^{-kt}$	ln [A] vs t	$-k$	sec^{-1}
Second	$-\frac{d[A]}{dt} = k[A]^2$	$[A] = \frac{[A]_0}{1 + kt[A]_0}$	1/[A] vs t	k	L $mole^{-1}$ sec^{-1}

(Table from a very good web site:
http://www.chm.davidson.edu/vce/kinetics/integratedratelaws.html)

b) What is the plasma elimination rate constant and, if calculable, the half-life for each drug?

The slopes of the logarithmic plots are –0.26 for A and –0.043 for B so the elimination rate constants are 0.26 hr^{-1} and 0.043 hr^{-1}, respectively. The half-lives are 2.7 hours for A and 16 hours for B.

c) What is the volume of distribution of Drug A? (Assume a dose of 80 mg and that rats have the same water compartments as humans.) Explain the physiological significance of this volume.

V_d = dose/peak blood concentration The peak blood concentration can be estimated by extrapolating the plot of ln(plasma concentration) versus time to t = 0 and using the Y-intercept to calculate the plasma concentration of the drug at t=0. This amounts to 2.5 and 2.0 mg/L for A and B, respectively (assuming the drug distributes evenly between red blood cells and plasma. Otherwise, we'd need to convert plasma volume to whole blood volume). The V_d values are thus 32 L and 40 L for A and B, respectively. These volumes lie some where between interstitial fluid (~15 L) and total body water (~40 L; interstitial + intracellular). Perhaps there are some cells into which the drug does not distribute?

Note: Plasma concentrations are usually measured as total drug concentration, with the analytical methods causing release of any protein-bound drug. However, some drugs are measured as protein-bound and "free" or unbound. For this problem, we will consider total drug concentration.

Drug A

Time, hr	[A], mg/L
1	2
3	1.13
5	0.7
7	0.43
10	0.2
18	0.025

Drug B

Time, hr	[B], mg/L
1	2
3	1.75
5	1.55
7	1.35
10	1.23
18	0.95

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9) What is the definition of half-life ($t_{1/2}$)? Derive the equation for calculating $t_{1/2}$.

The time it takes for the drug concentration to decrease by one-half. Derivation:

$$[A]_t = [A]_0 e^{-kt} \qquad \frac{[A]_t}{[A]_0} = 0.5 = e^{-kt_{1/2}} \qquad \therefore \ \ln(0.5) = 0.693 = -k \bullet t_{1/2}$$

20.201 Mechanisms of Drug Actions

Fall 2013