

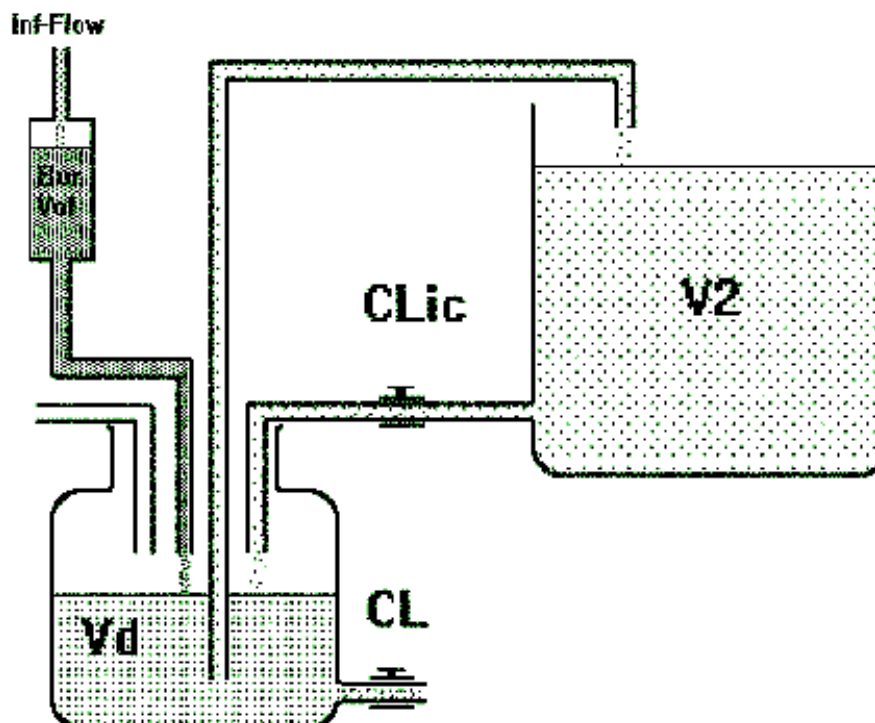
Beka 2 Cpt: Two Compartment Model Loading Dose and Maintenance Infusion

When drugs are administered rapidly and concentrations measured frequently the one-compartment model does not predict the time course of drug concentration very well, especially at early times. This is because it takes time for drug to distribute to the tissues and reach equilibrium. This experiment shows the time course of drug concentration in both central and tissue compartments after a single rapid injection (bolus) with and without a constant rate infusion using a two beaker system to represent a two compartment model. A third method of drug input, the first-order infusion, is added to the Bolus and constant rate infusion to show that it is possible to achieve and maintain a target concentration even when the one-compartment model is not appropriate. The first-order infusion method has been shown to be useful in achieving the target concentration for lignocaine when it is used as an anti-arrhythmic agent.

Experimental System

This experiment uses two beakers to simulate a central and a tissue compartment. The central compartment has a nominal volume of 1 L and a clearance of 0.1 L/min. The tissue compartment has a nominal volume of 4 L and an inter-compartmental clearance of 0.4 L/min. Water leaves both beakers under control of peristaltic pumps. These have been carefully set to achieve a flow of 0.1 L/min at a volume of 1 L for the central compartment and 0.4 L/min at a volume of 4 L for the tissue compartment. However, you may need to adjust the pump rate occasionally to keep the volume in each container close to the 1 L and 4 L marks. Water enters the central compartment at 0.1 L/min. During the infusion part of the experiment the pump flow is 0.09 L/min and the drip infusion is 0.01 L/min. Before starting the infusion and at its end this pump should be set at 0.1 L/min.

Diagram of the two compartment model system



Objectives

1. To observe the time course of drug concentration in the central and peripheral compartments of a two-compartment system.
2. To compare the time course after 3 types of input:
 - a. Single Bolus Dose
 - b. Single Bolus Dose and Constant Rate Infusion
 - c. Single Bolus Dose plus First-Order and Constant Rate Infusion

[The inputs studied will depend on student numbers in the course]

Overview

This experiment is quite demanding in terms of the number of concentration measurements that have to be made.

Each group member has a different responsibility:

Member A: takes a sample from each compartment at appropriate time points

Member B: constructs a calibration curve and performs concentration measurements for each sample using the spectrophotometer.

Member C: Ensures the other group members follow protocol during the initial stages of the experiment, then enters the results into a shared file (Excel) for analysis.

The class will be divided into groups with one group doing each experiment.

There are 3 different experiments:

Bolus

A single rapid injection into the central compartment beaker.

Bolus + Zero-order Infusion

A rapid injection is given into the central compartment followed immediately by starting a constant rate (zero-order) infusion for 2 hours.

Bolus + Zero-order + First-order Infusion

A rapid injection and constant rate (zero-order) infusion are given into the central compartment for 2 hours. A loading dose sufficient to fill the tissue compartment (larger beaker) is put into the burette of the giving set. The flow rate through the burette and its volume determine the first-order input rate. As the drip continues it slowly dilutes the solution in the burette and the rate at which drug enters the central compartment decreases until eventually the concentration in the burette is the same as that in the plastic bag providing the solution for the constant rate (zero-order) infusion.

Methods

DO NOT ADJUST THE BURETTE FLOW CONTROL (small plastic device with wheel located below the burette). This has been pre-adjusted to a flow rate of 0.1 L/min.

1. Sampling: Take 4 mL samples (with large pipette) from the central compartment and tissue compartment beakers at the times shown in Table 2. The -1 time sample can be taken any time **before** the amaranth is injected.
2. Dosing Scheme: The target concentration is 10 mg/L. Use the following doses for each experiment:

Bolus

A loading dose of 10 mg (0.4 mL of 25 mg/mL amaranth) should be injected into the central compartment beaker.

Bolus + Zero-order Infusion

Make up a solution of amaranth in the raised 2 L bottle to a final concentration of 100 mg/L. The flow rate from the drip chamber has been adjusted to 0.1 L/min. Unclip the flow stopper (not the flow controller) and make sure the flow rate is 0.1 L/min. Also check that the burette contains 100 mL of amaranth solution while the drip is running.

Add 0.4 mL of 25 mg/mL amaranth to the central compartment (10 mg bolus dose) then immediately start the zero-order infusion by placing the infusion tube into the central compartment. The amaranth infusion rate should be 1 mg/min.

Bolus + Zero-order + First-order Infusion

Make up a solution of amaranth in the raised 2 L bottle to a final concentration of 100 mg/L. The flow rate from the drip chamber has been adjusted to 0.1 L/min. Unclip the flow stopper (not the flow controller) and make sure the flow rate is 0.1 L/min. Also check that the burette contains 100 mL of amaranth solution while the drip is running.

Add 0.4 mL of 25 mg/mL amaranth to the central compartment (10 mg bolus dose) then immediately start the first-order infusion by adding 1.6 mL of 25 mg/mL amaranth (40 mg) to the 100 mL volume in the burette and placing the infusion tube into the central compartment. Shake the burette gently to mix the solution.

3. Calibration Curve: The spectrophotometer is set for absorbance at 518 nm. Calibrate the spectrophotometer using the amaranth standard solutions (0.1, 0.5, 2, 5, 10, 20, 25 mg/L). Use water as a zero concentration reference. You should do this approximately 30 minutes into the experiment, once everything is under control.

Repeat the calibration curve at 60 minutes after performing the first set of calibration measurements. These measurements should be used to calculate imprecision and bias (see below).

4. Concentration Measurement: Measure the absorbance of the central and tissue compartment samples and determine the concentration using the calibration curve. Enter the concentrations in Table 2.
5. Graphical Analysis: Enter the absorbances and calculate the measured concentrations in an Excel worksheet. Create a graph of time versus concentration in each compartment so that you can monitor the progress of your experiment.

6. Measurement of Physical Volumes and Flow Rates At **60 minutes** mark the level of the water in both beakers. When the experiment is over measure the volume of water that comes up to these marks. This is a measure of the volume of distribution of the central and tissue compartment. At 60 minutes collect all of the outflow from the central compartment beaker for 5 minutes and calculate the flow rate. This is a measure of the elimination clearance. After measuring the elimination clearance collect the water flowing from the central compartment beaker to the tissue compartment beaker for 1 minute and determine the flow rate between the beakers. This is a measure of inter-compartmental clearance. Return this water back to the tissue compartment beaker.
7. Infusion Concentration: When the infusion has stopped (after 120 minutes) take a sample out of the burette and measure the concentration after diluting it 10 fold e.g. put 1 mL of the burette contents into 9 mL of water in a volumetric flask.
8. Calculation of Concentrations
 - a. Plot a graph of amaranth standard concentration versus absorbance using the results from the first set of measurements.
 - b. Draw a best fit line 'by eye' through the points (should you force the line through zero?).
 - c. Use the slope and intercept of the line to calculate concentrations of amaranth sampled from the two compartment system and the 60 and 120 minute intra-assay controls.

$$Concentration_{PREDICTED} = \frac{(Absorbance_{MEASURED} - Intercept)}{Slope}$$

Assay Error - Imprecision, Bias and Lower Limit of Quantitation

An essential part of any scientific assay is an understanding of the measurement error. The error can be defined by imprecision and bias. The assay performance is also described by the lower limit of quantitation.

1. Imprecision: This is a measure of the error reproducing a measurement. It is calculated from the standard deviation of the replicates divided by their mean. When there are only two replicates (C_a and C_b) the standard deviation is calculated from:

$$\begin{aligned} \text{Mean} &= (C_a + C_b) / 2 \\ \text{Error}_a &= \text{Mean} - C_a \quad \text{Error}_b = \text{Mean} - C_b \\ \text{Imprecision} = \text{SD} &= \text{Square root of } (\text{Error}_a^2 + \text{Error}_b^2) \end{aligned}$$

$$\text{Relative Imprecision \%} = \text{SD} / \text{Mean} \cdot 100$$

2. Bias: This is a measure of how close a measurement is to its true value. Bias is calculated from the difference between the true and measured value. When there are only two replicates the Mean Bias is calculated from:

$$\begin{aligned} \text{Bias}_a &= \text{True} - C_a \quad \text{Bias}_b = \text{True} - C_b \\ \text{Bias}_{\text{mean}} &= (\text{Bias}_a + \text{Bias}_b) / 2 \end{aligned}$$

$$\text{Relative Bias \%} = \text{Bias}_{\text{mean}} / \text{True} \cdot 100$$

3. Lower limit of quantitation (LLOQ): Defined as the lowest intra-assay control concentration with an imprecision of 20 % or less.

Computer Analysis - Data Entry

1. Data: Export the time and concentration data from the Excel worksheet to .csv format for MONOLIX. The first column should be ID, the second DVID, the third TIME, and the fourth DV. The central compartment times and concentrations should be entered first and have the number 1 in the DVID column. Do not enter values at time 0. The tissue compartment times and concentrations should be entered beneath the central compartment values and have the number 2 in the DVID column. The format of the MONOLIX-ready csv file should look something like this:

ID	DVID	TIME	DV
1	1	1	2.03
1	1	3	2.34
1	1	4	2.42
1	1	5	2.59
1	1	7	2.84
1	1	10	2.98
1	1	15	3.46
1	1	20	3.82
1	1	25	4.38
1	1	30	4.21
1	1	35	3.74
1	1	40	3.74
1	1	50	2.42
1	1	60	1.75
1	1	75	1.78
1	1	90	1.28
1	1	105	0.39
1	1	120	0.83
1	2	1	0.30
1	2	3	0.55
1	2	4	0.69
1	2	5	0.61
1	2	7	0.41
1	2	10	0.44
1	2	15	0.72
1	2	20	1.34
1	2	25	1.61
1	2	30	1.36
1	2	35	1.50
1	2	40	1.67
1	2	50	1.78
1	2	60	1.87
1	2	75	1.67
1	2	90	1.67
1	2	105	0.94
1	2	120	1.25

2. In MONOLIX, select the data file and click 'Use header'.
3. Edit the beka model code (beka_mlxt.txt available from course website) to suit the experiment that you did (bolus, bolus with zero-order infusion, or bolus with first-order and zero-order infusion). Then compile it in MONOLIX and choose it as your model.

4. Enter your initial parameter estimates and fix the standard deviation of fixed effects to 0.
5. Use the comb2 residual error model for both compartment concentrations
6. Save as beak_Project.mat in your Pharmacometrics Data\BEKA 2 Cpt Experiment folder and run.

Graphical Analysis

1. Using Excel plot a graph of the measured concentrations for central and tissue compartments against time. Obtain a copy of this graph from other groups so that you can see the pattern for each of the 3 dosing experiments.
2. Calculate the AUC_{inf} from the measured central compartment concentrations using the trapezoidal rule. Derive the Clearance from the total dose administered (all inputs) and AUC_{inf} . The total dose includes the bolus loading dose into the central compartment and the amount of drug infused at a constant rate during the 30 minute infusion. The first-order infusion group will need to work out how much was given from the starting and final concentrations of amaranth in the 100 mL burette volume.

Assignment

Your assignment should include the following results:

1. Calibration curve graph
2. Table 1 (Assay Table)
3. Table 2 (Sample Measurements)
4. Table 3 (Parameter Estimates)
5. Graph of the measured concentrations (linear and log scales)
6. Graph showing the computer fit to the data
7. Table of Monolix parameter estimates
8. Beka.lib model
9. Answers to the following questions:
 - 9.1. Explain why the recommended doses are suitable for reaching the target concentration of 10 mg/L.
 - 9.2. What are potential sources of error in the calibration curve? How can these be reduced or allowed for?
 - 9.3. What are the sources of error in the intra-assay control measurements? Why are intra-assay controls used?
 - 9.4. Briefly describe the beka.lib model used by Monolix.
 - 9.5. Bolus Dose Group Only: Calculate the AUCinf from the measured central compartment concentrations using the trapezoidal rule. Derive the Clearance from the total dose administered (bolus) and AUCinf.
 - 9.6. Calculate the tissue compartment half-life from $0.7 \cdot V_2/CL_{ic}$ and the burette half-life from $0.7 \cdot \text{Burette Volume/Drip Flow Rate}$.
 - 9.7. If V_2 was known to be 6000 mL what loading dose is required in the burette for the first-order infusion?
 - 9.8. If V_2 was known to be 6000 mL what other change to the dose administration system would be needed to achieve and maintain a target concentration of 10 mg/L?

TABLE 1 - ASSAY ERROR

True Conc (mg/L)	Absorbance Replicates	Measured Conc (mg/L)	Mean (mg/L)	Error Mean-Conc (mg/L)	SD (mg/L)	Bias %	Imprecision %
0.1	a.	a.		a.			
	b.	b.		b.			
0.5	a.	a.		a.			
	b.	b.		b.			
1.0	a.	a.		a.			
	b.	b.		b.			
2.0	a.	a.		a.			
	b.	b.		b.			
5.0	a.	a.		a.			
	b.	b.		b.			
10.0	a.	a.		a.			
	b.	b.		b.			
15.0	a.	a.		a.			
	b.	b.		b.			
20.0	a.	a.		a.			
	b.	b.		b.			

a. = first calibration measurement b. = second calibration measurement (performed 60 minutes later)

TABLE 2 - TWO COMPARTMENT OBSERVATIONS

Time (min)	Actual Time (min:sec)	Absorb Central	Central (mg/L)	Absorb Tissue	Tissue (mg/L)	Time Interval (min)	AUC _i (mg/L•min)
Pre Dose							
0							
1							
2							
3							
4							
5							
7							
10							
15							
20							
25							
30							
35							
40							
50							
60							
75							
90							
105							
120							
SUM AUC _i							

TABLE 3 - TWO COMPARTMENT PARAMETER ESTIMATES

Parameter Name	Method	Value	Units
V1 (Central Beaker)	Measured		
CL (Central exit flow rate)	Measured		
V2 (Tissue Beaker)	Measured		
CLic (Tissue exit flow rate)	Measured		
T _½ Central	Graph		
T _½ Tissue	Graph		
T _½ .7•V1/CL	Computer		
AUC _{Tlast}	Bolus Group Only		
AUC _{extrap}	Calculate using Table 2		
AUC _{inf}			
CL			
V1		Computer	
CL	Computer		
V2	Computer		
CLic	Computer		
a C1 (residual error)	Computer		
b C1 (residual error)	Computer		
a C2 (residual error)	Computer		
b C2 (residual error)	Computer		
Lower Limit of Quantitation	Calibration Curve		